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RESEARCH ARTICLE

Associations of increased physical performance and change in body composition with molecular pathways of heart disease and diabetes risk

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Kettunen J, Joensuu A, Hagnäs M, Mikkola I, Wennerström A, Lee JH, Terwilliger JD, Borodulin K, Jousilahti P, Jauhiainen M, Jokelainen JJ, Keinänen-Kiukaanniemi S, Perola M. Associations of increased physical performance and change in body composition with molecular pathways of heart disease and diabetes risk. *Am J Physiol Endocrinol Metab* 316: E221–E229, 2019. First published November 13, 2018; doi:10.1152/ajpendo.00260.2018.—Higher physical activity is associated with a reduced hazard for a plethora of diseases. It has remained unknown how the two primary physical activity-associated health effects, improved physical performance and change in body composition, independently modulate metabolic profiles toward a reduced risk for adverse outcomes. Here, we utilized a prospective cohort of 664 young men undergoing military service. We studied the metabolic associations of changes in muscle performance and body composition during military service (range 6–12 mo). We subsequently replicated our results for body composition change in 234 population-based samples with a 7-yr follow-up. We found that increased physical performance was associated with reduced very-low-density lipoprotein (VLDL)-related measures [change in VLDL cholesterol: beta = −0.135; 95% confidence interval (CI) = −0.217, −0.054, $P = 1.2 \times 10^{-3}$] and lower inflammation (change in glycoprotein acetyls: beta = −0.138, 95% CI = −0.217, −0.059, $P = 6.5 \times 10^{-4}$), independent of change in body composition. Lower body fat percentage, independent of change in muscle performance, was associated with metabolic changes including lower low-density lipoprotein (LDL) cholesterol measures (change in LDL cholesterol: beta = −0.193, 95% CI = −0.295, −0.090; $P = 2.5 \times 10^{-4}$), increased high-density lipoprotein (HDL) cholesterol measures (change in large HDL cholesterol: beta = 0.316, 95% CI = 0.205, 0.427; $P = 3.7 \times 10^{-8}$), and decreased concentrations of amino acids (change in leucine concentration: beta = −0.236, 95% CI = −0.341, −0.132; $P = 1.0 \times 10^{-5}$) that are type 2 diabetes biomarkers. Importantly, all body fat percentage associations were replicated in a general population-based cohort. Our findings indicate that improved muscle performance showed weaker associations on the metabolic profiles than change in body composition and reduction in body fat percentage reduces cardiometabolic risk mediated by atherogenic lipoprotein particles and branched-chain and aromatic amino acid concentrations.

body composition; cardiometabolic diseases; metabolic measures; muscle performance

INTRODUCTION

When a person has an increased risk for cardiovascular diseases (CVD) or type 2 diabetes (T2D), it is recommended that they lose excess weight and exercise more. Higher physical activity (PA) has been associated with concrete health benefits, such as reduced risk for certain types of cancer, obesity, metabolic syndrome, T2D, CVD, and all-cause mortality (24). Increased PA is associated both with improved physical performance and change in body composition. In a large prospective study, an independent risk reduction for mortality was demonstrated from both weight loss and increased muscle performance. In addition, obese individuals with at least moderate physical performance, measured by a maximal exercise test, had a lower CVD mortality and all-cause mortality risk than lean, non-fit individuals (13). In their recent viewpoint, Kennedy et al. (8) summarized various studies where the independent associations of obesity (measured with BMI or weight gain) and cardiorespiratory fitness (CRF) for mortality were evaluated. It was found that increased CRF had independent beneficial associations in lowering mortality risk. Furthermore, the secondary analyses of the Look AHEAD trial showed that CVD risk reduction was proportional to the weight loss and improvement of physical fitness achieved by an intervention (14). However, changes in BMI or body weight are suboptimal measures of obesity, as they cannot separate fat mass from muscle mass. Also, change in body composition and

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improvement of physical performance are closely tied together. Thus our study aimed to understand the detailed statistically independent molecular underpinnings of risk reduction due to increased physical performance and change in body composition. To delineate the independent health benefits of fitness versus fatness on metabolism, we used a longitudinal study design in harmonized living conditions, in which study subjects underwent compulsory military service including an exercise intervention. These individuals took part in physical performance tests for both aerobic and muscular performance, body composition measurement, and assessment of cardiovascular and diabetic risk factors, and gave blood samples both at the start and at the end of their military service. For metabolomics profiling, nuclear magnetic resonance spectroscopy (NMR) analysis was performed to obtain detailed metabolic concentrations across many metabolic pathways both at baseline and at follow-up. Here, we studied how metabolic measures were modulated due to changes in physical performance and fat percentage, and replicated our findings on body fat percentage in an independent general population-based sample. Our aim was to answer a simple question: how do changes in physical performance and changes in body composition associate with metabolic pathways of cardiovascular, diabetic, and mortality risk?

