

Resistance Training Induces Antiatherogenic Effects on Metabolomic Pathways

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ABSTRACT

SARIN, H. V., J. P. AHTIAINEN, J. J. HULMI, J. K. IHALAINEN, S. WALKER, M. KÜÜSMAA-SCHILDT, M. PEROLA, and H. PELTONEN. Resistance Training Induces Antiatherogenic Effects on Metabolomic Pathways. *Med. Sci. Sports Exerc.*, Vol. 51, No. 9, pp. 1866–1875, 2019. **Introduction:** Arising evidence suggests that resistance training has the potential to induce beneficial modulation of biomarker profile. To date, however, only immediate responses to resistance training have been investigated using high-throughput metabolomics whereas the effects of chronic resistance training on biomarker profile have not been studied in detail. **Methods:** A total of 86 recreationally active healthy men without previous systematic resistance training background were allocated into (i) a resistance training (RT) group ($n = 68$; age, 33 ± 7 yr; body mass index, $28 \pm 3 \text{ kg}\cdot\text{m}^{-2}$) and (ii) a non-RT group ($n = 18$; age, 31 ± 4 yr; body mass index, $27 \pm 3 \text{ kg}\cdot\text{m}^{-2}$). Blood samples were collected at baseline (PRE), after 4 wk (POST-4wk), and after 16 wk of resistance training intervention (POST-16wk), as well as baseline and after the non-RT period (20–24 wk). Nuclear magnetic resonance–metabolome platform was used to determine metabolomic responses to chronic resistance training. **Results:** Overall, the resistance training intervention resulted in favorable alterations ($P < 0.05$) in body composition with increased levels of lean mass (~2.8%), decreased levels of android (~9.6%), and total fat mass (~7.5%). These changes in body composition were accompanied by antiatherogenic alterations in serum metabolome profile (false discovery rate < 0.05) as reductions in non–high-density lipoprotein cholesterol (e.g., free cholesterol, remnant cholesterol, intermediate-density lipoprotein cholesterols, low-density lipoprotein cholesterols) and related apolipoprotein B, and increments in conjugated linoleic fatty acids levels were observed. Individuals with the poorest baseline status (i.e., body composition, metabolome profile) benefitted the most from the resistance training intervention. **Conclusions:** In conclusion, resistance training improves cardiometabolic risk factors and serum metabolome even in previously healthy young men. Thus, suggesting attenuated risk for future cardiovascular disease. **Key Words:** RESISTANCE EXERCISE, OMICS, BIOMARKERS, LIPIDS, AMINO ACIDS, CARDIOVASCULAR DISEASE

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Submitted for publication November 2018.

Accepted for publication March 2019.

Supplemental digital content is available for this article. Direct URL citations appear in the printed text and are provided in the HTML and PDF versions of this article on the journal's Web site (www.acsm-msse.org).

0195-9131/19/5109-1866/0

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DOI: 10.1249/MSS.0000000000002003

Regular physical activity together with reduced adiposity is associated with beneficial modulation of biomarker profile, cardiometabolic health, and reduced risk of noncommunicable diseases (e.g., cardiovascular disease, type 2 diabetes, metabolic syndrome). This highlights why it is important to enhance detailed understanding of exercise-induced metabolic responses and to determine possible causal relationships between exercise, biomarker profile, and health effects (1). Developments in the field of systems biology, in particular high-throughput metabolomics, have made it possible to quantify and investigate a wide array of metabolites representing the direct signature of biochemical activities of the cell at a functional level (2). Through detailed quantification and interpretation of lipoprotein subclasses, serum free fatty acids, glycolysis precursors, amino acids, and inflammation biomarkers, it is

possible to distinguish how exercise regimens affect previously identified risk markers of cardiometabolic profile and noncommunicable diseases (2,3). Thus, understanding metabolite profile modulation and factors affecting it (e.g., exercise, body composition) will promote the identification of metabolic signatures that provide novel information about biomarkers that can be affected by exercise interventions and are relevant to health monitoring and disease prevention (4,5).

To date, resistance training interventions have been shown to effectively reduce fat mass (6,7)—a significant risk factor for cardiometabolic and overall health. Compared to interventions consisting of aerobic training and healthy diet, resistance training bears the benefit of more efficiently increasing lean mass, and preserving it in times of caloric restriction (e.g., weight loss) (8,9). The benefits of higher levels of lean mass in disease prevention has been relatively well covered in the elderly and individuals with chronic diseases where it has been shown to predict longevity and reduced mortality (10–12). However, it remains to be determined whether engagement in chronic resistance training leading to higher levels of lean mass promotes additional benefits even in previously healthy young individuals in terms of cardiometabolic health. Some studies have reported resistance training to promote cardiometabolically favorable alteration in cholesterol and lipid levels when measured with standard biomarker quantification (13). Detailed metabolomic profiling on the effects of resistance training has not been extensively studied. To date, only immediate responses to resistance training (12–14) have been investigated using high-throughput metabolomics, whereas the effects of chronic resistance training on metabolome profile have not been studied, thus, highlighting the importance of our study.

Moreover, it has been demonstrated that considerable individual variation exists regarding body composition changes in response to resistance training regimens (i.e., responders/nonresponders) (14). However, it has been questioned whether this concept actually holds true or is it masked by the individual variation (e.g., age, training adaptation, genetics) on how sensitive individuals are to different exercise regimens. Thus, it can be debated if it is more appropriate to call individuals high responders and low responders depending on their level of response, especially in studies allocating individuals to follow standard exercise regimen. Furthermore, previous findings on responder status and body composition imply that similar variation could be observed in biomarker profile responses of exercising individuals.

Understanding the metabolic signatures related to resistance training and body composition changes could provide tools to identify individuals most susceptible to the benefits of acute exercise and chronic resistance training. The aim of the present study was to examine changes in blood metabolome profiles in response to chronic resistance exercise training and associated changes in body composition in healthy young adult men. Based on findings from previous studies, we hypothesize that chronic resistance training has the potential to alter body composition and metabolome profiles in a cardiometabolically favorable manner.

