Disentangling the genetics of lean mass

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ABSTRACT

Background: Lean body mass (LM) plays an important role in mobility and metabolic function. We previously identified five loci associated with LM adjusted for fat mass in kilograms. Such an adjustment may reduce the power to identify genetic signals having an association with both lean mass and fat mass.

Objectives: To determine the impact of different fat mass adjustments on genetic architecture of LM and identify additional LM loci.

Methods: We performed genome-wide association analyses for whole-body LM (20 cohorts of European ancestry with $n = 38{,}292$) measured using dual-energy X-ray absorptiometry) or bioelectrical impedance analysis, adjusted for sex, age, age^2, and height with or without fat mass adjustments (Model 1 no fat adjustment; Model 2 adjustment for fat mass as a percentage of body mass; Model 3 adjustment for fat mass in kilograms).

Results: Seven single-nucleotide polymorphisms (SNPs) in separate loci, including one novel LM locus (*TNRC6B*), were successfully replicated in an additional 47,227 individuals from 29 cohorts. Based

on the strengths of the associations in Model 1 vs Model 3, we divided the LM loci into those with an effect on both lean mass and fat mass in the same direction and refer to those as "*sumo wrestler*" loci (*FTO* and *MC4R*). In contrast, loci with an impact specifically on LM were termed "*body builder*" loci (*VCAN* and *ADAMTSL3*). Using existing available genome-wide association study databases, LM increasing alleles of SNPs in *sumo wrestler* loci were associated with an adverse metabolic profile, whereas LM increasing alleles of SNPs in "*body builder*" loci were associated with metabolic protection.

Conclusions: In conclusion, we identified one novel LM locus (*TNRC6B*). Our results suggest that a genetically determined increase in lean mass might exert either harmful or protective effects on metabolic traits, depending on its relation to fat mass. *Am J Clin Nutr* 2019;109:276–287.

Keywords: body composition, skeletal muscle, body fat, meta-analysis of genome-wide association studies, metabolic profile

Introduction

Lean body mass (LM), consisting mostly of skeletal muscle, plays a role in mobility (1) and metabolic function. It is well established that high fat mass results in insulin resistance, increased risk of type 2 diabetes, and dyslipidemia. Observational studies indicate that lean mass adjusted for weight or fat mass is inversely associated with insulin resistance and metabolic abnormalities (2). However, the causal effects of lean mass on metabolic traits are unclear. Adipocytes and myocytes share common mesenchymal ancestry (3), and factors (genetic and/or environmental) stimulating the development of mesenchymal stem cells toward the myocyte lineage instead of the adipocyte lineage may lead to more favorable body composition.

In a recent large-scale study, we identified 5 loci associated with LM adjusted for fat mass in kilograms (4). In that study, we were primarily interested in genes contributing to lean mass independent of those regulating fat mass (4). Because lean mass is positively correlated with fat mass and may even be stimulated to increase by the mechanical demands of carrying more fat mass, our previous results were adjusted for fat mass in the statistical models. A potential limitation of this strategy of adjusting for fat mass is that the ability to identify genetic signals with an impact on both lean mass and fat mass will be reduced. Some studies suggested lean mass and fat mass have a substantial genetic correlation (5, 6).

Nevertheless, the *FTO* signal was found to be significantly associated with lean mass after fat adjustment, and the direction of this association was the same as the association with fat mass found in other studies (7). To identify additional lean mass loci and to gain more insight into the lean-fat mass relation and its health consequences, in this study we applied different statistical models with either no fat adjustment or 1 of 2 fat-adjustment models: fat as a percentage of body mass, or fat in absolute kilograms.

For identified lean mass single-nucleotide polymorphisms (SNPs), we also aimed to evaluate the associations with a variety of musculoskeletal and metabolic traits. Finally, we aimed to explore if the associations with musculoskeletal and metabolic parameters differed for significant loci identified in models without fat mass adjustment compared with those having the strongest association in models with fat mass adjustment.

Methods

We performed a genome-wide association study meta-analysis on whole body lean mass in a set of discovery cohorts (Stage I) and then meta-analyzed the discovery SNPs in replication cohorts (Stage II), followed by a combined analysis with discovery and replication cohorts. The total sample size for the combined analysis was 85,519 individuals of European ancestry from 47 studies. Because this research is not a clinical trial, it does not need to be registered.

Study population

The Stage I Discovery sample comprised 38,292 individuals of European ancestry drawn from 20 cohorts with a variety of epidemiological designs and participant characteristics (**Supplemental Table 1** and **Supplemental Note 1**). Whole body lean mass was measured using dual-energy X-ray absorptiometry (DXA) (10 cohorts, $n = 21{,}074$) and bioelectrical impedance analysis (BIA) (10 cohorts, $n = 17{,}218$). Of the 20 cohorts, 15 consisted of males and females, whereas 2 had males, and 3 had females only. In total, the cohorts included 22,705 women and 15,587 men.

Twenty-nine additional studies were used for replication with a total sample size of 47,227 subjects of European ancestry. The Stage II Replication included either cohorts with existing genome-wide association study (GWAS) data that were unavailable at the time of the Stage I Discovery or cohorts without GWAS data who agreed to undergo de novo genotyping. Because some of the replication cohorts performed de novo genotyping, there were fewer data points for SNPs that

Supplemental Tables 1–9, Supplemental Notes 1 and 2, Supplemental Figures 1 and 2, Sources of Support, and Acknowledgments are available from the Supplementary data link in the online posting of the article and from the same link in the online table of contents at https://academic.oup.com/ajcn/.

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Abbreviations used: AF, allele frequency; BIA, bioelectrical impedance analysis; BMD, bone mineral density; CAD, coronary artery disease; Chol, cholesterol; DM, diabetes mellitus; DXA, dual-energy X-ray absorptiometry; EQTL, expression quantitative trait loci; FDR, false discovery rate; FN, femoral neck; Fx, fracture; GWAS, genome-wide association study; GWS, genome-wide significant; HbA1C, glycated hemoglobin; HOMA-IR, homeostasis model assessment of insulin resistance; KASP, KBioScience Allele-Specific Polymorphism single-nucleotide polymorphism genotyping system; LD, linkage disequilibrium; LM, lean body mass; LM IA, lean mass increasing allele; LS, lumbar spine; MAF, minor allele frequency; Q-Q, quantile–quantile (plots); sGWS, suggestive genome-wide significance; SNP, single-nucleotide polymorphism; Trig, triglycerides.

Received March 22, 2018. Accepted for publication September 11, 2018.

First published online February 5, 2019; doi: https://doi.org/10.1093/ajcn/nqy272.

were newly genotyped than for SNPs that were imputed from already available GWAS studies. All studies were approved by their institutional ethics review committees, and all participants provided written informed consent.