METHODS

Subjects. We first examined the Sodankylä cohort, a group of young men in the Finnish military ($n = 664$) who underwent objective physical performance tests for aerobic and muscular performance, body composition measurements, as well as assessment of cardiovascular and diabetic risk factors at the start and the end of their military service (16, 17). We then examined a subset of the DIetary, Lifestyle, and Genetic determinants of Obesity and Metabolic syndrome (DILGOM) cohort ($n = 234$) for confirmation (2, 11). All participants gave written informed consent, and the study protocols have local ethical board approvals. Cohort descriptions are given in the APPENDIX.

Serum metabolites. Levels of circulating metabolites were analyzed using a high-throughput ^{1}H NMR metabolomics platform. The method has been described in detail elsewhere (9, 21, 22). In short, the NMR metabolomics platform offers metabolic measures from serum or plasma samples in concentration units that can be readily applied in epidemiological research. The platform can quantify various measures of lipoprotein metabolism, certain lipids, small molecules, and a few protein concentrations. We used in total 157 quantitative metabolic measures obtained by NMR profiling, all listed in Supplemental Table S9. (Supplemental Figs. S1–S7 and Supplemental Tables S1–S11 are all available online at the *American Journal of Physiology: Endocrinology and Metabolism* website.)

Physical performance during military service. All men attended an 8-wk basic training period at the beginning of their service. Subsequent military service included high-intensity physical exercise: running, Nordic walking, strength training, martial arts, orienteering, swimming, and cross-country skiing. To assess physical performance, the 12-min Cooper running test and muscle strength tests were administered at the baseline and at the follow-up. The Cooper running test evaluates maximal aerobic capacity by having the participants run for 12 min with maximal effort. The distance run represented their performance level (4). This test monitors the development of the participant's aerobic endurance and indirectly estimates the maximal oxygen uptake ($\dot{V}O_{2max}$). Muscular performance was measured using five tests: sit-ups, back muscles, push-ups, pull-ups, and standing long jump. For each test, conscripts were asked to perform a maximum number of repetitions within 60 s. Muscle performance was evaluated for each test on a 4-point scale: 0 = poor, 1 = satisfactory, 2 = good, and 3 = very good. The muscle performance index (MPI) was derived by summing the scores from the five tests (20). MPI of 0–4 was considered poor, 5–8 satisfactory, 9–12 good, and 13–15 very good.

Bioelectrical impedance measurements of body composition. Body fat percentage (PBF) was assessed by bioelectrical impedance measurements. For this purpose, the Sodankylä study used the InBody 720 Body Composition Analyzer (Biospace, Seoul, Korea). The electrode method of the device consists of a tetrapolar eight-point tactile electrode system [for more details, see Mikkola et al. (16, 17)]. The body composition measurements for DILGOM baseline and follow-up were obtained with Tanita TBF-300MA (Tanita Corporation of America, Arlington Heights, IL), which measures voltage drop from foot to foot when a small alternating current is applied through contact with two metal foot plates. The method has been shown to be comparable to a four-compartment method (7).

Statistical analysis. To facilitate comparisons, we first transformed changes in levels of metabolites and changes in PBF and MPI to SD units. We then performed multivariable linear regression to assess how reduction in PBF and increase in MPI alter the levels of metabolites in the two cohorts. Due to high correlation among several metabolites, we estimated the number of independent tests by calculating the number of principal components (PCs) that explained more than 95% of the variation in levels of metabolites, and this number was used to correct for multiple testing ($P < 0.05/24$ PCs; $P < 0.0021$). R (version 3.2.3, http://www.r-project.org/) was used for all statistical analysis.