MATERIALS AND METHODS

Participants and Study Design

A total of 86 recreationally active healthy men without previous systematic resistance training background formed the present research group; 68 men (age, 33 ± 7 yr, body mass index [BMI], 28 ± 3 kg·m⁻²) belonged to the resistance training (RT) group, and 18 nontraining peers (age, 31 ± 4 yr; BMI, 27 ± 3 kg·m⁻²) belonged to non-RT group to study whether similar responses in metabolome can also occur without resistance training (see Supplemental Fig. 1, Supplemental Digital Content 1, Flowchart of study design, <http://links.lww.com/MSS/B587>). The non-RT group was formed using data from two previously collected cohorts. The non-RT individuals were selected based on age and sex to match the characteristics of RT group participants. The participant characteristics and methods for this study have been reported in detail elsewhere (RT group: (15), (16); non-RT group: (17), (18)). All participants were informed of the potential risks associated with the study and they provided written informed consent before participation. The study was conducted according to the Declaration of Helsinki. Ethical approval for the study procedures were granted by the Ethical Committee at the University of Jyväskylä and where necessary by the Ethical Committee of the Central Hospital, Jyväskylä (for dual-energy X-ray absorptiometry (DEXA) measurements).

The duration of the fully supervised resistance training intervention for the RT group was 16 wk. Measurements were performed at baseline (PRE), after 4 wk (POST-4wk), and after 16 wk (POST-16wk) of resistance training. The non-RT group was measured at baseline (PRE) and after (POST-control) 20 wk ($n = 8$, (17)) or 24 wk ($n = 10$, (18)). Follow-up intervals were different for the RT and non-RT group as the non-RT group was formed using data from two previously collected cohorts. For all participants in both groups, all measurements regarding anthropometrics, muscle strength, and venous blood sample collection were performed at each time point (see Supplemental Fig. 1, Supplemental Digital Content 1, Flowchart of study design, <http://links.lww.com/MSS/B587>). For the RT group only, dietary information was measured once during the 12-wk RT period with 4-d food diaries. All participants, independent of group assigned, were advised to continue their normal dietary intake and habitual physical activities throughout the study period. In addition, the non-RT group was asked to maintain their preexperimental physical activity level throughout the study period.

Resistance Training Program

The resistance training program has been described in detail previously (15). The intervention for the RT group began with 4 wk of whole-body workouts twice-a-week. The participants performed 8 to 10 exercises within one workout, two to three sets for every exercise, and 10–15 repetitions in every set. Recovery time of 2 min was held constant between sets. Training loads were 50% to 80% of one repetition maximum

(1RM) increasing throughout this preparatory phase. Nine participants dropped out of the study after 4 wk of the preparatory RT phase. The remaining participants ($n = 59$) were divided after the first 4-wk training period into two groups: (i) training aiming for muscle hypertrophy and strength ($n = 33$) and (ii) training aiming for muscle hypertrophy, strength and power ($n = 26$) for the following 12 wk. The specific resistance training programs consisted of two to three training sessions per week and were divided into three 4-wk training blocks in which the volume of hypertrophic (75%–85% loads of 1RM), maximal-strength (86%–95% 1RM), and power-strength (50%–80% 1RM) training fluctuated nonlinearly according to the training goal. The training subgroups were pooled for the present study since from the point of view of this investigation there were no substantial differences detected in training adaptation between the allocated training subgroups in the primary outcomes of RT and, most importantly, to improve statistical power for the metabolite analyses.

Body Composition

Body composition was measured by dual-energy X-ray absorptiometry (DEXA) (LUNAR Prodigy Advance, GE Medical Systems, Madison, USA) from which lean mass of the upper and lower limbs were isolated from the trunk and estimated using the software-generated regions (enCORE 2005, version 9.3) (15,17). The DEXA measures were repeated in a similar fashion in a fasted state at the same time of the day for all study participants in both groups. Automatic analyses also provided the android region (the area between the ribs and the pelvis within the trunk region) that correlates with visceral fat measures. Measured total body lean and fat mass were normalized to body surface area (BSA) to calculate lean mass index (LMI_{BSA}) and fat mass index (FMI_{BSA}), respectively. The BSA was calculated by the following formula: $BSA (m^2) = \text{square root of } (\text{height (cm)} \times \text{weight (kg)})/3600$ (19).

Muscle Strength

Maximal strength was measured at baseline (PRE), after the 4-wk preparatory RT period (POST-4wk) and after the 12-wk RT period (POST-12wk) in the RT group or after the control period in the non-RT group (POST-control) (14–17). The subjects visited the laboratory once before the study to learn the appropriate techniques and practice the strength tests.

During the actual measurement protocols, the subjects were carefully familiarized with the test procedures and had several warm-up contractions on all used devices. Maximal bilateral dynamic concentric strength of the leg extensors (hip and knee extensors) was measured using a horizontal leg press device (D210; David Health Solutions Ltd., Helsinki, Finland). The device was set up so that the knee angle in the initial flexed position was on average 60° and a successful trial was accepted when the knees were fully extended (approximately 180°) (15). The highest load that the participant was able to lift to a full knee extension (180°) was accepted as the 1RM.

Dietary Intake

Subjects in the RT group kept 4-d food diaries during the second block of the 12-wk RT period (see above) ($n = 38$). The food diaries were analyzed by nutrient analysis software (Nutri-Flow; Flow-team Oy, Oulu, Finland). The subjects also received both verbal and written nutritional recommendations based on the dietary guidelines for normal healthy adults.

Venous Blood Sample Collection, Storage, and Analysis

Fasting blood samples were collected from the participants (i) PRE, POST-4wk, and POST-12wk in the RT group, and (ii) PRE and POST-control in the non-RT group. Blood samples were taken in the morning (07.00–09.00 h) after a 12-h overnight fast. Participants were asked to rest for at least 8 h during the preceding night and avoid strenuous physical activity for at least 48 h, including programmed training sessions. Blood samples were taken from the antecubital vein into serum tubes (Venosafe; Terumo Medical Co., Leuven, Hanau, Belgium) using standard laboratory procedures. Blood samples stood in room temperature for 10 min, after which they were centrifuged at 3500 rpm for 10 min (Megafure 1.0 R Heraeus; DJB Lab Care, Germany) to separate the serum. Serum samples were kept at –80°C for under a year for future metabolomics analyses. During transportation, handling, or storage of the serum samples, they were not allowed to thaw before metabolomic analyses. Thus, sources of major preanalytical bias/variance that could have otherwise affected the validity of the samples were eliminated.

