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Analytes and Metabolites Associated with Muscle Quality in Young, Healthy Adults

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Abstract

Purpose—Identification of mechanisms that underlie lower extremity muscle quality (leg press one repetition maximum/total lean mass; LP/Lean) may be important for individuals interested in optimizing fitness and sport performance. The purpose of the current study was to provide observational insight into mechanisms that may underlie muscle quality by characterizing the association between 286 mass spectrometry metabolites and 17 chemistry screen analytes with LP/Lean in young, healthy adults (N = 77 (49 women and 28 men); mean age, 24.4 ± 4.2 yr; BMI, 23.5 ± 2.6 kg·m^{-2}).

Methods—Principal components analysis (PCA) was used to reduce the 286 metabolites into 73 metabolite-containing PCA factors. Sex-adjusted linear regression was used to examine the association between PCA factors and chemistry screen analytes with LP/Lean. Q values were computed to account for multiple comparison testing. Stepwise linear regression and leave-one-out cross validation were used to identify a predictor set representative of LP/Lean and to assess internal validity, respectively.

Results—Metabolites or analytes related to dietary protein intake (albumin, branched-chain amino acids (BCAA)) and excitation-contraction coupling (calcium and magnesium) were positively associated, whereas metabolites related to gut bacterial metabolism (cinnamoylglycine, hydrocinnamate, hippurate, indolepropionate) and peroxisome proliferator–activated receptor-alpha (PPAR-α) (methylglutarylcarnitine and cinnamoylglycine) activation were negatively associated with LP/Lean. Use of leave-one-out cross validation identified magnesium, sex, and the PCA factors containing BCAAs and methionine and methylglutarylcarnitine to be present in more than 90% of the stepwise regression models, thereby explaining 26.7% of the variance (adjusted R^2) inherent in muscle quality.

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The authors have nothing to disclose related to this study.

Conclusion—Collectively, these data suggest that mechanisms related to dietary protein intake, excitation-contraction coupling, gut microbial metabolism, and PPAR-α activation may underlie lower extremity muscle quality in young, healthy adults.

Keywords

MUSCLE QUALITY; MASS SPECTROMETRY METABOLITES; CHEMISTRY SCREEN ANALYTES; MAGNESIUM; BRANCHED-CHAIN AMINO ACIDS; GUT BACTERIAL-RELATED METABOLITES

Components of physical fitness include body composition, aerobic capacity, and muscle strength, the sum of which is important for maintaining health and maximizing athletic and sport performance. Recently, mass spectrometry (MS)-based metabolomics, which aims to characterize and quantify all of the metabolites in a biological sample, thereby providing an analytical description of complex metabolic processes (12), and the standard chemistry screen (including electrolytes and circulating proteins that are not included in high-throughput metabolomic screens) have been used with the goal of identifying novel mechanisms that may underlie lean mass and aerobic capacity in young, healthy adults. For example, based on associations between serum metabolites or analytes with aerobic capacity (21), or with percent lean mass (22), mechanisms related to vitamin B_6, branched-chain amino acids (BCAA), uremia, inflammation, oxidative stress, or gut microbial metabolism have been suggested. To date, however, serum markers of muscle strength in young, healthy adults have yet to be published. Furthermore, because gains in strength are related to improvements in lean mass, muscle quality (leg press one repetition maximum/total lean mass; LP/Lean) may be a better indicator of muscle function than muscle strength (10); and therefore, identification of serum markers of muscle quality are of interest. To develop an improved understanding about mechanisms that may underlie muscle quality, the goal of the current study was to characterize the association between circulating MS metabolites and chemistry screen analytes with LP/Lean in young, healthy adults.