RESULTS

Characteristics of the Sodankylä study participants are presented in Table 1. Mean and SD for all 157 metabolic measures at baseline, follow-up, and change are provided in Supplemental Tables S10 and S11 for both the Sodankylä cohort and DILGOM, respectively. This study includes 664 young men with mean age of 19.2 yr. The mean percent body fat following the military service was reduced from 16.7% to 15.4%. The

Table 1. *The characteristics of the Sodankylä army cohort*

Sodankylä	*n*	Mean	SD	Range
Age	664	19.2	0.9	18–28
Weight at baseline	657	74.7	13.9	40.2–140.8
PBF baseline	657	16.7	8.3	3.0–47.4
PBF follow-up	625	15.4	5.8	3.6–37.9
PBF change	625	−1.3	4.6	−27.1
MPI baseline	645	7.9	3.8	0–15
MPI follow-up	663	9.4	3.5	0–15
MPI change	644	1.6	2.4	−19
Cooper test at baseline	664	2,453	360	1,000–3,380
Cooper test at follow-up	664	2,661	300	1,100–3,500
Cooper test change	664	207	262	−2,160
				n (*n*%)
Months in service				
6 mo				476 (72%)
9 mo				33 (5%)
12 mo				155 (23%)
Service started				
Winter				426 (64%)
Summer				238 (36%)
Smoking at baseline				
Yes				383 (58%)
No				281 (42%)

There were no obvious clinically relevant metabolic abnormalities in the army cohort as it in general consists of healthy young men. Type 1 diabetics are generally excluded from service. SD, standard deviation; PBF, percent body fat; MPI, muscle performance index.

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mean muscle performance index (created from five tests: sit-ups, back muscles, push-ups, pull-ups, and standing long jump, see METHODS for details) was improved from 7.9 at the baseline (range 0–15) to 9.4 after the military service, showing an average improvement of 1.6, regardless of the baseline body mass index (BMI) or baseline physical activity level. Aerobic fitness, as measured by the Cooper 12-min running test (4), improved during the military service by +208 m.

We studied the relations between changes in physical performance and changes in 157 metabolic measures. Detailed information on changes in physical performance and body composition in the whole Sodankylä cohort of 1,467 individuals was previously reported by Mikkola et al. (16, 17) (for details about metabolic measures, see METHODS). The metabolic associations with change in MPI were stronger than metabolic associations with change in aerobic fitness. Thus we focus here on the MPI associations. A comparison of the metabolite association effect estimates of MPI versus aerobic fitness is presented in Supplemental Fig. S1, and the change in aerobic fitness associations with change in metabolite levels are presented in Supplemental Table S1.

The mean body weight remained relatively stable during the military service: 74.7 kg at baseline and 74.0 kg at the end of service (average change −0.7 kg). The mean BMI at the beginning of the military service was 23.84 kg/m^2 and at the end of the service 23.46 kg/m^2 (average change in BMI −0.38 kg/m^2). However, the mean PBF, a more accurate descriptive measure of body composition than BMI, was 16.7% at the beginning of the military service and was reduced to 15.4% at the end of the service, representing an average change in PBF of −1.3%, i.e., 0.3 SDs reduction of the total mean PBF in the cohort.

After correcting for multiple testing (at a level of $P < 0.0021$), the change in circulating concentrations of 15 metabolic measures remained associated with an increase in MPI. The associations are corrected for age, length of service, starting concentration of the metabolic measure, starting weight, current smoking, season of entrance to service, and muscle performance index at the beginning. The association results are summarized in Supplemental Fig. S2 and Supplemental Table S2.

PBF reduction following the military service was significantly associated with 79 metabolic measures where the models were adjusted for age, length of service, starting concentration of the metabolic measure, baseline weight, current smoking, season of entrance to service, and PBF at the beginning of service. (For more detail, see Supplemental Fig. S2 and Supplemental Table S3 for the values of β coefficients in SD units and *P* values; and Supplemental Table S4 for the associations between PBF and metabolites in unscaled units.)

Statistically independent associations of increased MPI vs. reduced PBF on metabolic profiles. We observed that the associations of reduction in PBF on the metabolic measures are well correlated with the associations of increased MPI on the metabolic measures (Supplemental Fig. S3). However, the magnitude of effect size for reduction in PBF was generally stronger (beta = 1.65, SE = 0.1, correlation $r^2 = 0.64$) than that for increased MPI. To determine whether the two measures have statistically independent associations on levels of metabolites, we added reduction in PBF as a covariate in the model that assessed the associations of increased MPI on metabolic measures. For the model that assessed the associations of reduced PBF, MPI was added as a covariate. The cross correlation between change in MPI and change in PBF was −0.22 (Pearson correlation, Supplemental Fig. S4).