Detection, Quantification, and Analysis of Nuclear Magnetic Resonance Metabolomics

A nuclear magnetic resonance (NMR) metabolomics platform (Nightingale Health Ltd, Helsinki, Finland) was applied for the absolute detection and quantification of serum metabolites to identify affected metabolic pathways (1,2). Details of the experimentation and proton NMR spectrometer characteristics have been described previously (2,3). Shortly, the samples were measured using a Bruker AVANCE III HD NMR 500 MHz spectrometer equipped with a cryogenically cooled TCI CryoProbe Prodigy. The used measurement temperature was 310.1 K. Frozen EDTA plasma samples from a fasting state were used for metabolomic analyses. In total, the assay yielded 233 different biomarkers for further assessment, including a variety of 14 different lipoprotein subclasses (e.g., very-low density lipoprotein [VLDL], LDL, HDL), apolipoproteins, serum free fatty acids, and various low-molecular metabolites including glycolysis precursors, amino acids, and inflammation biomarkers (see Supplemental Table 1, Supplemental Digital Content 2, Full list of quantified metabolites with NMR platform, <http://links.lww.com/MSS/B588>). The complete process and methods of sample preparation, identification and quantification have been reported elsewhere in full detail (2,3).

Effect Size Estimation and Power Calculations

To date, for metabolic phenotyping studies, there is currently no accepted approach for estimation of statistical power and sample size, which is largely due to the unknown nature of the expected effect. In hypothesis generating metabolic phenotyping studies, similar to ours, neither the number or subclasses of important metabolites nor the effect size are known *a priori*. To limit possible problems with statistical power, as large of a sample size as feasible for the current study setting and design was adopted. Nevertheless, *post hoc* Cohen's *d* effect size estimates were calculated to guide sample size needed for future validation studies (see Supplemental Table 2, Supplemental Digital Content 2, Cohen's *d* effect sizes for the NMR metabolome platform metabolites, <http://links.lww.com/MSS/B588>).

Statistical Analyses

Physiological characteristics. All data are presented as mean and SD. Statistical analyses on physiological characteristics were carried out using SPSS 24.0 software for Windows (SPSS, Inc., Chicago, IL). The Kolmogorov–Smirnov test was used to test normality, and the Levene's test was used to analyze the homogeneity of variances. The differences in changes between the RT and non-RT groups following the intervention were assessed by using univariate ANOVA with corresponding baseline-value as covariate (i.e., ANCOVA). Changes within the RT and non-RT group were assessed by generalized linear model repeated measures with Bonferroni *post hoc* test. The independent-samples *t* test was used to assess the differences between the RT and non-RT groups at baseline. Statistical significance was accepted when $P \leq 0.05$.

Metabolomics. Standard data quality control protocol was applied to raw data extracted from the NMR metabolomics platform prior to further statistical analysis. At first, data skewness, normality, and outliers with dot plots and histograms were investigated to dispose of outliers that could otherwise expose bias to the data. Subsequently, final exclusion threshold was set to ± 4 SD difference from the mean to filter out outliers and poor-quality data. No further normalization was applied to the dataset to correct for variable distributions as the

selected statistical method, Generalized Estimating Equations, allows for deviations from normality. Generalized Estimating Equations with linear link and working independence correlation structure was used to perform statistical analysis of the serum metabolites. Initially, exploratory analyses were performed aiming to investigate (i) differences between RT and non-RT groups across time points and (ii) within RT group changes across time points. Exploratory analysis comparing RT and non-RT groups revealed relevant differences in baseline values that might introduce bias to the metabolomic analyses, which is why this study focuses mainly on RT group analyses. There were no differences detected in training adaptation between the allocated RT subgroups in any of the primary outcomes of RT, including strength or hypertrophy measures. In addition, no differences were observed in any of the investigated metabolomics variables between RT subgroups and therefore, these two RT subgroups were collapsed together and analyzed as a single RT group to improve statistical power (15).

Furthermore, *post hoc* tests were of most interest when examining whether there were differences in serum metabolome responses across time points when comparing high and low responders to resistance training. High and low responders were defined as the highest and lowest quartile based on the change in lean mass index (LMI_{BSA}) from the RT intervention. When needed, statistical analyses accounted for age, BMI, and metabolite baseline level to minimize the effect of confounding factors.

False discovery rate (FDR) was used to adjust *P* values for multiple testing for all analyses conducted on the NMR metabolome dataset. Significance threshold was set to $FDR \leq 0.05$. The software applied for statistical analyses of metabolome variables was R (version 3.3.3 or higher).

RESULTS

Overview: Resistance training reduces the risk of cardiovascular disease through body composition and metabolome profile modulation. Overall, favorable alterations in body composition were observed as a consequence of the RT regimen (PRE- to POST-16wk). As shown in Table 1, increased levels of total lean mass ($\sim 2.8\%$),

TABLE 1. Mean (SD) changes in physiological characteristics of the RT (from baseline to after 4 wk, $n = 68$; from baseline to postintervention, $n = 59$) and non-RT ($n = 18$) groups during the study.

| | | Baseline | After 4 wk | Postintervention | Change ^a from Baseline to Postintervention (95% CI) | <i>P</i> |
|---|--------|-------------|-------------|------------------|--|----------|
| Body mass (kg) | RT | 82.4 (10.5) | 82.6 (10.1) | 83.2 (10.5) | 0.44 (−0.07 to 0.95) | 0.560 |
| | non-RT | 80.0 (11.7) | | 80.9 (11.5) | 0.75 (−0.18 to 1.67) | |
| Lean mass normalized to BSA ($\text{kg}\cdot\text{m}^{-2}$) | RT | 29.4 (2.1) | 29.7 (2.1)* | 30.1 (2.1)* | 0.83 (0.65 to 1.00) | <0.001 |
| | non-RT | 30.2 (1.7) | | 30.0 (1.9) | −0.23 (−0.55 to 0.10) | |
| Fat mass normalized to BSA ($\text{kg}\cdot\text{m}^{-2}$) | RT | 9.4 (3.7) | 9.1 (3.6)* | 8.9 (3.5)* | −0.69 (−0.93 to −0.46) | 0.001 |
| | non-RT | 8.1 (3.1) | | 8.3 (3.1) | 0.18 (−0.25 to 0.61) | |
| Android fat mass (kg) | RT | 2.1 (1.0) | 2.0 (1.0)* | 2.0 (1.0)* | −0.20 (−0.26 to −0.13) | 0.001 |
| | non-RT | 1.7 (0.9) | | 1.8 (0.91) | 0.04 (−0.08 to 0.16) | |
| 1RM normalized to total body lean mass | RT | 3.5 (0.5)** | 3.7 (0.5)* | 4.1 (0.6)* | 0.54 (0.47 to 0.62) | <0.001 |
| | non-RT | 2.6 (0.4) | | 2.1 (0.4)* | 0.05 (−0.09 to 0.20) | |

^aCorresponding baseline values as covariate; *P* value represents between-group comparisons of changes.

