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5   Article type   : Original

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10 **Title Page:**

11 **Title**: Body composition and genetic lipodystrophy risk score associate with nonalcoholic fatty

12 liver disease and liver fibrosis (120/120 characters)

13

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28   **Keywords**: genetics; polygenic risk score; SNP; fibrosis; steatosis

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45   **List of Abbreviations**:

46   APRI, AST to platelet ratio index. BMI, body mass index. CT, computed tomography. DXA,

47   dual-energy X-ray absorption. FHS, Framingham Heart Study. LPR, liver-phantom ratio. LPRS,

48   lipodystrophy polygenic risk score. NAFLD, nonalcoholic fatty liver disease. MGI, Michigan

49   Genomics Initiative.

50

53

54   **Disclosures:**

55   Vincent Chen, Andrew Wright, Brian Halligan, Yanhua Chen, Xiaomeng Du, Samuel

56   Handelman, Michelle Long, Douglas P. Kiel, Elizabeth Speliotes: nothing to disclose

57

58   **Writing Assistance**: None

59

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Vincent Chen: study design, data analysis and interpretation, and drafting of the manuscript

Andrew Wright: study design, data analysis and interpretation, and drafting of the manuscript

Brian Halligan: data analysis and interpretation, and critical review of the manuscript

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Abstract:

Background: 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. Methods: 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 SNP 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. Results: Using sex-stratified multivariable linear regression, greater central body fat associated with increased hepatic steatosis, while greater lower extremity body fat associated

90   with decreased hepatic steatosis. Greater appendicular lean mass was associated with decreased

91   hepatic steatosis in men but not in women. A polygenic risk score for lipodystrophy (regional or

92   global loss of adipose tissue) was associated with increased hepatic steatosis, increased liver

93   fibrosis, and decreased lower extremity fat mass. Conclusions: Greater central body fat

94   associated with increased hepatic steatosis while greater lower extremity body fat and, in men,

95   greater appendicular lean mass were associated with decreased hepatic steatosis. A genetic risk

96   score for lipodystrophy was associated with NAFLD and liver fibrosis. Our results suggest that

97   buffering of excess energy by peripheral fat and muscle may protect against NAFLD and liver

98   fibrosis in the general population.

99

100   263/275 words

101   **Introduction**

102   Nonalcoholic fatty liver disease (NAFLD) is characterized by excessive triglyceride

103   accumulation in the liver in the absence of significant alcohol use or other underlying cause (1).

104   NAFLD is the most common chronic liver disease worldwide, affecting 20-40% of the general

105   population, and is associated with metabolic conditions such as obesity, diabetes, and

106   dyslipidemia (2-4). Unfortunately, treatment options remain limited and a better understanding

107   of the pathophysiology underlying NAFLD will be critical in developing more effective

108   treatments.

109

110   While NAFLD is associated with obesity, approximately 25% of patients with NAFLD are not

111   obese (5). This finding suggests that not all fat contributes equally to NAFLD risk: it may be

112   regional adiposity rather than overall adiposity that contribute to liver steatosis. Visceral fat is

113   associated with increased risk of NAFLD and progression to hepatic fibrosis (6-8). In contrast,

114   gluteofemoral and lower extremity fat correlate with decreased transaminases and may be

115   protective against hepatic steatosis (9-11). Despite the importance of different fat depots in

116   NAFLD, the literature on lower extremity fat in NAFLD is limited to studies of a few hundred

117   subjects, mostly in Asian populations.

118

119   Skeletal muscle mass may also protect against NAFLD. Sarcopenia, a condition of low skeletal

120   muscle mass, has been linked to increased risk of NAFLD and advanced fibrosis (12-14).

121   Skeletal muscle function too may influence NAFLD: greater hand grip strength has been linked

122   to decreased NAFLD prevalence (15). Further, NAFLD is associated with substitution of adipose

123   tissue in skeletal muscle (16) and increased insulin resistance of skeletal muscle (17). However,

124   again, the literature on muscle fat and NAFLD risk is limited to only small studies of a few

125   hundred subjects and has not been studied in a Western population.

126

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

128   body composition (18). For example, individuals with NAFLD in the presence of the PNPLA3

129   I148M variant are less frequently obese than those with the ancestral allele at *PNPLA3* (19, 20).

130   More recently, several genetic contributors to lipodystrophy have been identified. Lipodystrophy

131   is characterized by global or selective deficiency of adipose tissue in the absence of malnutrition

132   or a catabolic state (21). While most patients with NAFLD are not overtly "lipodystrophic",

133   NAFLD is itself a form of ectopic fat accumulation and is highly prevalent in patients with

134   familial lipodystrophy (21, 22). Lipodystrophy was previously viewed primarily through the lens

135   of rare familial diseases, but relative lipodystrophy may also exist in the general population as a

136   continuous trait (23). Non-familial lipodystrophy is heritable and a previously-reported

137   lipodystrophy polygenic risk score (LPRS) predicts insulin resistance and decreased lower

138   extremity adiposity, a feature of lipodystrophy (24). Whether people with an increased polygenic

139   lipodystrophy score store more fat in the liver is not known.