Lean mass measurements

Lean mass was measured in all cohorts using either DXA or BIA. DXA provides a 3-compartment body composition assessment based on specific X-ray attenuation properties: bone mineral, lipid (triglycerides, phospholipid membranes, etc.), and lipid-free soft tissue. Each pixel on the DXA scan is quantitatively partitioned into these 3 tissue types. For the cohorts with DXA measures, the phenotype used for these analyses was the lipid-free, soft tissue compartment that is referred to as lean mass and is the sum of body water, protein, glycerol, and soft tissue mineral mass. Two lean mass phenotypes were used: whole body lean mass and appendicular lean mass. The latter was obtained by DXA while considering only pixels in the arms and legs collectively (bone mineral excluded), which has been demonstrated to be a valid measure of skeletal muscle mass (8). Some of the cohorts estimated body composition using BIA, which has been detailed in our previous work (4). For BIA cohorts with specific resistance and reactance measures, we used the validated equation from Kyle et al. with an R^2 of 0.95 between BIA and DXA to calculate the appendicular lean mass (9).

Stage 1: Genome-wide association analyses in discovery cohorts

Genome-wide genotyping was carried out in each study on a variety of platforms following standard manufacturer protocols. Quality control was performed independently for each study. To facilitate meta-analysis, each group performed genotype imputation with IMPUTE (10) or MACH (11) software using HapMap Phase II release 22 reference panels (CEU or CHB/JPT as appropriate). Overall imputation quality scores for each SNP were obtained from IMPUTE ("proper_info") or MACH ("rsq_hat"). Details on the genotyping platform used, genotype quality control procedures, and software for imputation used for each study are presented in **Supplemental Table 2**. Because the project started before the creation of denser imputation panels, only Hap Map II based imputation was available.

Study-specific genome-wide association analyses with lean mass and different lean mass models.

Details about study-specific genome-wide association analyses and meta-analyses have been described previously (4). Briefly, in each study, a multiple linear regression model with additive genetic effect was applied to test for phenotype–genotype associations using ~2.0–2.5 million genotyped and/or imputed autosomal SNPs. Because lean mass is correlated with fat mass and height, we prespecified 3 models of adjustment: Model 1: adjustment for sex, age, age^2, height; Model 2: adjustment for sex, age, age^2, height, total body fat percentage; model 3: adjustment for sex, age, age^2, height, total body fat mass in kilograms. Because no fat mass adjustment was performed in Model 1, it will identify lean mass SNPs that also may have an effect on fat mass in the same direction. In contrast, because Model 3 is adjusted for fat mass (in kilograms), it will identify SNPs that are associated with lean mass independent of fat mass. Other covariates adjusted in the model included ancestral genetic background using principal components and, when appropriate, study-specific covariates such as clinical center for multicenter cohorts. For family-based cohorts, including the Framingham Study, ERF, UK-Twins, Old Order Amish Study, and the Indiana cohort, familial relatedness was taken into account in the statistical analysis (4).

Meta-analyses.

Meta-analyses were conducted using the METAL package (www.sph.umich.edu/csg/abecasis/metal/). We used the inverse variance weighting and fixed effect model approach. Before meta-analysis, we filtered out SNPs with a low minor allele frequency, MAF (<1%), and poor imputation quality (proper_info < 0.4 for IMPUTE and rsq_hat < 0.3) and applied genomic control correction where the genomic control parameter lambda (λ_{GC}) was >1.0.

We used quantile–quantile (Q-Q) plots of observed compared with expected $-\log_{10}$ (P value) to examine the genome-wide distribution of P values, Manhattan plots to report genome-wide P values, regional plots for genomic regions within 100 kb of top hits, and forest plots for meta-analyses and study-specific results of the most significant SNP associations. For all 3 models, a threshold of $P < 5 \times 10^{-8}$ was prespecified as being genome-wide significant (GWS), whereas a threshold of $P < 2.3 \times 10^{-6}$ was used to select SNPs for a replication study (suggestive genome-wide significant—sGWS).

Stage 2: Replication

In each GWS or sGWS locus, we selected the lead SNP with the lowest P value for replication. In addition, GWS or sGWS SNPs that had low linkage disequilibrium (LD) with the lead SNPs ($r^2 < 0.5$) were also selected for replication. Both in silico replication and de novo genotyping for replication were conducted. Replication was carried out in 24 cohorts that did not have data available at the time of the initial discovery efforts ($n = 47{,}227$ for whole body; $n = 42{,}360$ for the appendicular LM; **Supplemental Table 3**), of which 6927 had available in silico genome-wide genotyping. De novo replication genotyping was done using KBioScience Allele-Specific Polymorphism (KASP) SNP genotyping system (in OPRA, PEAK25, AGES, CAIFOS, DOPS cohorts), TaqMan (METSIM), Illumina OmniExpress + Illumina Metabochip (PIVUS and ULSAM), or Sequenom's iPLEX (WHI) (**Supplemental Table 4**). Samples and SNPs that did not meet the quality control criteria defined by each individual study were excluded. Minimum genotyping quality control criteria were defined as: SNP call rate > 90% and Hardy–Weinberg equilibrium $P > 1 \times 10^{-4}$.

Meta-analysis of replication and discovery studies

In the replication stage, we meta-analyzed results from individuals of European descent only. A successful replication

was considered if the association *P* value in the combined meta-analysis (Discovery plus Replication) was GWS and less than the discovery meta-analysis *P* value. Using the METAL package, we also estimated I^2 to quantify heterogeneity and *P* values to assess statistical significance for a total of 12 associations (3 SNPs from Model 1, 4 from Model 2, and 5 from Model 3) that were replicated in the cumulative-meta-analysis. Appendicular lean mass was available in a subsample of those with whole body lean mass (n = 70,690 from 38 studies), and models 1–3 for appendicular lean mass were evaluated for the replicated GWS associations from the whole body lean mass analyses.

Annotation and enrichment analysis of regulatory elements

We predicted the function of coding variants by PolyPhen-2. For all replicated variants, we annotated potential regulatory functions based on experimental epigenetic evidence including DNase hypersensitive sites, histone modifications, and transcription factor-binding sites in human cell lines and tissues from the ENCODE Project and the Epigenetic Roadmap Project. We first selected SNPs in high LD ($r^2 \geq 0.8$) with GWAS lead SNPs based on the approach of Trynka et al. (12) We then identified potential enhancers and promoters in the GWAS loci (GWAS SNPs and SNPs in LD with the GWAS SNPs) across 127 healthy human tissues/normal cell lines available in the ENCODE Project and the Epigenetic Roadmap Project from the HaploReg4 web browser (13) predicted by ChromHMM (14). To evaluate if replicated GWAS loci were enriched with regulatory elements in skeletal muscle tissue, we performed a hypergeometric test. Specifically we tested whether estimated tissue-specific promoters and enhancers in a GWAS locus were enriched in 8 relevant skeletal muscle tissues/cell lines compared with enrichment in nonskeletal muscle tissues (119 tissues/cell lines). The permutation with minimum *P* value approach was performed to correct for multiple testing. Permutation *P* values < 0.05 were considered statistically significant. In addition, we also performed enrichment analyses in smooth muscle tissues/cells, fat tissue, brain, blood cells, and gastrointestinal tract tissues. The 8 skeletal muscle relevant tissues/cells were excluded when conducting enrichment analyses for other tissue types. Detailed information for tissue types and chromatin state estimation is described in the Supplemental Materials (**Supplemental Note 2**).