*Statistically significant change from the baseline, Bonferroni adjusted.

**Statistically significant difference between the groups at baseline.

P value represents between-group comparisons of changes.

CI, confidence intervals.

TABLE 2. Results of short-term resistance training intervention on serum metabolome profile.

| Metabolite | Estimate | Standard Error | Wald | FDR |
|---|----------|----------------|-------|----------|
| Cholesterol esters to total lipids ratio in IDL | -1.100 | 0.262 | 17.59 | 1.27E-03 |
| Total cholesterol to total lipids ratio in IDL | -1.050 | 0.217 | 23.19 | 1.19E-04 |
| Cholesterol esters to total lipids ratio in large LDL | -0.799 | 0.228 | 12.23 | 1.16E-02 |
| Total cholesterol to total lipids ratio in large LDL | -0.676 | 0.223 | 9.18 | 3.20E-02 |
| Remnant cholesterol | -0.097 | 0.031 | 9.68 | 2.79E-02 |
| Free cholesterol | -0.069 | 0.019 | 13.42 | 7.27E-03 |
| Apolipoprotein B | -0.046 | 0.015 | 9.16 | 3.20E-02 |
| Total cholesterol in IDL | -0.044 | 0.015 | 8.50 | 4.36E-02 |
| Cholesterol esters in IDL | -0.037 | 0.011 | 11.55 | 1.31E-02 |
| Apolipoprotein B/apolipoprotein A1 -ratio | -0.035 | 0.011 | 10.71 | 1.77E-02 |
| Sphingomyelins | -0.018 | 0.006 | 8.27 | 4.48E-02 |
| Cholesterol esters in very small VLDL | -0.010 | 0.004 | 8.40 | 4.36E-02 |
| Tyrosine | 0.003 | 0.001 | 10.96 | 1.67E-02 |
| Phenylalanine | 0.005 | 0.001 | 23.10 | 1.19E-04 |
| Glutamine | 0.047 | 0.008 | 31.80 | 3.99E-06 |
| Conjugated linoleic fatty acids | 0.053 | 0.015 | 12.12 | 1.16E-02 |
| Phospholipids to total lipids ratio in small VLDL | 0.284 | 0.092 | 9.63 | 2.79E-02 |
| Triglycerides to total lipids ratio in medium LDL | 0.538 | 0.139 | 15.07 | 3.46E-03 |
| Triglycerides to total lipids ratio in large LDL | 0.600 | 0.149 | 16.21 | 2.20E-03 |
| Triglycerides to total lipids ratio in IDL | 0.777 | 0.181 | 18.34 | 1.08E-03 |
| Phospholipids to total lipids ratio in very large HDL | 1.710 | 0.495 | 11.92 | 1.17E-02 |

Results are derived from four SD from mean quality controlled data. Metabolites of resistance training group only -analysis (POST-16wk -PRE) depicted with FDR < 0.05. Final model used for Generalized Estimation Equations analysis: Metabolite ~ Time + Age + BMI.

decreased levels of android (~9.6%), and total fat mass (~7.5%) were evident ($P < 0.05$) in the RT group only (15–17). These favorable changes in body composition after the RT regimen were accompanied by significant (FDR < 0.05) cardiometabolically positive alteration of serum metabolome profile throughout the study period (Table 2; Fig. 1). As expected, a time-dependent modulation of metabolites was observed where responses after 4 wk (POST-4wk) and 16 wk (POST-16wk) of intervention differed slightly in various biomarkers (see Supplemental Tables 1 and 3, Supplemental Digital Content 2, Full list of quantified metabolites with NMR platform and Results of resistance training effects on biomarker profile during the study period, <http://links.lww.com/MSS/B588>). Average daily dietary intakes of the resistance-trained individuals are depicted in Supplemental Digital Content (see Supplemental Table 4, Supplemental Digital Content 2, Dietary intake of the RT group, <http://links.lww.com/MSS/B588>).

Chronic resistance training induces antiatherogenic effects of metabolome profile. Analysis revealed that the RT group had significant (FDR < 0.05) differences in the levels of 21 metabolites across time points (PRE- to POST-16wk) (Table 2). Overall, RT induced mainly antiatherogenic protective lipid changes in metabolome profile (Table 2; Fig. 1). After the RT intervention, decreased (FDR < 0.05) levels of non-high-density lipoprotein (HDL) cholesterol (e.g., low-density lipoprotein (LDL) cholesterol, intermediate-density lipoprotein (IDL) cholesterol, remnant cholesterol, free cholesterol) and subsequent apolipoproteins (e.g., apolipoprotein B (apoB), apolipoprotein B/apolipoprotein A1 [apoA1] ratio) were observed and these were accompanied by increased (FDR < 0.05) levels of non-HDL particle triglyceride content and conjugated linoleic fatty acids (Fig. 1; Table 2). In addition, elevations of aromatic amino acids, phenylalanine and tyrosine, and glutamine were detected (FDR < 0.05) in response to the short-term resistance training—modulation

previously associated with metabolic disorders (e.g., type 2 diabetes) (Table 2).