140

141   We hypothesize that NAFLD may be a marker of partial lipodystrophy in the population. We test

142   whether body composition—specifically, fat distribution and muscle bulk, strength, and fat

143   content—associate with NAFLD in a large, well-characterized European-ancestry cohort, the

144   Framingham Heart Study (FHS). Further, we test whether individuals with higher lipodystrophy

145   polygenic scores have higher prevalence of NAFLD and liver fibrosis using FHS and another

146   cohort, Michigan Genomics Initiative (MGI).

147

148   **Methods**

149

150   *Ethics statement*

151 All FHS participants provided written informed consent approved by the Boston University

152 Institutional Review Board and Hebrew SeniorLife Institutional Review Board. All MGI

153 participants provided written informed consent approved by the institutional review board of the

154 University of Michigan (Ann Arbor, MI). All research performed in this paper was approved by

155 the institutional review board of the University of Michigan (Ann Arbor, MI).

156

157 *Cohorts*

158 This study included two cohorts. The first was FHS, a multigenerational prospective cohort study

159 of residents in and around Framingham, Massachusetts characterizing a broad array of

160 phenotypes related to cardiovascular health (25). We included the FHS Offspring and Generation

161 3 sub-cohorts. Between 1995 and 1998, 3492 participants from the Offspring cohort completed

162 the seventh clinical examination (exam 7). Between 2008 and 2011, 3399 participants from

163 Generation 3 completed the second clinical examination (exam 2). These examinations included

164 a detailed medical history, physical examination, collection of blood specimens, and

165 measurement of anthropometric data including hand grip strength assessment (for the Offspring

166 cohort, hand grip strength measurements were collected separately) (25). Selected subjects

167 participated on sub-studies that involved additional testing including multidetector CT scan,

168 whole-body DXA scan, and quadriceps strength testing. We excluded participants who reported

169 excess alcohol use (>21 alcoholic drinks per week for men and >14 alcoholic drinks per week for

170 women). The physical activity index is a composite score calculated based on participant

171 responses to questions regarding different levels of physical activity and sleep patterns over a 24

172 hour period (26). Grip and quadriceps strength were measured as described previously (27, 28).

173

174 In FHS, a subset of participants underwent genotyping with a 550K SNP array (Affymetrix 500K

175 Dual GeneChip and 50K gene-centered MIP set) (29). Imputation was performed using the 1000

176 Genomes cosmopolitan panel March 2012(v3) on the Michigan Imputation Server

177 (https://imputationserver.sph.umich.edu/index.html) (30).

178

179 MGI is a prospective cohort with ongoing enrollment; all patients undergoing elective surgery at

180 Michigan Medicine (Ann Arbor, Michigan) are potentially eligible for enrollment in this cohort.

181 Enrollment involves genotyping of peripheral blood on the Illumina HumanCoreExome v.12.1

182   array, a GWAS and exome array consisting of >500,000 SNPs (31). In addition, full laboratory

183   information and billing codes are available.

184

185   *Hepatic steatosis and muscle attenuation assessment*

186   Between 2008 and 2011, multidetector abdominal CT scans (64-slice, General Electric Health

187   Care) were performed as described previously (32). The mean attenuation (Hounsfield units)

188   from three regions in the liver as well as from a calibration control (phantom) was calculated.

189   The liver-phantom ratio (LPR) was calculated by dividing the mean hepatic attenuation by the

190   attenuation of the calibration control ("phantom"). LPR ≤ 0.33 was used to define NAFLD as

191   reported previously (33). Muscle attenuation was measured at the left and right paraspinous

192   muscles at the mid-abdominal level as previously described (34).

193

194   *Body composition assessment*

195   Whole-body and regional measures of lean mass and fat mass were obtained by DXA scan (GE

196   Lunar Prodigy fan beam densitometer) as described previously (28, 35). For the Offspring

197   cohort, these DXA scans were obtained from 1996-2001. For Generation 3, they were obtained

198   in 2010 and 2011. The DXA protocol was the same between the two cohorts. Lower extremity

199   fat mass was a reported measure that combined the fat mass in both legs. Total fat mass was also

200   reported. Appendicular lean mass was calculated by combining bilateral upper and lower

201   extremity lean mass. Central fat mass was calculated by subtracting the bilateral upper and lower

202   extremity fat mass from the whole body total fat mass. These measures were scaled to body

203   weight by dividing the respective values by each participant's weight in kilograms and reported

204   as a percentage.