CIS-expression quantitative trait loci

We looked up *cis*-expression quantitative trait loci (EQTL) information from GTEx data on the 7 replicated GWS loci, SNPs rs2943656, rs9991501, rs2287926, rs4842924, rs9936385, rs10871777, and rs 733,381 with gene expression within 2Mb of the SNP position. Multiple testing was corrected by using a false discovery rate (FDR) *q* value of <0.05 to account for all pairs of SNP-gene expression analyses in multiple tissues.

Look-ups of replicated SNPs in GWAS of metabolic and musculoskeletal traits

For the 7 replicated lean mass SNPs, we performed look-ups of relevant metabolic and musculoskeletal traits using available results from published GWAS meta-analyses. The metabolic and musculoskeletal traits evaluated included % fat (15), BMI (7), coronary artery disease (16), type 2 diabetes (17), homeostasis model assessment of insulin resistance (HOMA-IR), (18), triglycerides (19), total cholesterol (19), LDL cholesterol (19), HDL cholesterol (19), hand grip strength (20, 21), bone mineral density (BMD) (22), and fractures (23).

Genetic correlation in LD score regression

We applied LD score regression to estimate genetic correlations across several muscle-related traits from summary-level data of publicly available GWAS. We used LD Hub (24), which is a centralized database of summary-level GWAS results for hundreds of diseases/traits from multiple consortia and online resources, as well as a web interface that automates the LD score regression analysis pipeline (25). According to Bulik-Sullivan et al. (26), the genetic correlation for a set of SNPs *S* is calculated as $r_s(y_1, y_2) = \rho_s(y_1, y_2)/\sqrt{h^2S(y^1)h^2S(y^2)}$, where ρ_S is genetic covariance among SNPs in *S*, y_1 and y_2 denote phenotypes, and $h^2{}_S$ is the heritability explained by SNPs in *S*.

Results

GWAS meta-analyses for discovery and replication

Descriptions and characteristics of the study populations in the discovery stage and the replication stage are listed in Supplemental Table 1, **Supplemental Table 5**, and Supplemental Note 1. The age of the participants ranged from 18 to 100 years. In the GWAS discovery set, comprising 38,292 participants for whole body lean mass, a substantial excess of low *P* values compared with the null distribution was observed after genomic control adjustment of the individual studies before meta-analysis: $\lambda_{GC} = 1.078$, $\lambda_{GC} = 1.075$ and $\lambda_{GC} = 1.076$, for Model 1 (not adjusted for fat mass), Model 2 (adjusted for percentage fat), and Model 3 (adjusted for fat mass in kilograms), respectively (**Supplemental Figure 1A–C**).

Table 1 shows the GWS and suggestive (sGWS) results for the 3 models in the discovery set (see also **Supplemental Figure 2**). In Model 1, we observed 3 independent GWS results (in/near *FTO*, *MC4R*, and *CALCR*) and 4 sGWS results (in/near *HSD17B11*, *GMPPA*, *CMTM8*, and *C10orf39;* Table 1; Supplemental Figure 2A). In Model 2, we observed 3 independent GWS results (in/near *HSD17B11*, *FTO*, and *CALCR*) and 10 sGWS results (in/near *MC4R*, *TNRC6B*, *RHOC*, *GMPPA*, *NUDT3*, *AKR1B1*, *ANGPT2*, *ZBTB16*, *ADAMTSL3*, *SMG6*; Table 1; Supplemental Figure 2B).

Data for Model 3 have already been presented in a previous publication (4), but for comparison we display it in Table 1. To reiterate, in Model 3, we observed one independent GWS result in/near *HSD17B11* and 10 sGWS results (in/near *IRS1*, *VCAN*, *ADAMTSL3*, *FTO*, *RHOC*, *PRR16*, *FRK*, *AKR1B1*, *CALCR*, *KLF12*; Table 1; Supplemental Figure 2C).

We selected all GWS and sGWS associations for all 3 models (Table 1) to conduct a replication study in a set of 27 cohorts comprising up to 47,227 participants of European descent. Owing to limited resources, 5 of the sGWS signals were evaluated only in the cohorts available for in silico replication (Table 1).

The upper parts of each panel (Models 1–3) in Table 1 show the results for successfully replicated SNPs (defined as combined

TABLE 1 Discovery and replication meta-analyses for all SNPs taken into replication