The associations between increase in MPI during military service and changes in the metabolome, independent of reduction in PBF, are summarized for selected metabolic parameters in Fig. 1 (orange effect estimates). After correcting for the reduction in PBF and other covariates from the previous model, the change in circulating concentrations of six metabolic parameters were associated with an increase in MPI (Supplemental Table S5). Significant inverse associations were demonstrated with total cholesterol in VLDL particles, and cholesterol esters in large VLDL particles. In addition to lipoprotein measures, increase in MPI was associated with a lower ratio of monounsaturated fatty acids to total fatty acids, higher serum glutamine concentration, and lower abundance of acetylated glycoproteins (GlycA).

The associations between a reduction in PBF during military service and changes in the serum metabolic profile, independent of increase in MPI, are summarized for selected metabolic measures in Fig. 1 (blue effect estimates). After correcting for the increase in MPI and other covariates as above, reduction in PBF was associated with a change in circulating concentrations of a total of 60 metabolic measures (Supplemental Table S6). The broad hallmarks of associations with reduced PBF showed a significant decrease in LDL cholesterol levels, an increase of cholesterol associated with large HDL particles, and a decrease in apoB-100 concentration and in the apoB-100/apoA-I ratio. A marked decrease in the branched-chain amino acids leucine and isoleucine, as well as a decrease in the aromatic amino acids phenylalanine and tyrosine, was observed.

The largest difference after the cross adjustment was observed in the low-density lipoprotein-related measures, where an increase in muscle performance was not associated with the metabolic measures, whereas reduction in body fat percentage showed a strong statistically independent association. In addition, the large HDL particle measures showed associations with reduced PBF but not with increased MPI. There were also some differences in the strength of association in the branched-chain and aromatic amino acids, where PBF showed a stronger association than change in MPI.

The association of changes in PBF and changes in the metabolic profile in the population-based DILGOM cohort. As the change in PBF appears to capture the change in muscular performance well in the Sodankylä cohort, we further examined a cohort of the general population to determine whether the findings from young men exposed to rigorous physical activity can be generalized to a general population. In the DILGOM cohort, longitudinal measurements with 7-yr follow-up of metabolic profiles and bioimpedance measurements were available for 234 individuals after excluding pregnant women, oral contraception users, and lipid-lowering medication users from both time points (Table 2). For women, the age range was set to 25–40 yr (mean = 31.4, SD = 4.6) at baseline to avoid metabolic changes associated with menopause (1) during follow-up. For men, age range was set to 25–50 yr (mean = 38.0, SD = 8.2) to maximize power, but to limit possible nonlinear aging effects. The mean BMI of the studied participants at the baseline of the health survey was 25.8 kg/m^2 and at the end of the 7-yr follow-up 26.4 kg/m^2 (average