As expected, biomarker levels partially depicted different trends in metabolomics profile when examining immediate-term (PRE- to POST-4wk) and short-term (PRE- to POST-16wk) effects in the RT group, suggesting time-dependent effects of resistance training on the serum metabolome profile (see Supplemental Table 3, Supplemental Digital Content 2, Results of resistance training effects on biomarker profile during the study period, <http://links.lww.com/MSS/B588>). Of the detected 21 significantly (FDR < 0.05) altered metabolites (PRE- to POST-16wk), five metabolites were already modulated after 4 wk of RT intervention (PRE- to POST-4wk). Similar trends throughout the study were observed in non-HDL cholesterol, amino acids, non-HDL particle triglyceride content (see Supplemental Table 3, Supplemental Digital Content 2, Results of resistance training effects on biomarker profile during the study period, <http://links.lww.com/MSS/B588>).

Some of the observed significant (FDR < 0.05) time-dependent changes in the serum biomarker profile in the analysis of RT group was also reflected in the group-wise comparison. Particularly, we observed (i) lower levels of free cholesterol and (ii) increased levels of non-HDL particle triglycerides, conjugated linoleic acid, and glutamine in the RT group, when compared with the non-RT group across time points (FDR < 0.05, PRE- to POST-16wk) (see Supplemental Tables 3 and 5, Supplemental Digital Content 2, Results of resistance training effects on biomarker profile during the study period and comparison between the RT and non-RT group during the study periods, <http://links.lww.com/MSS/B588>).

Changes in body composition reflect alteration in metabolome profile. High- (the greatest quartile, change 5.9% [1.9%], $n = 15$) and low responders (the lowest quartile, change 0.3% [0.7%], $n = 14$) were detected based on the change in lean mass index (LMI_{BSA}) during the RT

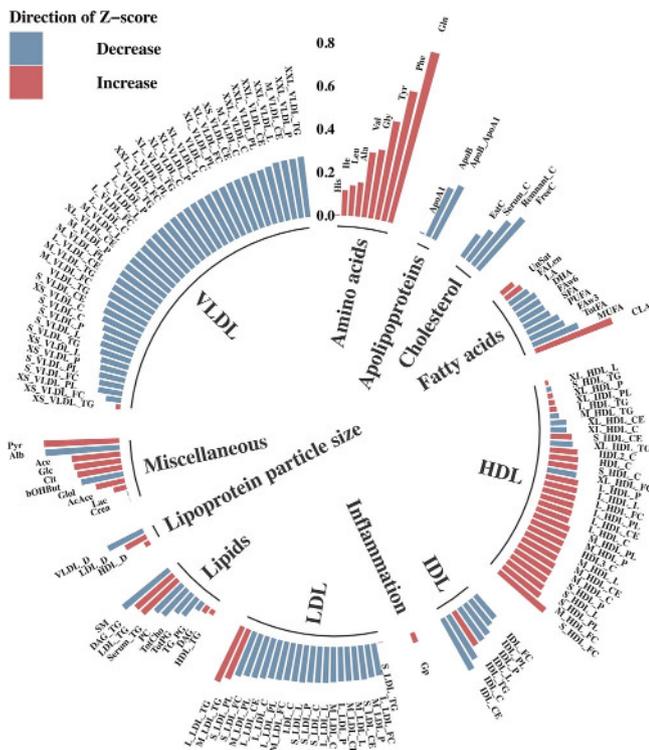


FIGURE 1—Overall changes in the metabolome profile after the resistance training intervention. For plotting, 155 health-related biomarkers were selected to demonstrate the overall effect of the 16-wk resistance training intervention ($n = 59$) on metabolome profile. The depicted polar plot is derived from metabolite raw values where outliers based on four SD from the mean have been excluded. Plotted metabolite values are represented as SD change from set reference Z-score. Baseline metabolite values were set as a reference. Red color indicates increase and blue represents a decrease compared to the reference Z score. Height of the bars depicts Z-score level and the scale is plotted on the figure vertically. Metabolites are ordered according to subclass and Z score values. Even short-term resistance training intervention was reflected in a cardiometabolically advantageous manner on overall biomarker profile (e.g., HDL \uparrow , LDL \downarrow , VLDL \downarrow , IDL \downarrow , triglycerides \downarrow , overall serum cholesterol \downarrow); although, the statistical analysis revealed significant difference in only some of the measured metabolites (Table 2).

intervention (PRE- to POST-16wk) (Fig. 2). Body composition of high responders was further altered in a favorable manner ($P < 0.05$) as increments in gained lean mass were accompanied by significant reductions ($P < 0.05$) in android fat mass ($\sim 20.6\%$) and overall adiposity ($\sim 17.7\%$) (see Supplemental Table 6, Supplemental Digital Content 2, Descriptives of responders to lean mass gains, <http://links.lww.com/MSS/B588>). Only minor differences were observed in adiposity among low responders in consequence to the RT intervention (see Supplemental Table 6, Supplemental Digital Content 2, Descriptives of responders to lean mass gains, <http://links.lww.com/MSS/B588>).

Discrepancies in body composition between high and low responders were distinctively ($FDR < 0.05$) reflected in the overall metabolome profiles as greater effects on HDL, LDL, and IDL subclasses was observed in high responders when compared with low responders (Fig. 3). Particularly, these differences in response were most evident in HDL particle levels as they increased substantially in the high-responder group

as opposed to low responders during the intervention period (PRE- to POST-16wk) (Fig. 3). In the end, increments in lean mass (LMI_{BSA}) explained most strongly these differences in HDL profile (Fig. 4; see Supplemental Table 7, Supplemental Digital Content 2, Lean mass responder status effect on metabolome profile modulation, <http://links.lww.com/MSS/B588>). Overall, we detected five different large HDL metabolites ($FDR < 0.05$) that were significantly modulated across time points between high and low responders (Fig. 4; see Supplemental Table 7, Supplemental Digital Content 2, Lean mass responder status effect on metabolome profile modulation, <http://links.lww.com/MSS/B588>). Interestingly; however, low responders seemed to have higher levels of HDL metabolites ($FDR > 0.05$) at baseline that might have contributed to some extent to the observed difference across time points between responder groups (Fig. 4). Discrepancies in overall adiposity did not further explain observed differences in HDL particle levels (see Supplemental Table 8, Supplemental Digital Content 2, Fat mass responder status effect on metabolome profile modulation, <http://links.lww.com/MSS/B588>).