Model of adjustment	SNP ID	Chr	Position	Gene	A1/A2	AF 1	Discovery ($n = 38,292$)			Replication ($n = 47,227$)			Combined ($n = 85,519$)			
							β	SE	P	β	SE	P	β	SE	P	I^2
Model 1	rs9991501	4	88,477,507	HSD17B11	T/C	0.04	–0.65	0.13	1.7×10^{-6}	–0.36	0.10	5.5×10^{-4}	–0.46	0.08	1.6×10^{-8}	6
Model 1	rs9936385	16	52,376,670	FTO	T/C	0.63	–0.38	0.04	1.0×10^{-19}	–0.28	0.04	4.5×10^{-13}	–0.32	0.03	1.8×10^{-30}	38
Model 1	rs10871777	18	56,002,743	MC4R	A/G	0.75	–0.28	0.05	4.1×10^{-10}	–0.27	0.04	4.2×10^{-10}	–0.28	0.03	1.0×10^{-18}	11
Model 1	rs2385539	2	220,075,197	GMPPA	C/G	0.62	0.24	0.05	7.3×10^{-7}	0.05	0.04	1.8×10^{-1}	0.13	0.03	3.2×10^{-5}	18
Model 1	rs6796318[1]	3	32,276,790	CMTM8	A/G	0.09	0.30	0.06	2.7×10^{-7}	0.02	0.15	9.2×10^{-1}	0.27	0.06	1.38×10^{-6}	14
Model 1	rs9641123	7	93,035,668	CALCR	C/G	0.40	0.24	0.04	4.6×10^{-9}	0.08	0.04	2.2×10^{-2}	0.15	0.03	2.6×10^{-8}	17
Model 1	rs902634[1]	10	133,793,941	C10orf39	A/G	0.30	0.21	0.04	1.5×10^{-6}	–0.09	0.10	3.6×10^{-1}	0.16	0.04	4.5×10^{-5}	16
Model 2	rs9991501	4	88,477,507	HSD17B11	T/C	0.04	–0.70	0.13	2.5×10^{-8}	–0.34	0.10	4.9×10^{-4}	–0.48	0.08	7.3×10^{-10}	6
Model 2	rs9936385	16	52,376,670	FTO	T/C	0.61	–0.28	0.04	6.3×10^{-13}	–0.21	0.04	2.8×10^{-9}	–0.24	0.03	2.4×10^{-20}	33
Model 2	rs10871777	18	56,002,743	MC4R	A/G	0.76	–0.22	0.04	2.7×10^{-7}	–0.20	0.04	3.1×10^{-7}	–0.21	0.03	4.2×10^{-13}	10
Model 2	rs733381	22	38,999,594	TNRC6B	A/G	0.79	0.22	0.04	2.6×10^{-7}	0.17	0.04	2.7×10^{-5}	0.20	0.03	4.6×10^{-11}	9
Model 2	rs2999156	1	113,048,650	RHOC	C/G	0.56	–0.19	0.04	2.4×10^{-7}	0.02	0.04	6.7×10^{-1}	–0.08	0.03	1.2×10^{-3}	14
Model 2	rs2385539	2	220,075,197	GMPPA	C/G	0.63	0.21	0.04	2.2×10^{-6}	0.06	0.04	9.5×10^{-2}	0.12	0.03	1.8×10^{-5}	21
Model 2	rs464553	6	34,423,057	NUDT3	T/G	0.20	0.21	0.04	2.1×10^{-6}	0.12	0.04	7.0×10^{-3}	0.16	0.03	1.6×10^{-7}	23
Model 2	rs9641123	7	93,035,668	CALCR	C/G	0.43	0.21	0.04	2.8×10^{-8}	0.08	0.03	2.2×10^{-2}	0.14	0.03	7.6×10^{-8}	19
Model 2	rs6944616[1]	7	133,739,304	AKR1B1	T/G	0.29	–0.19	0.04	2.2×10^{-6}	–0.06	0.10	5.7×10^{-1}	–0.17	0.04	4.4×10^{-6}	16
Model 2	rs2515418	8	6,355,630	ANGPT2	A/G	0.44	0.18	0.04	8.8×10^{-7}	0.07	0.03	5.1×10^{-2}	0.12	0.02	1.7×10^{-6}	16
Model 2	rs11214913	11	113,624,353	ZBTB16	A/C	0.12	–0.30	0.06	1.8×10^{-6}	–0.07	0.05	1.8×10^{-1}	–0.16	0.04	5.2×10^{-5}	24
Model 2	rs899926[1]	15	82,364,495	ADAMTSL3	A/G	0.47	–0.19	0.04	1.7×10^{-7}	0.52	–0.01	8.6×10^{-1}	0.52	–0.16	9.3×10^{-7}	17
Model 2	rs17834425	17	2,062,021	SMG6	T/C	0.17	0.25	0.05	3.8×10^{-7}	0.11	0.05	2.2×10^{-2}	0.18	0.03	2.0×10^{-7}	12
Model 3	rs2943656	2	226,830,162	IRS1	A/G	0.37	–0.17	0.03	2.5×10^{-7}	–0.13	0.03	8.0×10^{-6}	–0.14	0.02	1.5×10^{-11}	14
Model 3	rs9991501	4	88,477,507	HSD17B11	T/C	0.04	–0.61	0.11	2.9×10^{-8}	–0.26	0.08	1.9×10^{-3}	–0.39	0.07	5.8×10^{-9}	7
Model 3	rs2287926	5	82,851,164	VCAN	A/G	0.13	0.24	0.05	8.6×10^{-7}	0.15	0.04	8.5×10^{-4}	0.19	0.03	7.5×10^{-9}	7
Model 3	rs4842924	15	82,378,611	ADAMTSL3	T/C	0.52	–0.17	0.03	1.4×10^{-7}	–0.08	0.03	3.9×10^{-3}	–0.12	0.02	1.4×10^{-8}	23
Model 3	rs9936385	16	52,376,670	FTO	T/C	0.61	–0.17	0.03	1.1×10^{-6}	–0.11	0.03	1.6×10^{-4}	–0.14	0.02	1.4×10^{-9}	33
Model 3	rs2999156	1	113,048,650	RHOC	C/G	0.56	–0.17	0.03	4.0×10^{-7}	0.00	0.03	9.3×10^{-1}	–0.09	0.02	1.7×10^{-4}	12
Model 3	rs4404708[1]	5	119,680,527	PRR16	A/G	0.37	–0.18	0.04	1.9×10^{-6}	0.03	0.07	7.0×10^{-1}	–0.14	0.03	4.3×10^{-5}	15
Model 3	rs10223402	6	116,004,392	FRK	A/G	0.17	0.24	0.05	4.1×10^{-7}	0.04	0.04	3.4×10^{-1}	0.12	0.03	6.2×10^{-5}	19
Model 3	rs7795758	7	133,751,491	AKR1B1	T/G	0.73	0.17	0.04	1.9×10^{-6}	0.00	0.03	9.4×10^{-1}	0.08	0.02	8.6×10^{-4}	14
Model 3	rs9641123	7	93,035,668	CALCR	C/G	0.43	0.17	0.03	4.8×10^{-7}	0.06	0.03	3.3×10^{-2}	0.11	0.02	9.8×10^{-7}	21
Model 3	rs1028883	13	73,006,588	KLF12	T/G	0.43	–0.16	0.03	3.2×10^{-7}	–0.04	0.03	2.0×10^{-1}	–0.10	0.02	8.8×10^{-6}	20

[1]Replication number only 6927 (owing to limited resources, 5 of the suggestive genome-wide significance signals were replicated only in the cohorts available for in silico replication). Model 1: no adjustment for fat mass; Model 2: adjustment for fat mass as a percentage of body mass; Model 3: adjustment for fat mass in kilograms. $P < 5 \times 10^{-8}$ is significant. AF, allele frequency; I^2, measure of heterogeneity; SNP, single nucleotide polymorphism.

P value $< 5 \times 10^{-8}$ and lower than discovery P values) in participants, who were part of the discovery phase, replication phase, and the combined results. For Model 1, combined analysis of the discovery and replication cohorts successfully replicated 3 SNPs in/near *HSD17B11*, *FTO*, and *MC4R* (P values between 1.6×10^{-8} and 1.8×10^{-30}). For Model 2, the same 3 SNPs as reported for Model 1 were successfully replicated, and in addition one SNP in/near *TNRC6B* was also successfully replicated (P values between 7.3×10^{-10} and 2.4×10^{-20}). For Model 3, combined analysis of the discovery and replication cohorts successfully replicated 5 SNPs in/near *IRS1*, *HSD17B11*, *VCAN*, *ADAMTSL3*, and *FTO* (P values between 1.4×10^{-8} and 1.5×10^{-11}. Results of Model 3 SNPs have been previously reported (4) but are shown here for comparison (Table 1).

None of the 12 replicated associations (3 for Model 1, 4 for Model 2, and 5 for Model 3) had significant heterogeneity at $\alpha = 0.0042$ (0.05/12, Bonferroni-corrected for 12 tests). Only mild heterogeneity was indicated for the SNP in/near *FTO* in all 3 models (Model 1, $I^2 = 38\%$; Model 2, $I^2 = 33\%$; Model 3, $I^2 = 33\%$; Table 1).