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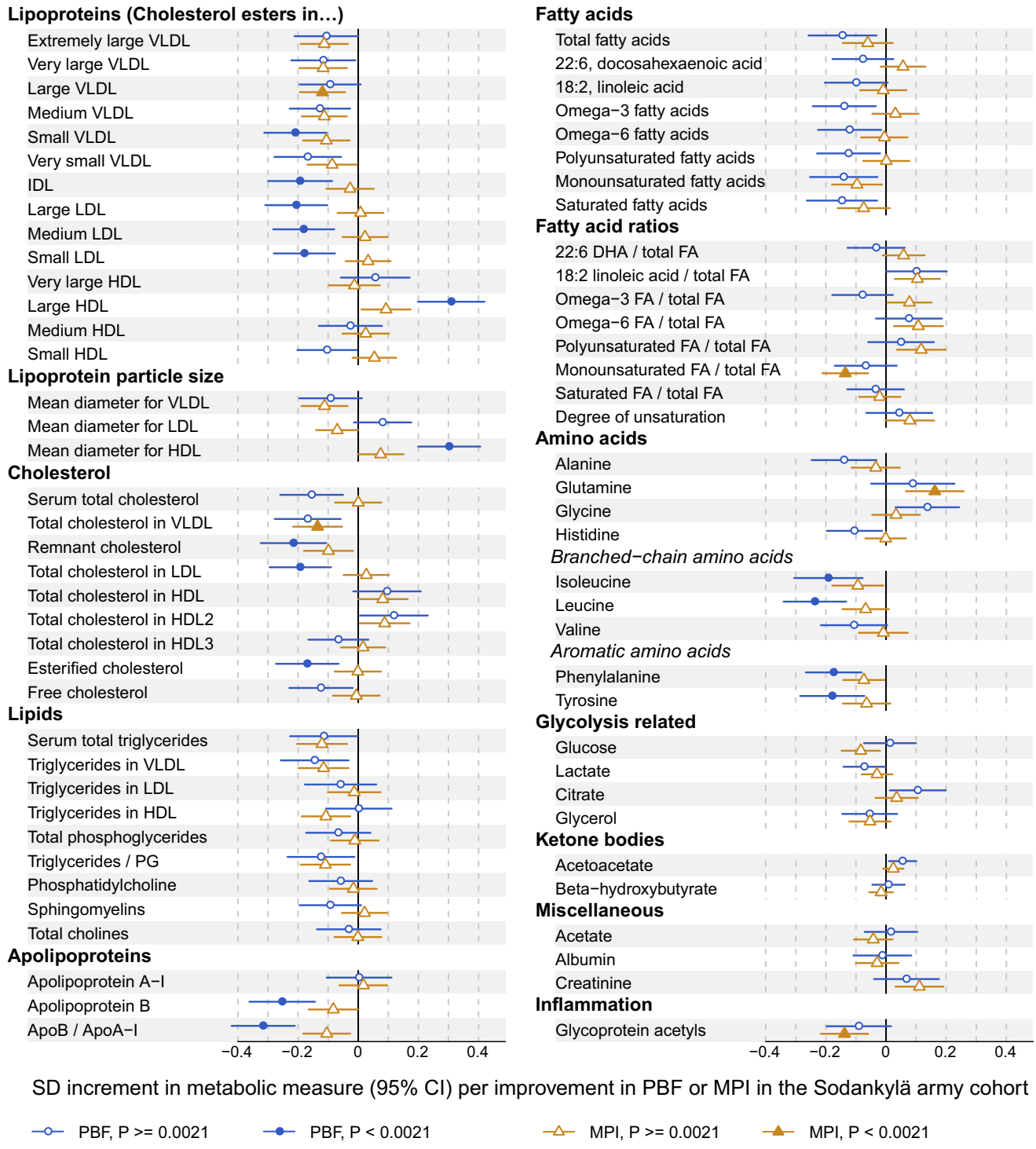

Fig. 1. The metabolite associations in standard deviations [95% confidence interval (CI)] from multivariable linear regression analysis of increase in muscle performance index (MPI) and reduction in body fat percentage (PBF) adjusted with each other in the Sodankylä army cohort. The models were further adjusted for age, length of service, starting concentration of the metabolic measure, starting weight, current smoking, season of entrance to service and muscle performance index, or body fat percentage at the beginning. Orange effect estimates are for the increase in muscle performance index and blue effect estimates are for reduction in body composition. Filled symbols denote significant associations after multiple testing correction. All association results are listed in Supplemental Tables S5 and S6.

increase in BMI of 0.54 kg/m^2). The mean PBF at baseline was 24.1% and at the end of follow-up 25.5% (average increase in PBF was 1.4%).

There were no differences in the metabolic associations with reduced PBF in women when compared with men (Supplemental Fig. S5). Therefore, we combined men and women, adjusting for sex in the main analyses. The associations between reduction in PBF during the 7-yr period and changes in the metabolome are summarized for selected metabolic measures in Fig. 2, which describes a representative subset of all

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Table 2. *The characteristics of the DILGOM cohort*

DILGOM	*n*	Mean	SD	Range
Age, men	174	37	7.7	25–50
Age, women	60	30.9	4.5	25–40
Weight at baseline	234	79.4	14	42.2–130.1
PBF baseline	234	24.1	7.1	8.8–52.10
PBF follow-up	234	25.5	7.3	10.8–48.4
PBF change	234	1.4	3.8	−27.9
Smoking at baseline				
Yes	53 (23%)			
No	181 (77%)			

As the DILGOM cohort has a generally low mean age, and we excluded lipid-lowering medication from both time points, there was no prevalent or incident coronary heart disease. There were three type 2 diabetes patients at baseline, and no incident diabetes cases. SD, standard deviation; PBF, percent body fat.

metabolic measurements. The associations were adjusted for age, sex, metabolic measures at baseline, weight at baseline, current smoking status, and PBF at the baseline. All association results are summarized in Supplemental Table S7. After correcting for multiple testing, the change in circulating concentrations of 128 metabolic parameters was associated with change in PBF. Supplemental Table S8 contains the effect estimates in unstandardized units.