205

206   *Clinical and laboratory measurements*

207   The age of the participant documented at the time of the clinical examination was used for the

208   analysis. Body mass index (BMI) was defined as weight (kg)/height (m^2). Diabetes was defined

209   by the presence of a fasting glucose ≥ 126 mg/dL, hemoglobin A1c ≥ 6.5%, medical history of

210   physician-diagnosed diabetes, or receiving medication for the treatment of diabetes.

211   Hypertension was defined as a systolic blood pressure ≥ 140 mmHg, diastolic blood pressure ≥

212   90 mmHg, physician-diagnosed hypertension, or receiving antihypertensive therapy. Metabolic

213 syndrome was diagnosed based on the National Cholesterol Education Program's Adult

214 Treatment Panel III guidelines (36).

215

216 In both FHS and MGI, the AST to platelet ratio index (APRI) score was used for noninvasive

217 assessment of liver fibrosis. In MGI, we defined cirrhosis based on presence of an ICD-9 code

218 (571.5, 571.2, and 571.6), ICD-10 code (K74.X, K70.2-4, and K71.7), or a text search for

219 cirrhosis. Text search of radiology and pathology reports was performed for the character

220 "cirrho," and participants with that character were flagged as having cirrhosis with the following

221 exceptions: (1) if the word "without" or "no" appeared in the same sentence as "cirrho," subjects

222 were considered to not have cirrhosis; (2) if the words "primary biliary cirrhosis" appeared in a

223 sentence, that sentence was ignored for text search purposes; and (3) if the words "evaluate,"

224 "assess," or "rule out" appeared in a sentence with "cirrho," that sentence was ignored for text

225 search purposes. A gastroenterologist (V.L.C.) manually reviewed 200 randomly-selected text

226 strings and identified no false positive cirrhosis diagnoses.

227

228 *Statistical analysis: non-genetic*

229 Differences in characteristics between participants with and without NAFLD were determined

230 using a t-test for continuous variables and chi-square test for proportions.

231

232 For the graphs of % NAFLD vs. central fat mass, we computed sex-specific percentiles of central

233 body fat mass and identified the percentage of participants within each percentile with NAFLD,

234 as defined by LPR < 0.33. These percentages were then stratified separately by high vs. low

235 appendicular lean mass or lower extremity fat mass (i.e. above vs. below sex-stratified median).

236 Univariable linear regression was performed on % NAFLD vs. central fat mass and graphed as a

237 smoothed linear model (Fig. 2). Addition of quadratic terms did not improve the regression ($p >$

238 0.05 for all comparisons).

239

240 Multivariable linear regression analysis was performed to determine the relationship between

241 liver steatosis (as measured by negative LPR) and measures of body composition and strength.

242 For these analyses negative LPR (increased liver steatosis) was treated as the dependent

243 (outcome) variable. Note that a higher LPR is associated with decreased liver steatosis, so that

244 *positive* beta values for covariates actually imply *decreased* liver steatosis. Since this is

245 counterintuitive, to increase readability, we used negative LPR as the dependent variable, so that

246 positive beta values imply *increased* liver steatosis. The primary independent (exposure)

247 variables were (1) appendicular lean mass, (2) lower extremity fat mass, (3) central fat mass, (4)

248 grip strength, (5) quadriceps strength, and (6) muscle steatosis (negative muscle attenuation, for

249 reasons similar to those for LPR, as above). These were inverse normally transformed in order to

250 improve interpretability and treated as continuous independent variables (18). β-values for body

251 composition parameters were reported as the effect of one rank unit (1/6th of the total variation of

252 that trait) on LPR. In sensitivity analyses, we ran these regressions with non-transformed

253 covariates and the results were qualitatively the same (data not shown). Regression analyses

254 were stratified by sex. Proportion of variation explained by variables was estimated by

255 comparing sums of squares for individual variables in the model with the total sum of squares.

256

257 Analyses were performed using R version 3.4.4 (R Foundation for Statistical Computing,

258 Vienna, Austria; www.r-project.org) with the tidyverse package (www.tidyverse.org). A two-

259 sided *p* value of 0.05 was used to determine statistical significance.

260

261 *Genetic analysis*

262 Only participants of European ancestry were included in genetic analyses. First, principal

263 components were calculated based on LASER/TRACE (https://laser.sph.umich.edu), using the

264 World imputed reference panel (37). To exclude individuals who did not cluster with the

265 European group, individuals with | Z score | > 3 for any of the first three principal components

266 were removed. Then, the principal components were recalculated on remaining individuals using

267 the European panel.