In total, 7 SNPs in independent loci (in/near *IRS1*, *HSD17B11*, *VCAN*, *ADAMTSL3*, *FTO*, *MC4R*, and *TNRC6B*) were successfully replicated in any of the 3 models, and the results for these 7 SNPs in the 3 different models for whole body lean mass are given in **Table 2**. The 7 SNPs were nominally ($P < 0.05$) significant in all 3 models except for the SNP in/near *IRS1*, which was not associated with lean mass unadjusted for fat mass in Model 1. Very similar associations were observed when these 7 SNPs were evaluated for their associations with appendicular lean mass available in up to 70,690 subjects of European descent (**Supplemental Table 6**).

We next evaluated the associations for 97 known BMI associated SNPs (7) with whole body lean mass in the discovery cohorts. As many as 42 of the known BMI associated SNPs were also associated ($P < 0.05$) with lean mass in the same direction as observed for BMI when evaluated in lean mass Model 1 not adjusted for fat mass (**Supplemental Table 7**). In general, the lean mass associations for the BMI SNPs were less pronounced after fat mass adjustment in lean mass Model 3.

Impact of fat mass adjustment for lean mass loci—"*sumo wrestler loci* " and "*body builder loci*"

In general, the results from Model 1 and Model 3 differed most from each other, whereas the associations for Model 2 were intermediate. Therefore, in the studies evaluating the impact of fat mass adjustment for lean mass loci, we mainly compared the results between Model 1 and Model 3. Six of the 7 loci (*FTO*, *MC4R*, *TNRC6B*, *HSD17B11*, *VCAN*, and *ADAMTSL3*) had an impact both on the absolute amount of lean mass (Model 1) and on the amount of lean mass adjusted for fat mass (Model 3). However, the strengths of the associations in Model 1 compared with Model 3 varied substantially. The *FTO* and *MC4R* signals had high Model 1/Model 3 ratios (a measure of the degree of attenuation of the LM association after fat mass adjustment) of beta values for the association with lean mass (M1/M3 ratio 222–234%), demonstrating that the strengths of the associations were reduced after fat mass adjustment. This suggests that these 2 loci have an impact on both lean mass and fat mass in the same direction, and this is also supported by the fact that they are associated with BMI and fat mass in the same direction as with lean mass (**Table 3; Supplemental Table 7**). Because the alleles of the *FTO* and *MC4R* signals that were associated with greater lean mass also were associated with increased fat mass, we named them "*sumo wrestler*" loci (Table 3; Supplemental Table 7).

In contrast, there were 2 lean mass loci that had a low Model 1/Model 3 ratio of beta values for the association with lean mass (M1/M3 ratio 64–67%), including the *VCAN* and *ADAMTSL3* loci. For these loci, the lean mass associations were stronger after adjustment for fat mass. This means that these 2 loci have a substantial impact specifically on lean mass with associations in the opposite direction or no association with fat mass (Table 3; Supplemental Table 7). Because the alleles of the *VCAN* and *ADAMTSL3* loci that were associated with greater lean mass were associated with slightly reduced fat mass, we named them "*body builder*" loci. The *TNRC6B* and *HSD17B11* loci had intermediate Model 1/Model 3 ratios of beta values for the association with lean mass (M1/M3 ratio 120–125%), suggesting that their impact on lean mass did not appear to be influenced by fat mass, so we called them "*intermediate*" loci (Table 3; Supplemental Table 7).

The signal in/near *IRS1* was not associated with lean mass without adjustment for fat mass. As shown in Table 3 and Supplemental Table 7, the lean mass increasing allele in/near *IRS1* was associated with lower fat mass. This association with lower fat mass may indirectly make the association with fat mass adjusted lean mass to be significant in the opposite direction. It is indeed a locus with an impact on the ratio between lean and fat mass but with no significant association with the absolute amount of lean mass when the effect of fat mass is not taken into account. The lean mass increasing allele was associated with reduced BMI and fat mass (Table 3; Supplemental Table 7), suggesting that its inverse association with fat mass is dominant for its effect on BMI, which is influenced by both lean mass and fat mass. We, therefore, named the *IRS1* locus a "*fat-mediated lean mass*" locus, because it primarily appears to impact the amount of fat mass (Table 3; Supplemental Table 7).

Metabolic associations for lean mass increasing alleles

We next evaluated the associations with metabolic traits for the 7 replicated lean mass SNPs, using available results from GWAS-meta-analyses of these traits (Table 3; Supplemental Table 7). The lean mass increasing alleles of SNPs in/near the 2 *sumo wrestler* loci (*FTO* and *MC4R*) were in general associated with an *adverse metabolic profile* regarding both carbohydrate metabolism (higher fasting insulin, higher HOMA-IR and increased risk of diabetes mellitus) and lipid metabolism (higher serum triglycerides and lower HDL cholesterol; Table 3; Supplemental Table 7). In addition, the lean mass increasing allele of the SNP in/near *FTO* was associated with increased risk of coronary artery disease (Table 3). In contrast, the lean mass increasing alleles of the SNPs in the 2 *body builder* loci (*VCAN* and *ADAMTSL3*) were in general associated with some *metabolic protection* regarding both carbohydrate metabolism (lower fasting insulin or reduced risk of diabetes mellitus)

TABLE 2 Comparison of models for GWS hits[1]

						Whole body lean mass								
						Model 1 ($n = 85,519$)			Model 2 ($n = 85,519$)			Model 3 ($n = 85,519$)		
SNP ID	Chr	Position	Gene	A1/A2	AF 1	β	SE	P	β	SE	P	β	SE	P
rs2943656	2	226,830,162	IRS1	A/G	0.37	–0.04	0.03	9.1×10^{-2}	–0.11	0.02	7.4×10^{-6}	–0.14	0.02	1.5×10^{-11}
rs9991501	4	88,477,507	HSD17B11	T/C	0.04	–0.46	0.08	1.6×10^{-8}	–0.48	0.08	7.3×10^{-10}	–0.39	0.07	5.8×10^{-9}
rs2287926	5	82,851,164	VCAN	A/G	0.13	0.12	0.04	3.2×10^{-3}	0.17	0.04	6.2×10^{-6}	0.19	0.03	7.5×10^{-9}
rs4842924	15	82,378,611	ADAMTSL3	T/C	0.52	–0.08	0.03	2.3×10^{-3}	–0.12	0.02	9.7×10^{-7}	–0.12	0.02	1.4×10^{-8}
rs9936385	16	52,376,670	FTO	T/C	0.61	–0.32	0.03	1.8×10^{-30}	–0.24	0.03	2.4×10^{-20}	–0.14	0.02	1.4×10^{-9}
rs10871777	18	56,002,743	MC4R	A/G	0.76	–0.28	0.03	1.0×10^{-18}	–0.21	0.03	4.2×10^{-13}	–0.12	0.03	6.2×10^{-7}
rs733381	22	38,999,594	TNRC6B	A/G	0.79	0.19	0.03	5.2×10^{-9}	0.20	0.03	4.6×10^{-11}	0.15	0.03	5.5×10^{-9}

[1]Model 1: not adjusted for fat; Model 2: adjusted for fat mass as a percentage of body mass; Model 3: adjusted for fat mass in kilograms. $P < 5 \times 10^{-8}$ is significant. AF, allele frequency; Chr, chromosome; SNP, single nucleotide polymorphism.

and lipid metabolism (lower serum triglycerides or higher HDL cholesterol; Table 3 presents the general direction of associations; Supplemental Table 7 actual beta coefficients). The lean mass signals in the *intermediate* lean mass loci (*TNRC6B* and *HSD17B11*), not influenced by fat mass adjustment, did not have any major impact on metabolic traits.