METHODS

Subjects

Subjects were recruited from the Boston area through local newspaper and internet advertisements and through postings at local area universities. Potential subjects were initially screened by telephone and were considered eligible if they were between the ages of 18 and 35, took no prescribed medication (oral contraceptives excluded), and had a body mass index (BMI) between 19 and 32 $kg \cdot m^{-2}$. Subjects who passed the initial telephone screening completed a preadmission informed consent form and medical history questionnaire, and underwent physical examination and medical screening by the study physician. Exclusion criteria included the presence of acute or terminal illness, upper or lower extremity fracture in the past 6 months, or an unwillingness to complete the study requirements. All volunteers were made aware of the potential risks and benefits associated with the procedures of the study before enrollment. Seventy-seven subjects, including 28 men and 49 women, were admitted into the study. All participants signed an informed

consent. The Tufts University Health Sciences Campus Institutional Review Board reviewed and approved the study protocol.

Measurement of mass spectrometry metabolites and chemistry screen analytes

Ten milliliters of blood was collected under standardized conditions between 8:00 and 10:00 a.m. after an overnight fast. After collection, blood was allowed to clot for 1 h at room temperature and was centrifuged at 2135*g* for 10 min at 4°C. Serum was derived by removing the supernatant and was stored in 1-mL aliquots at −80°C before analysis. Serum metabolomic data acquisition was performed by Metabolon Inc. (Research Triangle Park, NC). Small molecule metabolites were extracted from serum, and the reconstituted extracts were resolved using untargeted mass spectrometry platforms, including ultrahigh-performance liquid chromatography/tandem mass spectrometry (UHPLC/MS/MS) and gas chromatography/mass spectrometry (GC/MS). Chromatographic separation of all ions in each sample was followed by full scan mass spectra to record retention time and molecular weight (*m/z*) of all detectable ions present in the samples. The quant mass of the standard, the associated retention time, and the MS/MS fragmentation of the quantion coupled with the percent of the predominant peak are shown in the Supplemental Digital Content (SDC1, Metabolites identified with use of GC/MS, http://links.lww.com/MSS/A472; SDC2, Metabolites identified with use of UHPLC/MS/MS, http://links.lww.com/MSS/A473). Library matching of these ions to Metabolon's reference library of standards (2000 authentic standards plus thousands of additional library entries of unknown biochemicals based on unique characteristics of retention time, nominal mass, and fragmentation pattern) was then performed. The identity of metabolites was determined by matching the combination of chromatographic retention index and mass spectra signatures compared to the reference library entries (11). With the use of this technique, data for 350 metabolites were obtained, with the full list reported by Evans et al. (2009) (11). The median relative standard deviation (RSD) for the internal standards (measurement of instrument variability) was 6%, whereas the RSD for endogenous biochemicals (measurement of the total process variability) was 12%.

After a compound is identified in a sample, one of the characteristic and stronger ions is used to determine a relative concentration of that compound in each sample, thereby assuring that the compound will be represented only once in statistical analyses. Relative quantitation was based on peak integration and was expressed as scaled intensity. After peak identification and quality control filtering, integrated peak ion counts for each compound in each sample were normalized to correct for variation resulting from instrument interday tuning differences. Normalized peak area counts were divided by the median value for each run day, therefore setting the medians equal to 1 for each day's run. Median scaled data were natural log transformed before statistical analysis. Use of this method removes the variation that exists between each instrument run day, whereas the variation that exists across experimental samples remains (23).

A clinical chemistry automated analyzer (Olympus AU400, Olympus America Inc, Melville, NY) was used to measure 17 chemistry screen analytes using reagents, calibrators, and standard operating procedures as specified by the manufacturer.

Measurement of total lean mass and leg press one repetition maximum

Total lean mass and the bilateral leg press one-repetition maximum (LP 1 RM) measurements were performed after blood sampling. Dual-energy x-ray absorptiometry (DXA) was performed using a Hologic Discovery A densitometer (Hologic, Inc, Bedford, MA) to measure whole body lean mass. Dual-energy x-ray absorptiometry scans were analyzed with Hologic QDR software version 12.3 in array mode (22). The LP 1 RM (K400, Keiser Sports Health Equipment Inc, Fresno, CA) was used to measure lower extremity muscle strength. The 1 RM is defined as the maximum load that can be moved one time only throughout the full range of motion (ROM) while maintaining proper form (25). The examiner progressively increased the resistance for each repetition until the subject could no longer move the lever arm one time through the full ROM, as previously reported (13). To account for the strong correlation between the LP 1 RM with total lean mass ($r = 0.82$; $P < 0.0001$), the LP 1 RM was divided by total lean mass (LP/Lean) and has previously been defined as muscle quality (9).