Overall, in accordance with the exploratory RT group analysis, only positive changes were detected that focused on cardioprotective biomarkers ($FDR < 0.05$) when assessing the effects of RT and following lean mass gain in high responders throughout the study (PRE- to POST-16wk) (Fig. 1; see Supplemental Table 7, Supplemental Digital Content 2, Lean mass responder status effect on metabolome profile modulation, <http://links.lww.com/MSS/B588>). Furthermore, nominal findings ($FDR < 0.25$) also depicted similar positive trends in several cardioprotective metabolites, where the increase in other HDL-cholesterol-related particles and the decrease in apoB/apoA1 ratio was evident, thus, reinforcing our perception of the existing lean mass gain associated cardioprotective changes on serum metabolome (Fig. 3; see Supplemental Table 7, Supplemental Digital Content 2, Lean

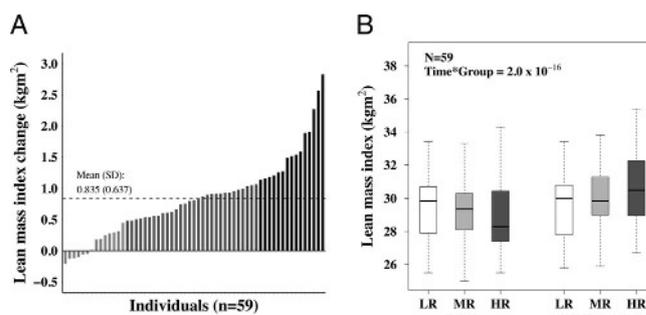


FIGURE 2—Responder status effect on lean mass change in the resistance training group. The figure represents resistance training effect on lean mass change in different responder groups. Panel A depicts individual data for lean mass index ($\text{kg}\cdot\text{m}^{-2}$) change during the resistance training intervention ($n = 59$) (PRE- to POST-16wk). Different color bars represent different responder groups where individuals were divided into three groups, i) black, high responder ($n = 15$); ii) dark gray, medium responder ($n = 30$); and iii) light gray, low responder ($n = 14$), based on lean mass index change as described in the methods. Panel B shows differences in different groups of responders (LR, low responders; MR, medium responders, HR, high responders) at baseline (PRE) and at the end of the study period (POST-16wk).

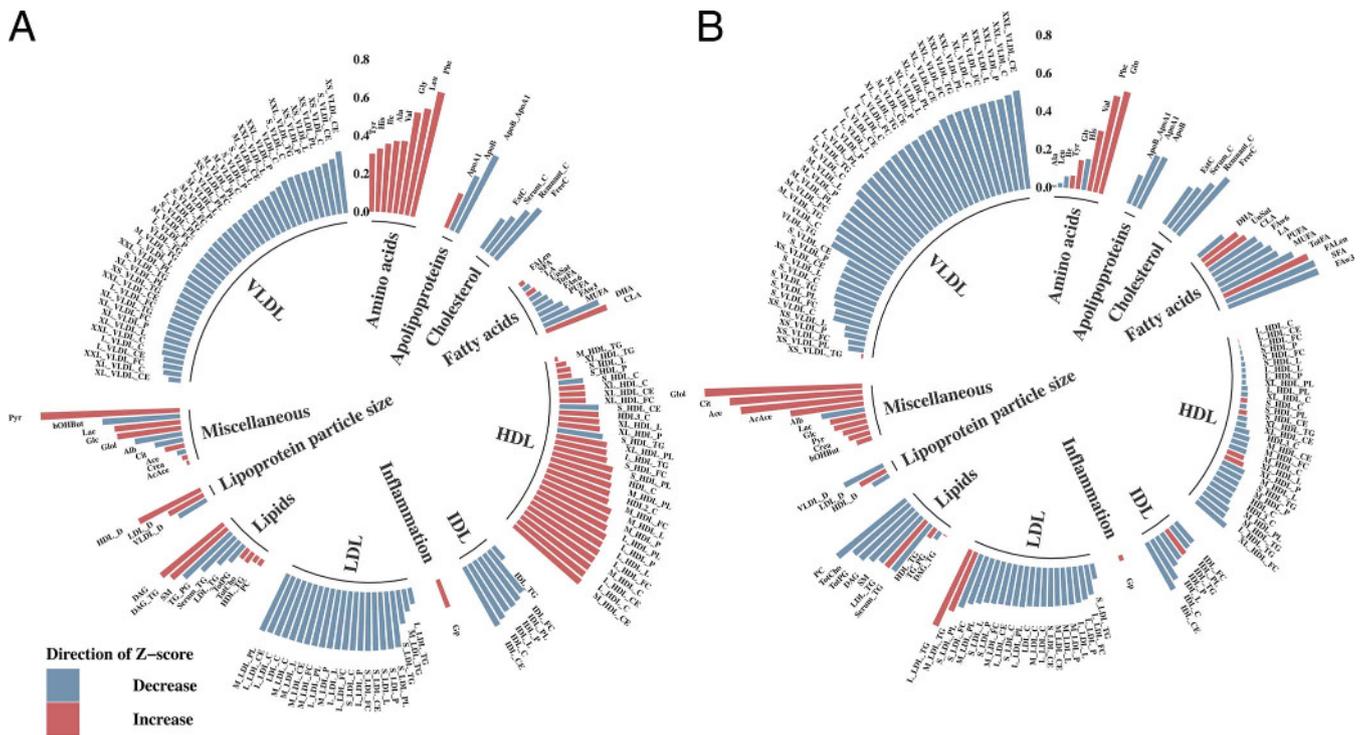


FIGURE 3—Overall differences in the metabolome profile after the resistance training intervention between LR and HR. For plotting, 155 health-related biomarkers were selected to demonstrate the overall differences in metabolome profile between LR and HR in response to 16-wk resistance training intervention. Panel A depicts the changes in HR ($n = 15$) whereas panel B demonstrates the changes in metabolome profile in LR following the intervention ($n = 14$) (PRE- to POST-16wk). Depicted polar plots are derived from metabolite raw values where outliers based on four SD from mean have been excluded. Plotted metabolite values are represented as SD change from set reference Z score. Baseline metabolite values were set as a reference. Red color indicates increase and blue decrease compared to reference Z score. Height of the bars depicts Z-score level and the scale is plotted on the figure vertically. Metabolites are ordered according to subclass and Z-score values. Responder status was notably reflected on the overall metabolome profile. Resistance training intervention had greater impact on HDL, IDL, and LDL subclasses among HR. Particularly, LR and HR showed differences in HDL metabolites as HR depicted increased levels of HDL metabolites as opposed to LR in which HDL metabolites had a tendency to decrease.

mass responder status effect on metabolome profile modulation, <http://links.lww.com/MSS/B588>).