As reported previously (27), the lean mass increasing allele of the SNP in the *fat-mediated* lean mass locus *IRS1* was associated with an adverse metabolic profile (Table 3; Supplemental Table 7).

Musculoskeletal associations of lean mass increasing alleles

We also evaluated the associations between the 7 replicated lean mass SNPs and musculoskeletal traits. Importantly, the lean mass increasing alleles of the SNPs in/near *TNRC6B* and in/near *ADAMTSL3* were robustly associated with greater hand grip strength (**Table 4; Supplemental Table 8**). In general, the associations with the other musculoskeletal traits (Table 4; Supplemental Table 8) were less pronounced than the associations with metabolic traits (Table 3 and Supplemental Table 7), and no general pattern for the signals in the *sumo wrestler* loci compared with the signals in the *body builder* loci was observed for the musculoskeletal traits (Table 4; Supplemental Table 8). Surprisingly, the lean mass increasing allele of the SNP in/near *TNRC6B* was associated with lower lumbar spine BMD and increased risk of fractures.

Genetic correlations with lean mass by LD score regression

We next determined the genetic correlations between lean mass phenotypes and a variety of parameters with a focus on metabolic and musculoskeletal phenotypes using LD score regression (**Table 5**). *Obesity traits*, including both extreme phenotypes, such as childhood obesity and extreme BMI, and quantitative traits, such as BMI and waist-to-hip ratio, demonstrated a strong positive genetic correlation with lean mass in the model not adjusted for fat mass (r_g from 0.45 to 0.98, Model 1), and as expected these genetic correlations were attenuated after fat mass adjustment (from 0.17 to 0.76 and –0.16 to 0.51, respectively, for Model 2 and Model 3; Table 5).

For all carbohydrate-metabolism-related traits (type 2 diabetes mellitus, fasting glucose, fasting insulin, fasting proinsulin, glycated hemoglobin, and HOMA-IR) positive genetic correlations with lean mass in Model 1 were observed (r_g from 0.19 to 0.48, $P < 0.005$). All these correlations were substantially attenuated after fat mass adjustment in Models 2 and 3 (0.15–0.33 and 0.05–0.26, respectively; some nonsignificant).

TABLE 3 Associations of lean mass increasing alleles with metabolic phenotypes[1]

						Cross-phenotype										
SNP	LM IA	Gene	Effect in M1	M1/M3 ratio, %	Type of locus	Fat, %	BMI	CAD	DM	Insulin	HOMA-IR	Trig	Chol	LDL	HDL	Summary (metabolism)
rs9936385	C	FTO	Yes	234	Sumo Wrestler	++	++	+	++	++	++	++	0	0	– –	Adverse metabolic profile
rs10871777	G	MC4R	Yes	222	Sumo Wrestler	++	++	0	++	+	+	++	0	0	– –	
rs733381	A	TNRC6B	Yes	125	Intermediate	0	++	0	0	0	0	0	0	0	–	No metabolic phenotype
rs9991501	C	HSD17B11	Yes	120	Intermediate	0	0	0	?	0	0	?	?	?	?	
rs2287926	A	VCAN	Yes	64	Body Builder	0	0	0	?	–	0	– –	0	0	+	Metabolic protection
rs4842924	C	ADAMTSL3	Yes	67	Body Builder	– –	0	0	–	0	0	0	0	0	++	
rs2943656	G	IRS1	No	NA	Indirect/Fat locus	– –	– –	++	++	+	+	++	0	0	– –	Adverse metabolic profile

[1]The threshold for a statistically significant association with Bonferroni correction for 10 traits is $P = 0.005$ (0.05/10). For further details, see Supplemental Table 7. +, nominal significant increase; ++, multiple testing corrected significant increase; –, nominal significant decrease; – –, multiple testing corrected significant decrease; 0, no significant effect; ?, not included; CAD, coronary artery disease; Chol, cholesterol; DM, diabetes mellitus; HOMA-IR, homeostasis model assessment of insulin resistance; LM IA, lean mass increasing allele; M1, Model 1 not adjusted for fat; M3, Model 3 adjusted for fat mass (kg); M1/M3 ratio, a measure of the degree of attenuation of the LM association after fat mass adjustment; NA, not applicable because no effect in M1; SNP, single nucleotide polymorphism; Trig, triglycerides.

TABLE 4 Associations of lean mass increasing alleles with musculoskeletal phenotypes[1]

SNP	LM IA	Gene	Effect in M1	M1/M3 ratio, %	Type of locus	Cross-phenotype			
						Grip strength	FN-BMD	LS-BMD	Fx
rs9936385	C	FTO	Yes	234	Sumo Wrestler	0	0	0	−−
rs10871777	G	MC4R	Yes	222	Sumo Wrestler	0	0	0	0
rs733381	A	TNRC6B	Yes	125	Intermediate	++	0	−−	+
rs9991501	C	HSD17B11	Yes	120	Intermediate	0	0	+	0
rs2287926	A	VCAN	Yes	64	Body Builder	0	0	0	0
rs4842924	C	ADAMTSL3	Yes	67	Body Builder	++	0	0	0
rs2943656	G	IRS1	No	NA	Indirect/Fat locus	0	0	0	0

[1]The threshold for a statistically significant association with Bonferroni correction for 4 traits is $P = 0.0125$ (0.05/4). For further details, see Supplemental Table 8. +, nominal significant increase; ++, multiple testing corrected significant increase; −−, multiple testing corrected significant decrease; 0, no significant effect; BMD, bone mineral density; FN, femoral neck; Fx, fracture; LM IA, lean mass increasing allele; LS, lumbar spine; M1, Model 1 not adjusted for fat; M3, Model 3 adjusted for fat mass in kilograms; M1/M3 ratio, a measure of the degree of attenuation of the lean mass association after fat mass adjustment; NA, not applicable because no effect in M1.

When lipid-metabolism-related traits were evaluated in lean mass Model 1, a positive genetic correlation was observed for serum triglycerides, and inverse genetic correlations were observed for total cholesterol and HDL cholesterol (–0.14 and –0.40; Table 5). The significant genetic correlation with triglycerides was lost in lean mass Model 3 adjusted for fat mass in kilograms. Although the genetic correlations with HDL cholesterol was attenuated after fat mass adjustment (–0.28, Model 3), the correlation was still significant at $P = 0.000002$. Of note, the genetic correlations of lean mass with coronary artery disease were not significant (0.02–0.08, $P > 0.05$).