Statistical analysis

Given the large number (350) and potential collinearity of metabolites, principal components analysis (PCA) was performed to decrease these multiple metabolites into a smaller set of constructed latent variables (principal components or factors) and to control for type I error, as previously reported in studies of metabolic intermediates (14). Principal components analysis–obtained factors account for common variance in the original measurements, with metabolites that correlate highly with one another, forming common factors. The total number of metabolites was greater than the number of the subjects, and therefore, it was first necessary to divide these multiple metabolites into groups smaller than the sample size ($N = 77$). For example, PCA has previously been performed separately for amino acids, fatty acids, and acylcarnitines (20,32). However, the definition of what constitutes an amino acid or fatty acid metabolite is not always clear, whereas the amino acid leucine is clearly defined as an amino acid; its deamination product, 4-methyl-2-oxopentanoic acid, can be classified as both an amino acid and as a branched-chain fatty acid. Therefore, before using PCA, we separated metabolites based on their carbon chain length. Two hundred eighty-six metabolites, including amino acids, fatty acids, acylcarnitines, lysolipids, bile acids, sugars, purines, pyrimidines, and vitamins, were separated into five groups, including two separate one to six carbons (C1–C6) groups (groups 1 and 2 containing 76 and 21 metabolites, respectively), 7–11 carbons (C7–C11; 76 metabolites), 12–19 carbons (C12–C19; 53 metabolites), and 20–30 carbons (C20–C30; 60 metabolites). Sixty-four metabolites including xenobiotics, steroids, fibrinogen, and bilirubin-related metabolites were not included. With use of orthogonal varimax rotation (SAS Enterprise Guide 4.3, SAS Institute Inc, Cary, NC), PCA was performed separately on each of these five metabolite groups, resulting in 73 PCA factors (C1–C6, 26 factors; C7–C11, 21 factors; C12–C19, 12 factors; and C20–C30, 14 factors) with an eigenvalue greater than 1. Individual metabolites with component loadings values 0.5 or greater or −0.5 or less were considered as being contained within that factor. After PCA analysis, factor scores for each subject were computed using SAS Enterprise Guide 4.3.

Sex-adjusted linear regression was used to investigate the association between PCA factors and chemistry screen analytes with LP/Lean. False discovery rates (2) were computed using the *q*-value method (36) to account for multiple comparison testing. Principal components analysis factors and analytes that were associated with LP/Lean at $P \leq 0.05$ and $q \leq 0.20$ were considered statistically significant. A q value of 0.20 indicates that the result is likely to be valid eight of 10 times, which we suggest is reasonable in the setting of exploratory discovery.

To develop a LP/Lean predictive model, PCA factors and analytes that were significantly associated with LP/Lean at $P \leq 0.05$ and $q \leq 0.20$ were entered, with sex as a candidate variable for stepwise linear regression (SAS Enterprise Guide 4.3). Statistical significance to be retained in the stepwise model was set at $P < 0.10$ (4). Leave-one-out cross validation was used to assess the internal validity of the stepwise model. Seventy-seven data sets were created, each with one participant excluded, and the stepwise procedure was run on each data set. The percent of models for which a given variable was retained was calculated for each variable.

RESULTS

Characteristics of the 77 (49 women and 28 men) young (mean age, 24.4 ± 4.2 yr), healthy (mean BMI, 23.5 ± 2.6 $kg \cdot m^{-2}$) adults of the current study including percent female, age, LP 1 RM, total lean mass, and LP/Lean are shown in Table 1.