The broad signatures of associations with reduced PBF included a significant decrease in VLDL (including VLDL remnants) and LDL cholesterol levels, a pronounced increase in cholesterol and especially cholesterol ester content in large-sized HDL particles, a marked decrease in triglyceride concentration in all major lipoprotein classes, especially of triglycerides carried in VLDL particles, and a significant decrease in apoB-100, and also in apoB-100/apoA-I ratio. For serum lipid concentrations, we observed a decrease in serum total fatty acids, especially for monounsaturated fatty acids and saturated fatty acids. The relative concentration increase of omega-6 fatty acids and 18:2 linoleic acid to total fatty acids was also associated with reduction in PBF. We observed a marked decrease of all branched-chain amino acids as well as a decrease in the aromatic amino acids phenylalanine and tyrosine. In addition, a decrease in serum alanine, glycerol, glucose, and GlycA levels was observed. Glycine was the only small molecule that showed an increase with reduced PBF.

We demonstrated that *1*): the directional effect of the estimates was in good agreement between the two cohorts; but *2*) the associations between PBF and the metabolic parameters in the DILGOM cohort were much stronger than those in the Sodankylä cohort (Fig. 2, Supplemental Fig. S2, and Supplemental Tables S3 and S7). The strongest differences were observed in the inflammation-related marker GlycA, glucose, and several VLDL-related measures that relate to triglyceride-rich lipoprotein metabolism.

Fat loss vs. fat gain in the general population-based DILGOM cohort. A total of 150 participants gained PBF and 84 lowered PBF during the 7-yr follow-up period. To test whether fat percentage gain would be metabolically more disturbing than fat percentage loss, we compared the relations between changes in PBF and changes in metabolic parameters in those who gained fat vs. those who lost fat during the 7-yr follow up (Supplemental Fig. S6). We did not observe differences between the two groups.

Change in PBF associations when further adjusted for change in BMI. As BMI can be a suboptimal measure for change in body composition, we further tested the associations of change in PBF with change in metabolic measures when further taking into account the change in BMI in the DILGOM cohort. The associations were therefore adjusted for age, sex, metabolic measures at baseline, weight at baseline, current smoking status, PBF at the baseline, and change in BMI. We observed that change in PBF still showed association with 20 metabolic measures when taking into account multiple testing (Supplemental Fig. S7). In particular, the atherogenic lipoprotein cholesterol-related measures (from small VLDL to small LDL) showed stronger association when taking into account the change in BMI.

DISCUSSION

The present study is, to the best of our knowledge, the first longitudinal investigation that attempts to tease apart the potential independent health benefits of objectively measured increase of physical performance versus changes in body composition. In addition, this is the first large study that compared 157 serum metabolic parameters of young men before and after an intensive lifestyle change. Using high-throughput metabolite profiling, we identified six independent parameters as biomarkers for muscle performance measured by MPI. However, the change in PBF demonstrated stronger independent associations on metabolic markers totaling 60 parameters. These findings in the discovery study were not only replicated in the general population-based cohort, but the strengths of associations were even stronger than those in the cohort of young men.

Increase in physical performance generally followed a similar, but weaker, association pattern as the decrease in PBF in the Sodankylä cohort. The largest differences between physical performance and PBF change were displayed for LDL cholesterol metabolism. We observed that increased physical performance did not show a statistically independent effect for lowering LDL cholesterol, and the lowering effect was targeted to loss of body fat. Our results thus underscore that reduction in body fat is essential to reduce LDL cholesterol, a known causal risk factor for CVD. Branched-chain amino acids have been implicated as risk factors for T2D (15), and a recent study using Mendelian Randomization found that the branched-chain amino acids are likely new markers for insulin resistance (23). We observed that change in PBF showed stronger associations with these amino acids than change in MPI, indicating that again loss of body fat is a more important factor in reducing diabetes risk. These differences suggest that the reduction of PBF is essential to reduce the levels of these cardiovascular and diabetic biomarkers and subsequently reduce the risk for CVD and T2D through reducing the levels of LDL and aromatic amino acids, as they have been proposed to be causal for the disease development.