268

269 LPRS was calculated as previously reported (24). In brief, LPRS was the total number of

270 disease-causing alleles at each of 53 previously-reported single nucleotide polymorphisms

271 (SNPs) each individual carried. These SNPs were selected based on being associated with

272 increased serum insulin, decreased high-density lipoprotein cholesterol, and increased

273 triglycerides; they were tested and shown to associate with decreased lower extremity fat

274 indicative of lipodystrophy (24). In cases when the genotype at that SNP was imputed rather than

275   directly genotyped, we used dose, i.e. probability of having that given genotype at the SNP. First,

276   we calculated in FHS the percentage of participants with NAFLD (defined as LPR ≤ 0.33 (38))

277   as a function of LPRS, and performed logistic regression using proportion NAFLD as the

278   dependent variable and number of risk alleles as the independent variable. This was graphed as a

279   smoothed linear model (Fig. 3A). There was no improvement in the model after addition to

280   quadratic terms for number of risk alleles. Next, LPRS was used as an independent variable for

281   phenotypes including the continuous traits of hepatic steatosis (negative LPR) and APRI, as well

282   as the binary traits of cirrhosis or NAFLD. These models were adjusted for age, age^2, and the

283   first ten principal components (to account for ethnic differences) and either stratified by or

284   adjusted for sex as well.

285

286   **Results**

287   *Study population*

288   Fig. 1 illustrates FHS participant selection for this study. Data on CT-measured hepatic steatosis,

289   whole body DXA scan, quadriceps and hand grip strength, physical activity index, and clinical

290   examination were available from 1,389 individuals from Generation 3 and 1,032 from Offspring.

291   After excluding individuals with excess alcohol intake (Methods), 1,300 individuals from

292   Generation 3 and 949 individuals from Offspring remained for a total of 2,249 individuals.

293

294   Overall, the cohort was 49% male with mean age 58.5 ± 11.8 years (Supp. Table 1). Prevalence

295   of NAFLD was 28.3%. Table 1 shows clinical parameters stratified by presence vs. absence of

296   NAFLD. Participants with NAFLD were older and more frequently male, and had higher

297   prevalence of diabetes, hypertension, and the metabolic syndrome, as well as expected

298   differences in biochemical profiles ($p < 0.05$ for all comparisons).

299

300   *Body composition and muscle strength*

301   Table 1 depicts body composition and muscle strength in FHS participants with or without

302   NAFLD in univariate analyses. In the overall cohort, participants with NAFLD had greater

303   amounts of total fat and central fat, and smaller amounts of total lean mass and appendicular (i.e.

304   arms and legs) lean mass (Table 1; $p < 0.0001$ for all). There was no difference in lower

305   extremity fat mass ($p = 0.26$). Grip strength was greater in participants with NAFLD ($p = 0.007$)

306   while there was no difference in quadriceps strength based on NAFLD status ($p = 0.92$, Table 1).

307   Sex-stratified analysis was fairly similar overall. However, among women, grip strength no

308   longer differed based on NAFLD status (Supp. Table 2). Among men, lower extremity fat was

309   higher and grip strength lower in those with NAFLD(Supp. Table 3).

310

311   *Effect of body composition on NAFLD and fibrosis*

312   Next, we sought to identify whether differences in body composition associated with increased

313   risk of NAFLD in FHS. Because central fat, lower extremity fat, and appendicular lean mass are

314   correlated with one another, we investigated whether they independently affected hepatic

315   steatosis after adjustment for one another. Fig. 2 shows percentage of participants with NAFLD

316   as a function of percentile of central fat mass, stratified by sex and either lower extremity fat

317   mass or appendicular lean mass status. In all analyses, higher central body fat associated with

318   greater NAFLD prevalence ($p < 0.0001$). High lower extremity body fat associated with lower

319   NAFLD prevalence in both men and women ($p < 0.05$). High appendicular lean mass associated

320   with lower NAFLD prevalence in men ($p < 0.05$), but not in women ($p = 0.16$).

321

322   We then performed multivariable linear regressions to determine whether body composition

323   parameters independently associated with hepatic steatosis as a continuous variable in FHS. We

324   used as minimal covariates in all models age, physical activity, alcoholic drinks per week, and

325   cohort. On multivariable analysis, in both men and women, greater central fat mass associated

326   with more liver steatosis, while greater lower extremity fat mass associated with less liver

327   steatosis (Table 2 and Supp. Table 4). In men, but not in women, greater appendicular lean mass

328   associated with less hepatic steatosis (Table 2 and Supp. Table 4). In both sexes, greater

329   paraspinal muscle fat associated with increased hepatic fat (Table 2 and Supp. Table 4). These

330   findings persisted in models adjusting for minimal covariates, central fat mass, lower extremity

331   fat mass, appendicular lean mass, and either hand grip strength, quadriceps strength, or muscle

332   attenuation (Supp. Table 4). In no model did quadriceps strength or hand grip strength associate

333   with hepatic steatosis (Supp. Table 4).