PCA factors and analytes associated with LP/Lean

Use of sex-adjusted linear regression identified positive associations for C1–C6 factor 3 (BCAA: isoleucine, leucine, valine; and methionine), magnesium, calcium, and albumin; whereas C12–C19 factor 12 (methylglutarylcarnitine) and C7–C11 factor 5 (gut bacteria-related metabolites: hydrocinnamate, cinnamoylglycine, hippurate, and indolepropionate) were negatively associated with LP/Lean (Table 2). Principal components analysis factors and analytes that were significantly associated with LP/Lean after adjustment for sex were additionally significantly associated with LP, after adjusting for sex and total lean mass (data not shown). Nonsignificant associations between PCA factors and analytes with LP/Lean are shown in the Supplemental Digital Content (SDC3, Table 1, Associations between PCA Factors with LP/Lean, http://links.lww.com/MSS/A474; SDC4, Table 2, Associations between analytes with LP/Lean, http://links.lww.com/MSS/A475).

Sex and PCA factors and analytes that were significantly associated with LP/Lean were considered as candidate variables for stepwise linear regression. Whereas sex explained 8.5% of the variability inherent in LP/Lean, the combination of magnesium, albumin, C7–C11 factor 5 and C1–C6 factor 3 explained an additional 24%, for a model-adjusted R^2 of 0.325 ($P < 0.001$; Table 3). With the use of leave-one-out cross validation, magnesium, sex, C1–C6 factor 3, C12–C19 factor 12, calcium, C7–C11 factor 5, and albumin were found in 100%, 99%, 99%, 90%, 21%, 17%, and 10% of the stepwise models, respectively. Covariates that entered in 90% or greater of the stepwise models including magnesium, sex, C1–C6 factor 3 and C12–C19 factor 12 explained 26.7% of the variance inherent in LP/Lean.

DISCUSSION

The main findings of the current study are that electrolytes involved in excitation-contraction coupling, metabolites or analytes previously related to gut bacterial metabolism, peroxisome proliferator–activated receptor-alpha (PPAR-α) activation, and dietary protein intake were associated with muscle quality in young, healthy adults.

The positive associations identified between serum levels of calcium (Ca^{2+}) and magnesium (Mg^{2+}) with LP/Lean suggests disruptions in excitation-contraction coupling in subjects with reduced muscle quality. Ca^{2+} ions bind to troponin in the actin-containing filaments of the muscle sarcomere, producing a change in the structure of actin that allows a force-generating interaction with adjacent myosin-containing filaments. Furthermore, cross-bridge formation and metabolism (i.e., NADH-dehydrogenase) are Ca^{2+}-dependent processes that can affect skeletal muscle function (34). Similarly, myosin conformational changes are induced by Mg^{2+} bound to ATP, an effect that mimics the molecular motion in a muscle's force generation process (37), and Mg^{2+} is involved in various physiological processes involved in muscle function, including energy production and Ca^{2+} balance (29). In support of our findings that levels of calcium and magnesium are related to muscle quality, reduced intramuscular levels of calcium and magnesium are associated with reduced contractile force production (8). Collectively, these data suggest a link between serum levels of calcium and magnesium, skeletal muscle excitation-contraction coupling, and muscle quality in young, healthy adults.

Cinnamoylglycine, hydrocinnamate, and indolepropionate (C7–C11 factor 5) are found in the serum (where hippurate is 17-fold elevated) or colonic lumen from conventional but not germ-free mice (24,38), evidence that suggests a negative role for gut bacterial metabolism on the maintenance of muscle quality. In support of this, the PCA factor containing cinnamoylglycine and hydrocinnamate was recently reported to be significantly negatively associated with LP/Lean in older adults (20). Alternatively, cinnamoylglycine, hippurate, and methylglutarylcarnitine (C12–C19 factor 12) increase during the progression from healthy to mild to chronic kidney disease (3), evidence that suggests decreased kidney function in subjects with reduced LP/Lean. In disagreement with this hypothesis, serum creatine (as a marker of kidney function; (19)) was not significantly associated with C7–C11 factor 5 or C12–C19 factor 12 (data not shown). Similarly, although dietary polyphenols including chlorogenic acid and catechin are degraded by gut bacteria to yield cinnamic acid, hydrocinnamate, and benzoic acid (18) (glycine conjugation of cinnamic and benzoic acids produces cinnamoylglycine and hippurate [5]), catechin supplementation is positively associated with physical function (17), evidence that argues against the hypothesis of increased catechin degradation in subjects with reduced muscle quality. Therefore, we suggest that an altered gut microflora may be responsible for the presence of these metabolites in serum. For example, phenylalanine and tryptophan degradation by the gut bacterium *Clostridium sporogenes* produces cinnamic acid and hydrocinnamate, and indolepropionate, respectively (1). Accordingly, future studies aimed at identifying the association between gut bacteria with circulating metabolites and with physical function in young, healthy adults are of interest.