Studies from as early as the 1980s showed that exercise training can alter HDL subfractions, providing a clue that exercise is capable of modulating HDL metabolism (18). Würtz et al. (26) showed that the large HDL particle concentration was the strongest predictor of future cardiovascular events when using these same metabolic measurements and adjusting for nonlaboratory measures. It is well known that physical activity, especially aerobic exercise, is associated with

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Fig. 2. The metabolite associations in standard deviations (95% CI) from multivariable linear regression analysis of reduction in percent body fat (PBF) in the DILGOM cohort. The analysis was adjusted for age, sex, starting concentration of the metabolic measure, starting weight, current smoking and PBF at baseline. Filled symbols denote significant associations after multiple testing correction. All association results are listed in Supplemental Table S7. For associations between PBF and metabolites in unscaled units, see Supplemental Table S8.

an increase in circulating HDL cholesterol concentration (10). Our results suggest that the main effect of physical activity-associated change in large HDL measures is due to change in body composition, rather than increased physical performance. Our results suggest that HDL could be a biomarker of body composition dynamics and therefore reflects the cardiovascular risk as a noncausal biomarker. However, this intriguing finding requires further validation.

It is important to note that an increase in physical performance is tied to changes in body composition, and we cannot rule out any beneficial effects from the results of this study. We propose that the focus for treating CVD or diabetes risk should

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be in reducing PBF with all possible means including increased physical exercise. Moreover, increased muscle performance showed an independent association with VLDL metabolism, suggesting that increased muscle performance could also increase lipoprotein lipase-facilitated VLDL triglyceride hydrolysis and lipid uptake by muscle cells. As shown in a recent report by Catoire et al. (3), during endurance exercise, selective induction of the protein angiopoietin-like 4 (ANGPTL4) in nonexercising muscle reduces local fatty acid uptake via ANGPTL4-mediated lipoprotein lipase (LPL) inhibition, presumably preventing muscle fat overload while directing fatty acids to the active skeletal muscle as fuel where LPL is fully active. However, this interesting observation needs further studies.

Our work stands apart from previous studies on the associations of physical activity and obesity with these same metabolic parameters (12, 27). Our study is unique because of its longitudinal design, objective measurement of physical performance, and by showing the statistically independent associations that were beyond reach in the previous work with physical activity and BMI. In addition, BMI does not separate between muscle and fat mass change, and therefore the present work enables more detailed conclusions of body composition and its associations on circulating metabolites. We showed that adjusting with change in BMI did not explain the associations completely, further corroborating that BMI is not a perfect proxy for obesity-related associations in circulating metabolism. Our study showed that increased MPI is generally weakly associated with the metabolic profile compared with decreased PBF. In particular, we did not observe any changes in the LDL metabolism, contradicting earlier studies comparing individuals with high and low physical activity levels (12). The associations observed with physical activity in the previous study may arise from more subtle differences in body composition that are generally poorly captured by BMI and the cross-sectional nature of the study. It may be that when BMI is accompanied with a physical activity or fitness test in the same multivariable model for adverse events, the fitness measures may proxy more fine-grained information about the ratio of fat versus muscle mass, as fit or physically active individuals likely have a lower fat percentage. Usually a physical activity measurement in observational studies relies on self-reporting, which correlates poorly with objective measures or performance tests (5, 25).

This study also has some limitations. Although the Sodankylä cohort went through a harmonized lifestyle change, the dietary control of the individuals is still limited. The study participants were served harmonized meals in regard to quality, but the amount consumed could not be controlled. In addition, dietary consumption outside of service hours could not be controlled. The study included only a relatively young part of the general population, and these results do not apply to elderly, as weight loss is detrimental for the elderly. The study is not a randomized intervention, and some of the metabolic associations may still be due to residual confounding. However, we used longitudinal study design in an attempt to minimize the potential confounding as well as possible. We used only metabolic measures from circulation and did not test for metabolic changes in other tissues of interest, such as muscle or adipose tissue, and this may hinder our ability to provide mechanistic insight into the observed associations. Additionally, although bioimpedance measurement describes better body composition than for example BMI, it does not provide accurate information about the localization of body fat, which is likely also an important factor with respect to the observed associations.

Many of the lipid and lipoprotein associations were stronger in the population-based cohort than in the military cohort; however, the largest differences were observed in glucose levels and the inflammation marker GlycA. GlycA has been shown to be elevated in individuals with a higher risk of all-cause mortality in the general population (6). A recent study showed that GlycA levels were stable within individuals for up to a decade, and were associated positively with various cytokines, indicating that GlycA is likely to be a marker of low-grade chronic inflammation (19). GlycA was lowered with increased muscle performance in the Sodankylä cohort, which may explain the mortality risk associated with cardiorespiratory performance. Taken together, the impaired glucose metabolism and increased inflammation are likely to take a longer time to develop; thus the observed relations may appear at an older age when fat accumulates rather than in younger individuals with a relatively short follow-up.