334

335   For reference, among women, one standard deviation of liver steatosis corresponds to an LPR of

336   0.06. Thus, the fact that in women each rank unit of central fat mass was associated with a

337   change in liver steatosis of 0.02 implies significant explanatory power. Among men, the liver

338   steatosis standard deviation was 0.07, and the β values associated in men with each inverse-

339   normalized unit of central fat mass (0.03), lower extremity fat (-0.01), and appendicular lean

340   mass (-0.01) were relatively large. The combination of central fat, lower extremity fat,

341   appendicular lean mass, and muscle attenuation accounted for 14.4% of variation in liver

342   steatosis in women and 18.1% in men.

343

344   We also tested whether these fat depots associated with APRI, a noninvasive marker of fibrosis,

345   in FHS. In men, greater central body fat associated with increased APRI: each rank unit increase

346   was associated with 0.031 increase in APRI (95% CI 0.003-0.061). There were no other

347   associations between body composition and APRI in men. In women, there was no association

348   between body composition and APRI.

349

350   *Genetic lipodystrophy risk score*

351   We further explored whether genetic predisposition to partial lipodystrophy influences liver-

352   related phenotypes in FHS and MGI, using a lipodystrophy polygenic risk score (LPRS)

353   (Methods). In FHS, mean (SD) LPRS was 52.8 (4.3) and in MGI, 55.1 (4.6). Participants with

354   NAFLD had higher LPRS than those without NAFLD (53.2 vs. 52.7, $p = 0.006$). We validated

355   that, consistent with conferring a partial lipodystrophy phenotype, higher LPRS associated with

356   dyslipidemia and insulin resistance and decreased lower extremity fat ($p < 0.001$ for all), but did

357   not affect central fat or overall body mass index (Fig. 3A). These findings held when men and

358   women were analyzed separately (Supp. Figs. 1-2).

359

360   Unadjusted NAFLD prevalence increased significantly with increasing LPRS in the overall

361   cohort (Fig. 3B; $p < 0.005$). This association remained in women ($p < 0.005$) but not in men ($p =$

362   0.16; Supp. Figs. 1 and 2). On multivariable linear regression, higher LPRS was associated with

363   increased liver steatosis in the overall FHS cohort (Fig. 3A) and among women (Supp. Fig. 1A).

364   In men, LPRS did not associate with liver steatosis but the trend was in the same direction as in

365   women (Supp. Fig. 2A). Adjusted odds ratio for NAFLD per allele of LPRS in the overall cohort

366   was 1.04 (95% CI 1.01-1.06); overall, individuals in the 90th percentile for LPRS were 36% more

367   likely to have NAFLD than those in the 10th percentile.

368

369　Finally, we examined the effect of the LPRS on liver fibrosis. We first performed linear

370　regression with APRI as the dependent and LPRS as the independent variable in FHS, but the

371　association was not significant. Therefore, we tested this hypothesis in a hospital based cohort,

372　the Michigan Genomics Initiative (MGI) (n = 19,239). In the overall MGI cohort, each allele of

373　LPRS was associated with OR 1.02 for cirrhosis diagnosis (95% CI 1.00-1.03, p = 0.03; Fig.

374　3C). Individuals in the 90th percentile of LPRS were 22% more likely to have cirrhosis than those

375　in the 10th percentile. Adjusted OR among men was 1.02 (95% CI 1.00-1.04, p = 0.03; Supp. Fig.

376　2C) and among women there was no significant association though consistent direction of effect

377　(OR 1.01, 95% CI 0.99-1.03; Supp. Fig. 1C). After adjustment, higher LPRS was associated with

378　greater APRI (Fig. 3A), indicating increased fibrosis. While the association was not significant

379　when men and women were analyzed separately, the directions of effect trended in the same

380　direction (p = 0.06 and 0.10 in women and men, respectively; Supp. Figs. 1-2).

381

382　**Discussion**

383　In summary, we show that greater central fat mass associated with increased hepatic steatosis,

384　while greater lower extremity fat mass and appendicular lean mass associate with less hepatic

385　steatosis. In addition, greater paraspinal muscle fat was associated with increased hepatic

386　steatosis. Overall, these four body composition parameters accounted for a substantial proportion

387　of variation in hepatic steatosis: 14% in women and 18% in men. Finally, higher LPRS led to

388　increased hepatic steatosis and fibrosis in the population, with a 36% and 22% increased risk,

389　respectively, in individuals with high vs. low LPRS.

390

391　Our findings suggest that NAFLD may be a marker of partial lipodystrophy in the population.

392　Lipodystrophy is classically thought of as a rare monogenic disease, but partial lipodystrophy (or

393　even differences in fat depot distribution) may exist as a continuous trait in the population (21,

394　24). We found that LPRS associates with increased hepatic steatosis and fibrosis. Further, among

395　participants with NAFLD, non-obese participants had a lower appendicular fat than did obese

396　subjects. Together, these findings imply that inadequate appendicular adipose tissue may

397　contribute to NAFLD and fibrosis.