Findings for methylglutarylcarnitine, cinnamoylglycine, valine, and methionine suggest decreased PPAR-α activation in subjects with reduced muscle quality. Peroxisome proliferator–activated receptor-α is a nuclear hormone receptor family transcription factor (15) that is involved in the transcriptional activation of genes that regulate energy metabolism (26), a potentially important finding because PPAR-$\alpha^{-/-}$ mice have decreased muscle function when compared with wild type mice (27). Methylglutarylcarnitine accumulates when HMG-CoA lyase is deficient (33). *Hmgcl*, the gene that encodes HMG-CoA lyase, is increased when PPAR-α is activated (31), evidence that suggests that subjects with elevated methylglutarylcarnitine have reduced PPAR-α activation. Furthermore, cinnamoylglycine is elevated in PPAR-$\alpha^{-/-}$ mice (39), and plasma levels of valine and methionine are elevated in response to PPAR-α activation (30), findings that collectively suggest a role for PPAR-α activation on influencing muscle quality in young, healthy adults.

The positive associations identified between albumin and C1–C6 factor 3 (BCAA and methionine) with LP/Lean suggests an increased dietary protein intake in subjects with elevated muscle quality. In support of this, dietary protein intake is positively associated with serum albumin (16) and with plasma BCAAs (28,35). Furthermore, in support of the hypothesis that elevated serum BCAA may be related to an increased dietary protein intake, serum urea nitrogen, as a marker of dietary protein intake (6), was positively associated with C1–C6 factor 3 ($\beta \pm$ SE, 1.2 ± 0.5; $P = 0.02$). Although a meta-analysis of 10 studies in young adults found that protein supplementation significantly improved the gain in mean 1 RM leg press strength during prolonged resistance exercise training (7). However, independent of gains in muscle mass, mechanisms underlying dietary protein-induced strength increases are currently unknown.

Limitations

Limitations of the current study include use of PCA on the five separate metabolite groups (instead of performing PCA on all metabolites together in one group), use of a relatively small sample size ($N = 77$), unmeasured associations between circulating metabolites with muscle-specific metabolites, and the absence of diet standardization. First, because the total number of metabolites was larger than the sample size, PCA was performed separately on five metabolite-containing groups, potentially missing clustering between metabolites in different groups. In contrast, in the presence of a sample size that is greater than the number of measured metabolites, PCA can be performed on all metabolites together as one group, thereby maximizing metabolite clustering and potentially discovering additional associations with LP/Lean. Second, although use of leave-one-out cross validation identified magnesium, sex, C1–C6 factor 3, and C12–C19 factor 12 to be present in more than 90% of the stepwise regression models, we acknowledge that because of our relatively small sample size, validation of these results in a larger cohort is necessary. Alternatively, challenge experiments that measure differential metabolism before and after a leg press 1 RM test may provide a better way to discover markers of muscle quality that may be appropriate for smaller cohorts due to increased statistical power. Third, it is important to note that circulating metabolites reflect not only muscle status but also metabolism by all other organs and tissues in the body. Therefore, future studies aimed at identifying associations between muscle and serum metabolites with muscle quality are of interest. Fourth, although we

obtained blood samples under standardized conditions, inclusion of meals with a defined nutrient composition for a period of time before performing metabolomic measurements may reduce biologic variability, thereby potentially increasing the number of metabolites significantly associated with muscle quality.