DISCUSSION

This is the first longitudinal study to show that a short-term resistance training regimen has a significant effect on serum biomarker profile in the level of NMR metabolome. The resistance-training regimen resulted in improved body composition (e.g., fat mass ↓, visceral fat mass ↓, lean mass ↑) and subsequent positive alterations in serum lipids and lipoproteins (e.g., apoB ↓, apoB/apoA1 –ratio ↓, free-, remnant-, IDL and LDL-cholesterol ↓, conjugated linoleic acid ↑), thus suggesting a cardiometabolically favorable alteration of serum metabolome. These changes in lipid profile were also accompanied by a prominent increase in the levels of amino acids (e.g., phenylalanine, tyrosine, glutamine). Furthermore, we detected a uniform positive effect on HDL-cholesterol-related metabolites in a subsample of lean mass high responders. Ultimately, our study supports previous more general biomarker quantification studies on resistance training, but advances current knowledge and gives more detailed insight with high-throughput quantification, especially regarding lipoprotein subclasses, serum free fatty acids, and amino acids profile. Detailed understanding of biomarkers is essentially

warranted, as arising evidence suggests that similar metabolites with small differences in characteristics (e.g., size, composition, electric charge) can have substantial effects on their biological activity and functions.

It has been thoroughly covered that negative modulation of cholesterol and lipoprotein metabolism promotes atherogenic actions, and increases the risk of future cardiovascular disease (20,21). Particularly, increased levels of non-HDL cholesterol have associated strongly with negative atherogenic effects on cardiovascular health (21). Consequently, the short-term resistance training intervention induced a reduction in the majority of metabolites related to non-HDL cholesterol (Fig. 1; Table 2). Furthermore, lower levels of apoB and apoB/apoA1 ratio were detected that have also associated with attenuated risk of cardiovascular/heart outcomes (22,23). These antiatherogenic effects were further promoted by increased levels of conjugated linoleic acids that has been previously associated with lower risk of heart failure (24). Altogether, our significant findings on lipid metabolites were further supported by the nominal changes that suggested widespread beneficial modulation of lipoprotein and cholesterol levels (Fig. 1).

In the past, in accordance with our findings, resistance training has been shown to elicit beneficial changes in serum cholesterol levels in response to short- and long-term interventions (25–27). Previous resistance training interventions

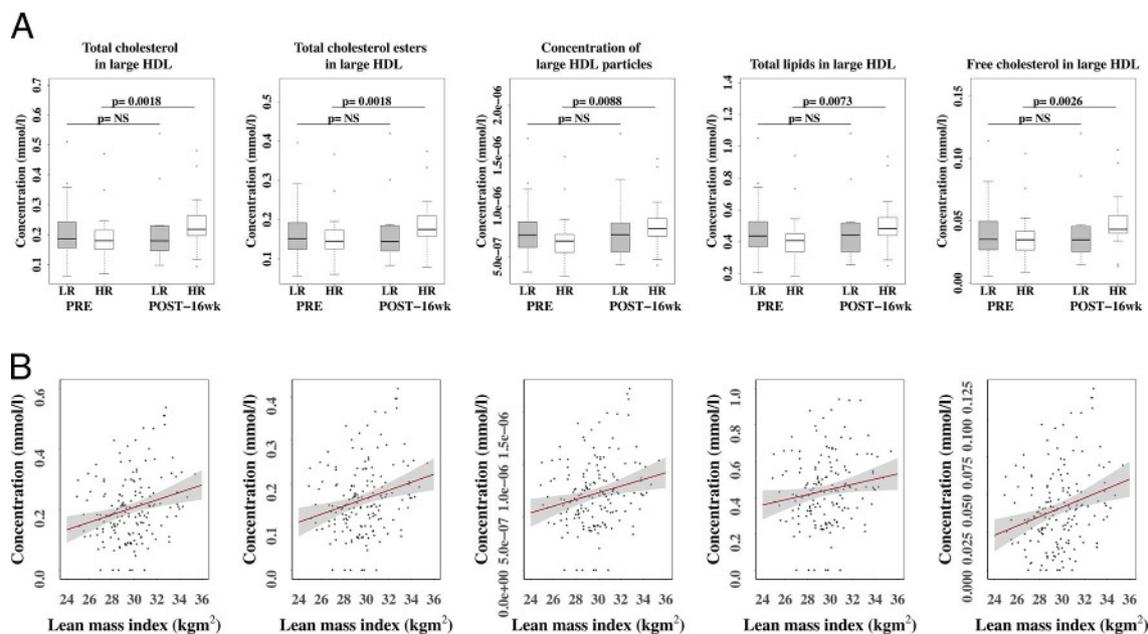


FIGURE 4—Most significant metabolite changes between HR and LR relative to lean mass change during intervention. Figure represents most significant metabolites changes (PRE- to POST-16wk) in the resistance training group when divided into high- ($n = 15$) and LR ($n = 14$) based on highest and lowest quartile of lean mass index change. Panel A *boxplots* show overall trends where HR depict greater increase in HDL metabolite concentration compared with LR. High-responders also depict lower levels of HDL metabolites at the baseline. NS, not significant. Panel B plots indicate trends where individuals with higher lean mass index is accompanied by higher levels of depicted HDL metabolite concentration. Significant ($r \approx -0.2, P < 0.05$) correlation was detected for all five large HDL metabolites. Panel B is plotted from all available study points from resistance training ($n = 59$) and nonresistance training groups ($n = 18$). *Boxplots* and *line plots* are derived from four SD quality-controlled data.

have depicted reduced levels of total cholesterol and LDL as well as increased levels of HDL in humans (28). Overall, regular moderate intensity exercise has been shown to modulate lipid profile by increasing HDL-cholesterol levels and preventing the increase of LDL-cholesterol and triglyceride levels (28). Interestingly, some of the favorable changes on body composition and lipid profile were already observed in the present study after only 4 wk of resistance training, whereas the remaining 12 wk of resistance training further enforced positive alterations in these parameters (see Supplemental Table 3, Supplemental Digital Content 2, Results of resistance training effects on biomarker profile during the study period, <http://links.lww.com/MSS/B588>). These findings together with previous studies suggest that resistance training modulates serum metabolome in a time-dependent manner, where even short-term engagement (~4–12 wk) in resistance training is sufficient in accomplishing favorable modulation of serum metabolome, especially lipid profile, and thus promoting antiatherogenic effects and cardiometabolic health. Our findings on antiatherogenic effects of resistance training were corroborated by a recent study showing resistance training association with reduced cardiovascular morbidity and mortality independent of aerobic training (29).