398

399   Interestingly, we found LPRS associates with increased hepatic steatosis in women but not men.

400   This may be because men have lesser lower extremity fat (7.7% vs. 13.2%) and consequently

401   greater total lean mass (67% vs. 56%) and appendicular lean mass (31% vs. 24%) than women.

402   We note that while there was no statistical association between appendicular lean mass and

403   hepatic steatosis in women, the direction of effect was the same in both men and women

404   suggesting that in both genders muscle mass is protective against NAFLD. Thus, it may be that

405   in men muscle may be able to buffer excess calories more than in women who have less mass

406   and thus use fat to buffer excess calories. A genetic decrease in lower extremity fat may

407   therefore confer proportionally greater risk for NAFLD in women than in men who already have

408   a small amount of lower extremity fat depot.

409

410   We also found that muscle steatosis associates with NAFLD, likely because when excess energy

411   cannot be buffered by adipose tissue it may be stored in ectopic fat depots such as muscle and

412   liver. Unlike muscle steatosis however, muscle strength as measured by quadriceps and hand

413   grip strength did not in this study correlate with NAFLD. Thus, while it appears that muscle fat

414   is associated with increased hepatic steatosis and muscle mass with decreased steatosis, muscle

415   strength does not appear to associate with NAFLD.

416

417   Consistent with earlier findings, in our study greater central (visceral) adiposity associates with

418   increased prevalence of NAFLD while greater lower extremity adiposity associates with

419   decreased NAFLD prevalence (10, 39). The mechanisms underlying these differences in disease

420   risk based on fat location remain incompletely-characterized but may relate to differences in

421   macrophage and cytokine profiles in visceral fat and direct blood flow from visceral fat to the

422   liver via the portal circulation (40, 41). In both men and women, body composition metrics

423   explained a substantial proportion of variation in hepatic steatosis (18% and 14%, respectively).

424   Additional studies will be required to better understand the biology underlying these

425   relationships.

426

427   Our study is limited by including only participants of European ancestry. The association

428   between hepatic steatosis and LPRS may only reflect an association with one particular form of

429   lipodystrophy and may not be generalizable to all lipodystrophy. Finally, DXA cannot

430   distinguish between subcutaneous lower extremity fat and deeper lower extremity fat layers, or

431   between muscle and other lean tissues such as skin and connective tissue, though there is no

432   clear pathophysiologic reason non-muscle lean tissue would be related to NAFLD.

433

434   Strengths of the study include that it is a large, population-based study, which increases the

435   generalizability of our findings. In addition, CT and DXA are excellent quantitative noninvasive

436   measurements of hepatic steatosis and body composition, respectively, allowing rigorous testing

437   of how body composition relates to NAFLD. Full genotypic information was available for

438   genetic analysis. We also were able to assess for effects of the LPRS on fibrosis using two

439   independent methods.

440

441   In conclusion, we demonstrated a novel association between partial lipodystrophy and liver

442   steatosis and fibrosis in the population. We also report a connection between CT-measured

443   hepatic steatosis and muscle steatosis, and to our knowledge this is the first such report in a

444   Caucasian population. These results suggest that interventions directed at increasing muscle

445   quantity, decreasing overall fat burden, or shifting fat distribution toward appendicular fat may

446   be beneficial in reducing NAFLD and preventing its complications.

447

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571

572 **Figure Legends**

573

574 **Figure 1**: Study design flowchart. CT, computed tomography. DXA, dual-energy X-ray

575 absorptiometry. PAI, physical activity index.

576

577 **Figure 2**: Effect of central body fat, lower extremity body fat, and appendicular lean mass on

578 nonalcoholic fatty liver disease prevalence. Percentage of participants with nonalcoholic fatty

579 liver disease (NAFLD) based on sex-specific percentile of central fat mass. (A-B) Stratified

580 based on lower extremity fat (LEF) mass above or at the median ("high") vs. below the median

581 ("low"), for women (A) and men (B). (C-D) Stratified based on appendicular lean mass (ALM)

582 above or at the median ("high") vs. below the median ("low"), for women (C) and men (D).