CONCLUSIONS

In summary, we report serum analytes and metabolites that are significantly associated with muscle quality in young, healthy adults. Collectively, our results suggest that future studies aimed at testing the causative role of electrolytes involved in excitation-contraction coupling, metabolites related to gut bacterial metabolism and PPAR-α activation, and dietary protein on influencing muscle quality are of interest.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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REFERENCES

1. Barker HA. Amino acid degradation by anaerobic bacteria. Annu Rev Biochem. 1981; 50:23–40. [PubMed: 6791576]
2. Benjamini YHY. Controlling the false discovery rate: a practical and powerful approach to multiple testing. Journal of the Royal Statistical Society, Series B. 1995; 57:289–300.
3. Boelaert J, t_Kindt R, Schepers E, et al. State-of-the-art non-targeted metabolomics in the study of chronic kidney disease. Metabolomics. 2013; 10(3):425–42.
4. Bursac Z, Gauss CH, Williams DK, Hosmer DW. Purposeful selection of variables in logistic regression. Source Code Biol Med. 2008; 3:17. [PubMed: 19087314]
5. Caldwell J, Moffatt JR, Smith RL. Post-mortem survival of hippuric acid formation in rat and human cadaver tissue samples. Xenobiotica. 1976; 6(5):275–80. [PubMed: 936647]
6. Campbell WW, Johnson CA, McCabe GP, Carnell NS. Dietary protein requirements of younger and older adults. Am J Clin Nutr. 2008; 88(5):1322–9. [PubMed: 18996869]
7. Cermak NM, Res PT, de Groot LC, Saris WH, van Loon LJ. Protein supplementation augments the adaptive response of skeletal muscle to resistance-type exercise training: a meta-analysis. Am J Clin Nutr. 2012; 96(6):1454–64. [PubMed: 23134885]
8. Chonkar A, Hopkin R, Adeghate E, Singh J. Contraction and cation contents of skeletal soleus and EDL muscles in age-matched control and diabetic rats. Ann N Y Acad Sci. 2006; 1084:442–51. [PubMed: 17151321]
9. Choquette S, Dion T, Brochu M, Dionne IJ. Soy isoflavones and exercise to improve physical capacity in postmenopausal women. Climacteric. 2013; 16(1):70–7. [PubMed: 22338607]
10. Dutta C, Hadley EC, Lexell J. Sarcopenia and physical performance in old age: overview. Muscle Nerve Suppl. 1997; 5:S5–9. [PubMed: 9331374]
11. Evans AM, DeHaven CD, Barrett T, Mitchell M, Milgram E. Integrated,nontargeted ultrahigh performance liquid chromatography/electrospray ionization tandem mass spectrometry platform for the identification and relative quantification of the small-molecule complement of biological systems. Anal Chem. 2009; 81(16):6656–67. [PubMed: 19624122]