APPENDIX

Cohort Descriptions

The Sodankylä army cohort. As part of the Sodankylä army cohort collected in 2005 (16), a total of 1,467 men (mean age at baseline 19.2 yr; SD 1.0; range 18–28 yr) participated in the current study. The Sodankylä samples represented a population-based cohort of Finnish men in their 20s, as military service is compulsory in Finland for all men, except for about 20% of an age cohort who chose to serve in nonmilitary civil service or had health or other issues that precluded them from serving in the armed forces. Thus these Finnish army recruits represent young Finnish men, unselected for socioeconomic background, baseline physical performance, geographical area, education, or employment. All participants gave written informed consent, and the study protocol was approved by the Ethics Committee of Lapland Central Hospital, Rovaniemi, Finland.

The period of military service offers us an opportunity to investigate the effect of continuous physical training on young men's health. Moreover, these young men shared a relatively homogeneous sleeping, diet, and physical activity environment during the service (17). All data including venous blood samples, anthropometrical measurements, body composition measurements (InBody 720 Body Composition Analyzer; Biospace, Seoul, Korea), physical performance measurements, and questionnaires were collected twice: once at the beginning and another at the end of their military service either at 6, 9, or 12 mo. The duration of service depended on the type of training and tasks. All biosample measurements were performed after an overnight fast. Information on participants' background, demographics, and smoking was obtained by questionnaires. Of 1,467 original study participants, 664, who had complete longitudinal data as well as metabolic measures for both the baseline and follow-up assessments, were included in the current study.

DILGOM cohort. One thousand three-hundred twelve individuals from the original population-based DIetary, Lifestyle and Genetic determinants of Obesity and Metabolic syndrome (DILGOM) study participated in the current study. Briefly, DILGOM included 25- to 74-yr-old Finnish men and women who took part in the study at baseline (n = 5,024) in 2007 (2, 11). In the health examination, trained research nurses measured the participants' height and weight and obtained blood samples from them. Between April and June of 2014, all living baseline participants were invited to take part in a follow-up study, and 3,735 responded (response rate = 82%). The current study includes a subset of participants who lived in the areas

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of Turku and Loimaa and in the cities of Helsinki and Vantaa. The research protocol of the DILGOM baseline and follow-up studies was designed and conducted in accordance with the guidelines of the Declaration of Helsinki and have been approved by the Ethics Committee of Helsinki and Uusimaa Hospital District (Decision Nos. 229/E0/2006 and 332/13/03/00/2013, respectively). In addition, written informed consent was obtained from all participants.

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DISCLOSURES

J. Kettunen possesses stock options of Nightingale Health Ltd, a company offering NMR metabolomics profiling. None of the other authors has any conflicts of interest, financial or otherwise, to disclose.

AUTHOR CONTRIBUTIONS

P.J., S.K.-K., and M.P. performed experiments; J.K. and A.W. analyzed data; J.K., A.J., M.H., J.H.L., K.B., M.J., J.J.J., S.K.-K., and M.P. interpreted results of experiments; A.J. prepared figures; J.K., A.J., M.H., I.M., A.W., J.H.L., M.J., S.K.-K., and M.P. drafted manuscript; J.K., A.J., M.H., I.M., J.H.L., J.D.T., K.B., P.J., M.J., J.J.J., S.K.-K., and M.P. edited and revised manuscript; and J.K., A.J., M.H., I.M., A.W., J.H.L., J.D.T., K.B., P.J., M.J., J.J.J., S.K.-K., and M.P. approved final version of manuscript.

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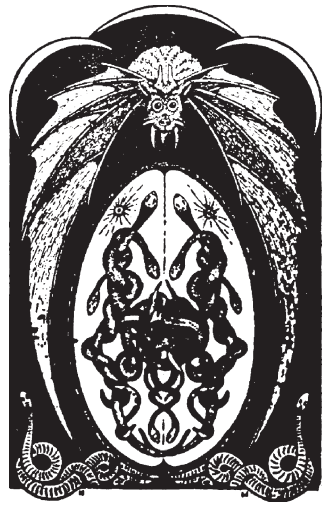

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