There was a modest positive genetic correlation between BMD parameters and lean mass in all 3 models, whereas the genetic correlation with grip strength, a proxy for muscle function, was observed in Model 3 but not in Model 1 (0.28, $P = 0.0036$ and 0.16, $P > 0.05$, respectively; Table 5).

Age at menarche and age at menopause can be regarded as indicators of lifetime sex steroid exposure. Age at menarche but not age at menopause displayed inverse genetic correlations with lean mass in all 3 models, although this was most pronounced in Model 1 (r_g –0.36; Table 5).

Annotation and enrichment analysis of regulatory elements

In the enrichment analysis of tissue-specific regulatory elements using experimental epigenetic evidence (DNase hypersensitive sites, histone modifications, and transcription factor-binding sites in human cell lines and tissues from the ENCODE Project and the Epigenetic Roadmap Project), SNPs in the *TNR6CB* locus were significantly enriched in these regulatory elements in blood cells, but not in muscle or other selected tissues after multiple testing correction (**Supplemental Table 9**). There was no significant tissue-specific enrichment of regulatory elements for the *MC4R* locus. The enrichment results for the other loci have previously been presented (4).

Expression quantitative trait loci

No significant association was found between rs733381 and *TNRC6B* gene expression in the skeletal muscle tissue ($P = 0.13$, $N = 491$) from GTEx data, although individuals with homozygosity of minor allele G appear to have relatively lower *TNRC6B* gene expression in the skeletal muscle tissue. We also looked at EQTLs of rs733381 in other tissues from GTEx data, but none of the associations attained statistical significance after multiple testing correction. *MC4R* gene expression is not detectable in the skeletal muscle tissue, whole blood, and many other tissue types, except for brain tissues, esophagus, and testis from GTEx data. Among those tissues with detectable *MC4R* gene expression, the smallest P value between rs10871777 and *MC4R* gene expression was found in the frontal cortex brain tissue ($P = 0.017$, $N = 118$). However, no statistical significance was found after multiple testing correction. The EQTL results for the other loci have previously been presented (4).

Discussion

Body weight consists of LM, fat mass, and bone mass, each with substantial heritable components and each playing important roles in physical function and metabolism. Because LM is correlated with fat mass, it is difficult to identify genetic determinants specific for LM. In addition, this makes it challenging to determine the metabolic health consequences of LM independent of fat mass. In the present study, we performed large-scale GWAS for LM without or with different fat mass adjustments (as well as sex, age, age^2, and height), and we identified genetic variants in 7 separate loci, including one novel locus (*TNRC6B*), associated with LM. Based on the relative strengths of the associations in the model without fat mass adjustment (Model 1) and the model with fat adjustments in kilograms (Model 3), we divided the LM loci that we identified into those with an effect on both LM and fat mass in the same direction (named *sumo wrestler loci with a high Model 1:Model 3* ratio) and those with an impact specifically on LM (named *body builder loci with a low Model 1:Model 3* ratio). Of note, both Model 2—relative amount of fat (percentage fat)—and Model 3—absolute amount of fat (kilograms of fat)—produced similar results; however, we did identify and successfully replicate the novel locus (*TNRC6B*) using Model 2.

LM increasing alleles of SNPs in *sumo wrestler loci* were associated with an adverse metabolic profile, whereas LM

TABLE 5 Genetic correlations of lean mass phenotype with other traits and conditions[1]

		Model 1		Model 2		Model 3	
Trait		r_g	P	r_g	P	r_g	P
Obesity	BMI	0.96	1.1×10^{-91}	0.76	1.2×10^{-53}	0.49	3.3×10^{-24}
	Childhood obesity	0.60	1.4×10^{-19}	0.47	4.4×10^{-13}	0.30	2.6×10^{-6}
	Extreme BMI	0.98	4.8×10^{-40}	0.76	4.1×10^{-26}	0.51	9.4×10^{-13}
	Overweight	0.93	2.1×10^{-70}	0.72	1.3×10^{-43}	0.46	1.1×10^{-16}
	Waist-to-hip ratio	0.48	4.1×10^{-20}	0.29	3.3×10^{-9}	0.15	4.0×10^{-3}
	Body fat	0.45	3.7×10^{-13}	0.17	1.2×10^{-2}	–0.16	1.4×10^{-2}
Carbohydrate	Type 2 diabetes	0.27	1.0×10^{-4}	0.18	1.0×10^{-2}	0.10	1.9×10^{-1}
	Fasting glucose	0.21	4.9×10^{-3}	0.20	4.6×10^{-3}	0.15	3.6×10^{-2}
	Fasting insulin	0.48	2.2×10^{-9}	0.28	2.0×10^{-4}	0.20	1.1×10^{-2}
	Fasting proinsulin	0.35	9.8×10^{-3}	0.21	9.6×10^{-2}	0.05	7.1×10^{-1}
	HbA1C	0.19	4.3×10^{-2}	0.15	1.0×10^{-1}	0.08	3.5×10^{-1}
	HOMA-IR	0.48	8.2×10^{-6}	0.33	5.0×10^{-4}	0.26	8.7×10^{-3}
Lipid	Triglycerides	0.15	6.5×10^{-3}	0.12	2.3×10^{-2}	0.08	1.8×10^{-1}
	Total cholesterol	–0.14	1.8×10^{-2}	–0.17	3.0×10^{-3}	–0.20	6.0×10^{-4}
	HDL cholesterol	–0.40	7.5×10^{-11}	–0.35	4.6×10^{-9}	–0.28	2.2×10^{-6}
	LDL cholesterol	–0.05	4.4×10^{-1}	–0.10	1.1×10^{-1}	–0.16	1.9×10^{-2}
Cardiovascular	CAD	0.08	1.7×10^{-1}	0.07	2.0×10^{-1}	0.02	7.3×10^{-1}
Musculoskeletal	FN-BMD	0.15	9.5×10^{-3}	0.11	4.3×10^{-2}	0.12	2.9×10^{-2}
	LS-BMD	0.14	2.4×10^{-2}	0.15	1.8×10^{-2}	0.17	8.9×10^{-3}
	Grip strength	0.16	1.1×10^{-1}	0.2704	4.4×10^{-3}	0.2797	3.6×10^{-3}
Reproductive	Age at menarche	–0.36	2.9×10^{-18}	–0.32	1.9E-15	–0.24	1.9×10^{-8}
	Age at menopause	–0.11	7.0×10^{-2}	–0.04	4.7×10^{-1}	0.05	3.8×10^{-1}

[1]BMD, bone mineral density; CAD, coronary artery disease; FN, femoral neck; HbA1C, glycated hemoglobin; HOMA-IR, homeostasis model assessment of insulin resistance; LS, lumbar spine; r_g, genetic correlation. $P < 0.05$ is significant.

increasing alleles of SNPs in *body builder* loci were associated with metabolic protection.