12. Fiehn O. Metabolomics—the link between genotypes and pheno-types. Plant Mol Biol. 2002; 48(1–2):155–71. [PubMed: 11860207]
13. Fielding RA, LeBrasseur NK, Cuoco A, Bean J, Mizer K, Fiatarone Singh MA. High-velocity resistance training increases skeletal muscle peak power in older women. J Am Geriatr Soc. 2002; 50(4):655–62. [PubMed: 11982665]
14. Huffman KM, Shah SH, Stevens RD, et al. Relationships between circulating metabolic intermediates and insulin action in over-weight to obese, inactive men and women. Diabetes Care. 2009; 32(9):1678–83. [PubMed: 19502541]
15. Issemann I, Green S. Activation of a member of the steroid hormone receptor superfamily by peroxisome proliferators. Nature. 1990; 347(6294):645–50. [PubMed: 2129546]
16. Kaysen GA, Chertow GM, Adhikarla R, Young B, Ronco C, Levin NW. Inflammation and dietary protein intake exert competing effects on serum albumin and creatinine in hemodialysis patients. Kidney Int. 2001; 60(1):333–40. [PubMed: 11422769]
17. Kim H, Suzuki T, Saito K, et al. Effects of exercise and tea catechins on muscle mass, strength and walking ability in community-dwelling elderly Japanese sarcopenic women: a randomized controlled trial. Geriatr Gerontol Int. 2013; 13(2):458–65. [PubMed: 22935006]
18. Lees HJ, Swann JR, Wilson ID, Nicholson JK, Holmes E. Hippurate: the natural history of a Mammalian-microbial cometabolite. J Proteome Res. 2013; 12(4):1527–46. [PubMed: 23342949]
19. Levey AS. Measurement of renal function in chronic renal disease. Kidney Int. 1990; 38(1):167–84. [PubMed: 2200925]
20. Lustgarten MS, Price LL, Chale A, Fielding RA. Metabolites related to gut bacterial metabolism, peroxisome proliferator-activated receptor-alpha activation, and insulin sensitivity are associated with physical function in functionally-limited older adults. Aging Cell. 2014; 13(5):918–25. [PubMed: 25041144]
21. Lustgarten MS, Price LL, Logvinenko T, et al. Identification of serum analytes and metabolites associated with aerobic capacity. Eur J Appl Physiol. 2013; 113(5):1311–20. [PubMed: 23184236]
22. Lustgarten MS, Price LL, Phillips EM, Kirn D, Mills J, Fielding RA. Serum predictors of percent lean mass in young adults. J Strength Cond Res. In press.
23. Macotela Y, Emanuelli B, Bang AM, et al. Dietary leucine—an environmental modifier of insulin resistance acting on multiple levels of metabolism. PLoS One. 2011; 6(6):e21187. [PubMed: 21731668]
24. Matsumoto M, Kibe R, Ooga T, et al. Impact of intestinal microbiota on intestinal luminal metabolome. Sci Rep. 2012; 2:233. [PubMed: 22724057]
25. McDonagh MJ, Davies CT. Adaptive response of mammalian skeletal muscle to exercise with high loads. Eur J Appl Occup Physiol. 1984; 52(2):139–55.
26. Michalik L, Auwerx J, Berger JP, et al. International Union of Pharmacology. LXI. Peroxisome proliferator-activated receptors. Pharmacol Rev. 2006; 58(4):726–41. [PubMed: 17132851]
27. Muoio DM, MacLean PS, Lang DB, et al. Fatty acid homeostasis and induction of lipid regulatory genes in skeletal muscles of peroxisome proliferator-activated receptor (PPAR) alpha knockout mice Evidence for compensatory regulation by PPAR delta. J Biol Chem. 2002; 277(29):26089–97. [PubMed: 12118038]
28. Nelson AR, Phillips SM, Stellingwerff T, et al. A protein-leucine supplement increases branched-chain amino acid and nitrogen turnover but not performance. Med Sci Sports Exerc. 2012; 44(1): 57–68. [PubMed: 21685813]
29. Nielsen FH, Lukaski HC. Update on the relationship between magnesium and exercise. Magnes Res. 2006; 19(3):180–9. [PubMed: 17172008]
30. Ohta T, Masutomi N, Tsutsui N, et al. Untargeted metabolomic profiling as an evaluative tool of fenofibrate-induced toxicology in Fischer 344 male rats. Toxicol Pathol. 2009; 37(4):521–35. [PubMed: 19458390]
31. Rakhshandehroo M, Sanderson LM, Matilainen M, et al. Comprehensive analysis of PPARalpha-dependent regulation of hepatic lipid metabolism by expression profiling. PPAR Res. 2007; 2007:26839. [PubMed: 18288265]

32. Redman LM, Huffman KM, Landerman LR, et al. Effect of caloric restriction with and without exercise on metabolic intermediates in nonobese men and women. J Clin Endocrinol Metab. 2011; 96(2):E312–21. [PubMed: 21123443]