583 Shaded areas represent 95% confidence intervals.

584

585 **Figure 3**: Effect of LPRS on multiple traits. (A) Percentage of FHS participants with NAFLD

586 based on number of LPRS alleles. (B) Percentage of MGI participants with cirrhosis based on

587 number of LPRS alleles. The shaded area represents the 95% confidence interval. (C) Forest plot

588 of associations between LPRS and multiple traits. The scale on the x axis is the allele effect size

589 (β) of one LPRS allele, divided by the standard deviation of the specific parameter; i.e. what

590 proportion of standard deviation is accounted for by each additional allele of LPRS. Error bars

591 depict 95% confidence interval. Liver steatosis represents negative liver-phantom ratio. Muscle

592 steatosis represents negative muscle attenuation in Hounsfeld units. All traits except APRI were

593 measured in FHS; APRI was measured in MGI. HDL, high-density lipoprotein. TRIG,

594 triglycerides. LDL, low-density lipoprotein. HOMA-IR, homeostatic model of insulin resistance.

595 BMI, body mass index.

Table 1: Clinical and laboratory characteristics

Characteristic	Overall (n = 2249)	No NAFLD (n = 1613)	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
Body mass index (kg/m^2)	28.1 (5.0)	26.9 (4.4)	31.1 (5.1)	< 0.0001
Systolic blood pressure (mmHg)	120.2 (15.9)	118.5 (15.8)	124.4 (15.3)	< 0.0001
Diastolic blood pressure (mmHg)	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
Liver-phantom ratio	0.34 (0.06)	0.37 (0.03)	0.27 (0.07)	< 0.0001

NAFLD, nonalcoholic fatty liver disease

Table 2: Body composition and strength metrics

Characteristic	Overall (n = 2249)	No NAFLD (n = 1613)	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	61.1% (9.5)	62.1% (9.6)	58.6% (8.8)	< 0.0001

(kg/kg*100%)

|				
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

NAFLD, nonalcoholic fatty liver disease

Table 3: linear regression on hepatic steatosis

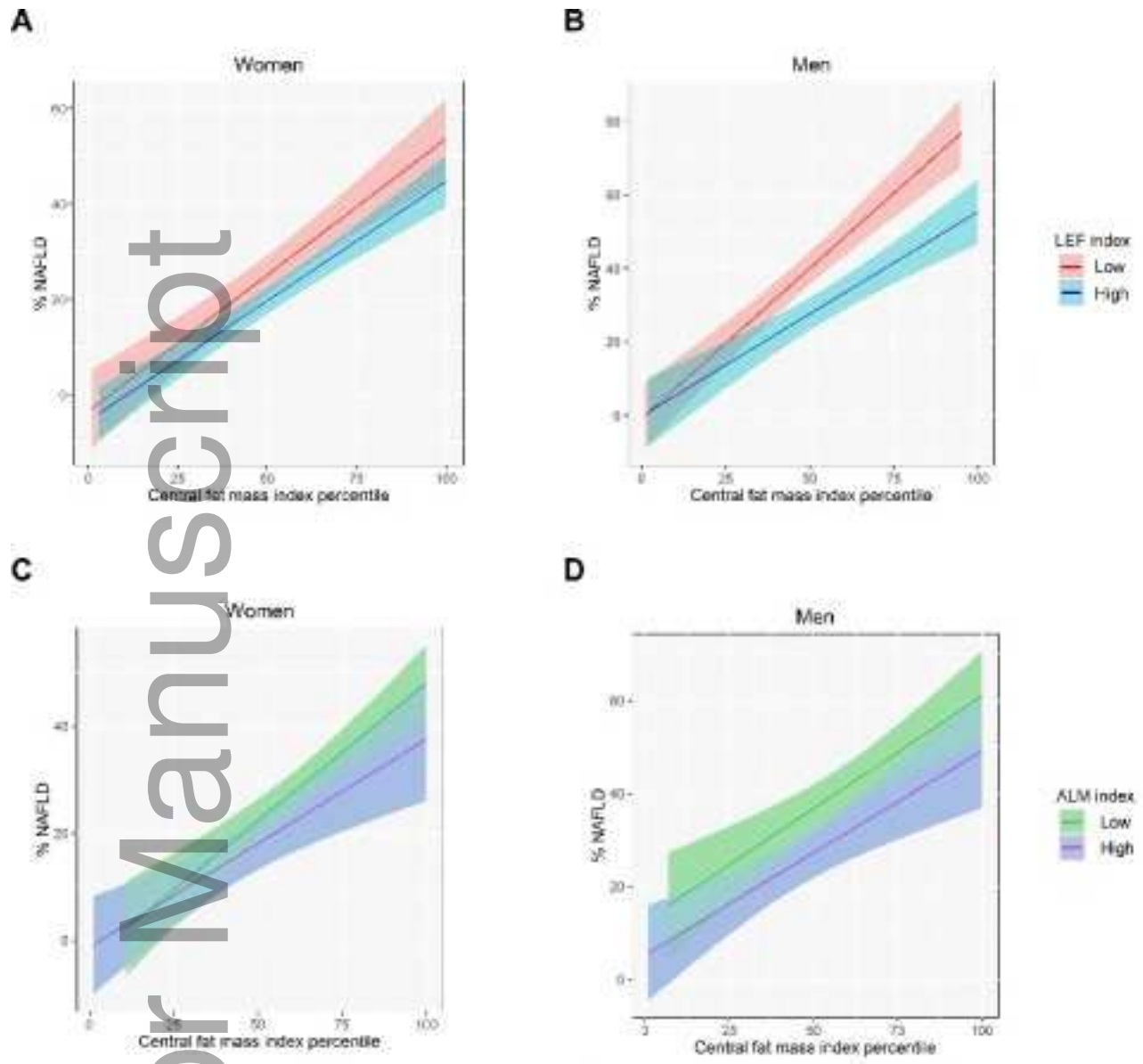| Parameter	Beta coefficient	
	Women	Men
Central fat index	0.0193 *	0.0272 *
Lower extremity fat index	-0.0072 *	-0.012 *
Appendicular lean mass index	-0.0038 NS	-0.0106 *
Muscle steatosis	0.0013 *	0.0009 *