The 7 SNPs that were reproducibly associated with LM in any of the 3 models used were all in independent loci (in/near *IRS1*, *HSD17B11*, *VCAN*, *ADAMTSL3*, *FTO*, *MC4R*, and *TNRC6B*). Five of these SNPs (in/near *IRS1*, *HSD17B11*, *VCAN*, *ADAMTSL3*, and *FTO*) were identified in the model adjusted for fat mass in kilograms, and the results from this model have been previously reported (4). However, in the present study, we could determine how the strengths of the LM associations for these 5 SNPs were affected in different models without or with fat mass adjustment, enabling us to divide them into *sumo wrestler loci* and *body builder loci*.

A genetic variant in the *MC4R* locus was in the present study GWS associated with LM in the model not adjusted for fat mass, whereas the association was weaker in the model adjusted for fat mass in kilograms, and consequently this locus was categorized as a *sumo wrestler* locus. The *MC4R* locus has not previously been identified as an LM locus in a GWAS on LM. However, in a GWAS on fat mass, the *MC4R* locus was found to be associated not only with fat mass but also in secondary analyses with LM in the same direction (28). These findings indicate that the *MC4R locus* has a pleiotropic effect, regulating both fat mass and lean mass in the same direction.

Importantly, the *TNRC6B* (*Trinucleotide Repeat Containing 6B*) locus was identified as a novel LM locus in the present study, and comparison of the strengths of the associations in the different models of fat mass adjustments demonstrated that its LM association was only modestly affected by different fat mass adjustments. *TNRC6B* is a protein coding gene in pathways related to cellular senescence, innate or adaptive immune system, *Wnt* signaling, and calcium modulating pathways (GO:0,007223). In addition to the LM, BMI, HDL, grip strength, LS-BMD, and fracture associations presented here, other GWAS have reported the *TNRC6B* locus GWS associated with a "chronotype" (defined as "Morningness" or "Eveningness") phenotype (29), uterine fibroids (30), and mammographic density (31). Understanding the mechanisms by which *TNRC6B* variants relate to body composition and this multitude of phenotypes may be useful for mitigating a wide range of aging and disease states.

The LM increasing allele of SNPs in the *sumo wrestler loci* (*FTO* and *MC4R*) was associated with higher fasting insulin, higher HOMA-IR, increased risk of diabetes mellitus, higher serum triglycerides, and lower HDL cholesterol. In addition, the LM increasing allele of the SNP in the *FTO* locus was associated with increased risk of coronary artery disease. Thus, a genetically determined increase in LM by genetic variants in *sumo wrestler loci* is clearly associated with an adverse metabolic profile. In contrast, the LM increasing alleles of SNPs in the *body builder loci* (*VCAN* and *ADAMTSL3*) were in general associated with a beneficial metabolic profile regarding both carbohydrate metabolism (lower fasting insulin or reduced risk of diabetes mellitus) and lipid metabolism (lower serum triglycerides or higher HDL cholesterol). The intermediate *loci* (*TNRC6B* and *HSD17B11*) were not associated with a clear metabolic profile. These findings suggest that the observation of genetically determined higher LM correlating with adverse metabolic consequences is being driven by the higher fat mass. Once adjustment for fat mass is made, the genetic correlation is more favorable. Our findings therefore support a protective effect of increased lean mass for glucose intolerance. Alternatively, the described associations with metabolic traits could be explained by pleiotropic effects of the respective genes.

Although we could divide the SNPs that we found to be associated with LM in the different models into categories based on a relation with LM and fat mass or LM only, we found that the SNP in *IRS1* behaved differently from the other genes. The LM increasing allele in/near IRS1 was associated with lower fat mass and lower BMI but had no significant effect on the absolute amount of LM when the effect on fat mass was not taken into account. We, therefore, named the *IRS* locus a "*fat-mediated lean mass*" *locus*, because it primarily appears to impact the amount of fat mass.

Besides cross-phenotype analyses, we determined the genetic correlations between LM phenotypes and a variety of parameters with a focus on metabolic and musculoskeletal phenotypes using LD score regression. Genetic correlation in LD score regression is (asymptotically) proportional to Mendelian randomization estimates (26). This method has an advantage, for several reasons: it does not require individual genotypes; it is not restricted to GWS SNPs; and there is no need for LD pruning (which loses information if causal SNPs are in LD) (26). LD score regression analyses revealed strong positive genetic correlations between LM and several obesity traits and carbohydrate-metabolism-related traits such as type 2 diabetes mellitus, fasting glucose, and fasting proinsulin. These genetic correlations were attenuated in models adjusted for fat mass (in kilograms), supporting the notion that genes that determine LM not adjusted for fat mass have a stronger genetic overlap with genes that determine obesity and glucose intolerance than genes that determine LM irrespective of fat mass. Similar findings, with stronger genetic correlations shown for models not adjusted for fat mass, were observed for the positive genetic correlations with serum triglycerides and the inverse genetic correlations with HDL cholesterol.

Cross-phenotype analyses revealed that the LM increasing alleles of the SNPs in/near *TNRC6B* and in/near *ADAMTSL3* were robustly associated with higher hand grip strength, suggesting that increased muscle mass resulted in increased muscle strength. This notion is supported by our finding of a positive genetic correlation between LM and grip strength in models adjusted for fat mass. In general, fat mass adjustment attenuated the genetic correlations between LM and metabolic traits, whereas the same adjustment enhanced or did not change the genetic correlations between LM and musculoskeletal traits.

Interestingly, age at menarche but not age at menopause displayed inverse genetic correlations with LM in all 3 models but was most pronounced in Model 1, implying that genes related to both fat mass and LM are correlated with genes determining age at menarche. Previous studies have demonstrated that high BMI is associated with early age at menarche, and the onset of menstruation may be initiated when body fat percentage levels exceed 22% (32). The present study indicates that also the amount of LM is involved in the onset of menarche. Alternatively, it is possible that sex hormone status might be the link between early menarche and high LM.

There are limitations to our study. The X chromosome, harboring the androgen receptor gene, was not included in the present meta-analysis, which is notable because androgens have a major impact on muscle mass. Another potential weakness of this study is our decision to meta-analyze body composition results using 2 different techniques (BIA and DXA). Nevertheless, the 2 methods are highly correlated (9), and by combining them, the power to detect GWS loci was greatly enhanced.

In conclusion, we identified one novel LM locus (*TNRC6B*), and our results suggest that a genetically determined increase in LM might exert either harmful or protective effects on metabolic traits, depending on its relation to fat mass.

Sources of Support and Acknowledgments are available from the Supplementary data link in the online posting of the article and from the same link in the online table of contents at https://academic.oup.com/ajcn/. All authors: read and approved the final manuscript. The authors had no conflicts of interest to declare.

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