33. Roe CR, Millington DS, Maltby DA. Identification of 3-methylglutarylcarnitine. A new diagnostic metabolite of 3-hydroxy-3-methylglutaryl-coenzyme A lyase deficiency. J Clin Invest. 1986; 77(4):1391–4. [PubMed: 3958190]

34. Smith LR, Meyer G, Lieber RL. Systems analysis of biological networks in skeletal muscle function. Wiley Interdiscip Rev Syst Biol Med. 2013; 5(1):55–71. [PubMed: 23188744]

35. Solon-Biet SM, McMahon AC, Ballard JW, et al. The ratio of macronutrients, not caloric intake, dictates cardiometabolic health, aging, and longevity in ad libitum–fed mice. Cell Metab. 2014; 19(3):418–30. [PubMed: 24606899]

36. Storey JD, Tibshirani R. Statistical significance for genomewide studies. Proc Natl Acad Sci U S A. 2003; 100(16):9440–5. [PubMed: 12883005]

37. Taniguchi M, Matsumoto O, Suzuki S, et al. MgATP-induced conformational changes in a single myosin molecule observed by atomic force microscopy: periodicity of substructures in myosin rods. Scanning. 2003; 25(5):223–9. [PubMed: 14748384]

38. Wikoff WR, Anfora AT, Liu J, et al. Metabolomics analysis reveals large effects of gut microflora on mammalian blood metabolites. Proc Natl Acad Sci U S A. 2009; 106(10):3698–703. [PubMed: 19234110]

39. Zhen Y, Krausz KW, Chen C, Idle JR, Gonzalez FJ. Metabolomic and genetic analysis of biomarkers for peroxisome proliferator-activated receptor alpha expression and activation. Mol Endocrinol. 2007; 21(9):2136–51. [PubMed: 17550978]

TABLE 1

Subjects' demographics.

N	77
% female	64
Age, yr	24.4 ± 4.2
LP 1 RM, kN	2.0 ± 0.7
Total lean mass, kg	49.7 ± 11.5
LP/Lean, $N \cdot kg^{-1}$	40.0 ± 7.3

Values shown represent mean ± SD.

TABLE 2

Principal components analysis factors and analytes significantly associated with LP/Lean.

	Component Loadings	$\beta \pm$ SE	*P* Value	*q* Value
PCA factors				
C12–C19 F12: Methylglutarylcarnitine	0.94	–2.0 ± 0.8	0.01	0.20
C7–C11 F5: Gut bacterial metabolites (hydrocinnamate, cinnamoylglycine, hippurate, and indolepropionate)	0.92, 0.85, 0.60, 0.58	–1.9 ± 0.8	0.02	0.20
C1–C6 F3: BCAA (isoleucine, leucine, valine), methionine	0.93, 0.89, 0.85, 0.63	1.9 ± 0.8	0.02	0.20
Chemistry screen analytes				
Magnesium	-	18.4 ± 4.6	0.0002	0.01
Calcium	-	6.2 ± 2.2	0.007	0.15
Albumin	-	7.9 ± 2.8	0.007	0.15

Significant ($P \leq 0.05$ and $q \leq 0.20$) sex-adjusted associations between metabolite-containing PCA factors and chemistry screen analytes with LP/Lean are shown with component loadings ≥0.5, with parameter estimates and standard errors ($\beta \pm$ SE), *P* values, and *q* values (false discovery rates).

TABLE 3

LP/Lean stepwise linear regression model.

	$\beta \pm$ SE	*P* Value
LP/Lean: Adjusted $R^2 = 0.325$; $P \leq 0.0001$		
Magnesium	13.9 ± 4.8	0.005
C7–C11 F5: Gut bacterial metabolites	–1.5 ± 0.7	0.04
Sex	3.2 ± 1.6	0.05
C1–C6 F3: BCAA, methionine	1.4 ± 0.7	0.06
Albumin	4.7 ± 2.8	0.09

Covariates significantly associated with LP/Lean are shown with parameter estimates and standard errors ($\beta \pm$ SE) in order of significance (*P* value).