Linear regression on hepatic steatosis, as defined as liver-phantom ratio (see Methods for details). Muscle steatosis was defined as negative muscle attenuation (Hounsfeld units). All four traits were inverse normalized. Beta coefficients correspond to the effect of one rank unit (approximately 1/6

of the total variation). Analysis was stratified by sex. Covariates were age, age^2, physical activity index, drinks per week, and cohort (i.e. Offspring vs. Generation 3), and the four parameters in the above table. NS, not significant; * $p < 0.05$

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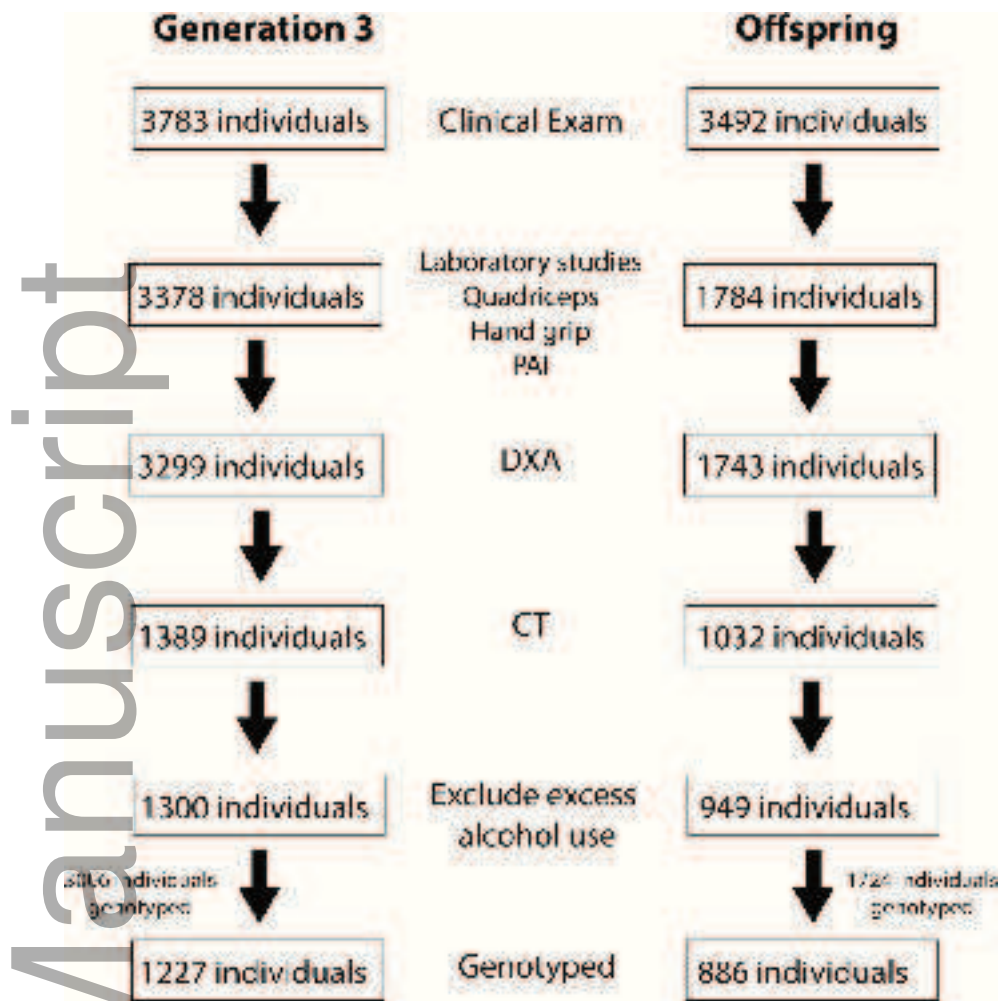
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