Furthermore, it has been well documented regarding resistance training that increased volume rather than increased intensity has greater impact on lipid profile (28). Consequently, increased volume of exercise is usually accompanied by increases in caloric expenditure and fat mass loss, which have also shown to positively impact serum lipid profile (30,31). Despite arising evidence, it is still debatable whether these

cardiometabolically beneficial changes in cholesterol and lipid levels are mediated more strongly through exercise itself or exercise-induced weight loss and subsequent improvements in body composition (32). After the 16-wk study period, our findings of beneficially modulated cholesterol levels (HDL, LDL, IDL, VLDL) and apolipoproteins were accompanied by an increased level of muscle mass, and decreased levels of android and overall fat mass (Table 1; Fig. 1). These all suggest that resistance-training transmitted cardiometabolic positive effects could emerge mainly from enhanced body composition (e.g., lean mass \uparrow , subcutaneous and visceral fat mass \downarrow). Consequently, this hypothesis was supported by significant positive association between lean mass gains and HDL-related metabolite levels (Fig. 4; see Supplemental Table 7, Supplemental Digital Content 2, Lean mass responder status effect on metabolome profile modulation, <http://links.lww.com/MSS/B588>). However, the increments in absolute levels of lean mass in the resistance training group were notably greater compared with the amount of total and visceral fat mass lost (Table 1). This could also contribute to the observed association between lean mass and HDL-cholesterol, but not with adiposity parameters and cholesterol levels (Supplemental Tables 7–8, Supplemental Digital Content 2, <http://links.lww.com/MSS/B588>). Overall, it seems that the effect of resistance training on serum metabolome is most likely mediated through enhanced body composition; although, more studies are warranted to determine whether fat mass and lean mass alteration affect serum metabolome profile subclasses in similar fashion.

Previously, higher levels of aromatic amino acids have associated with molecular pathways linked to increased risk of

metabolic disorders (e.g., type 2 diabetes, insulin resistance, cardiovascular disease) in large population-based cohorts (33,34). Interestingly, suggested adverse elevations of serum aromatic amino acids, phenylalanine and tyrosine, were observed in consequence to the short-term resistance training intervention (see Supplemental Table 3, Supplemental Digital Content 2, Results of resistance training effects on biomarker profile during the study period, <http://links.lww.com/MSS/B588>). However, long-term exercise has also been previously shown to induce similar elevations in circulating levels of aromatic amino acids, thus alleviating doubts of adverse health effects (35,36). As with aromatic amino acids, increased levels of branched-chain amino acids (e.g., isoleucine, leucine, alanine) have been strongly associated with insulin resistance and risk of diabetes, but no significant alterations in these parameters were detected after the present resistance training intervention (see Supplemental Table 3, Supplemental Digital Content 2, Results of resistance training effects on biomarker profile during the study period, <http://links.lww.com/MSS/B588>). Furthermore, evidence is growing on the efficacy of resistance training in glycaemia control and treatment of type 2 diabetics, thus promoting our view of unharmed alteration in circulating levels of amino acids (37,38). Our observation of increased amino acid concentrations was probably mediated through altered amino acid and protein metabolism during resistance training. Altogether, it seems that the use of circulating levels of amino acids as disease predictors cannot be generalized to all populations, as they are highly dependent on age, energy availability and exercise/activity level.

Previous studies have shown that most positively affected individuals by exercise regimens are the ones with previously low HDL-cholesterol levels, increased abdominal adiposity and elevated serum triglyceride levels (13,39). Subsequently, our findings on the beneficial modulation of HDL-cholesterol-related biomarkers and serum triglycerides in the high-responder group is probably mostly explained by the aforementioned suggestions (Fig. 4; see Supplemental Table 6, Supplemental Digital Content 2, Descriptives of responders to lean mass gains, <http://links.lww.com/MSS/B588>). High responders in our study had lower levels of lean mass and HDL-cholesterol levels, and higher adiposity compared with the low responders thus supporting the fact that improvements in HDL-cholesterol levels and body composition are most evident in people with the most unfavorable baseline levels (Fig. 2; see Supplemental Table 6, Supplemental Digital Content 2, Descriptives of responders to lean mass gains, <http://links.lww.com/MSS/B588>). Low responders had greater variance observed in the metabolic profile, which could have also contributed to the differences found when compared to the high-responder group. Overall, our findings enforce the perception that suitable exercise regimen interventions should be targeted to people with the

poorest health parameters concerning both body composition and metabolic profile.

The current study examining effects of resistance training on metabolome had considerable strengths. The study population for the controlled resistance training intervention was rather large. It also included shorter and longer training periods in addition to including a non-RT group as a comparison. The latter can be also considered as a limitation since the non-RT group was heterogenic when compared with the RT group. The non-RT group was formed using data from two previously collected cohorts where the follow-up intervals were different from the RT group, thus affecting on the overall comparability of the RT and non-RT group. Despite groups being similar in terms of age and sex, we detected significant differences and heterogeneity in the baseline metabolome levels between the RT and non-RT groups, which could explain our miscellaneous findings in the between-group testing. We also recognize the lack of dietary standardization as a limitation of our study. Dietary intake is known to affect, for instance, serum lipid and amino acid levels. Study participants were instructed to maintain their habitual dietary intake throughout the study period. Moreover, although body weight remained stable, it is not possible to entirely exclude the effect of diet from those of exercise. Finally, targeted H-NMR metabolomics platform has low coefficients of variation (<5%) for more than 75% of the metabolic measures (40). However, it has lower sensitivity and coverage when compared to metabolomics approaches utilizing mass spectroscopy. Also, it should be noted that the number of the measured lipoprotein species are difficult to interpret as they are comprised of heterogeneous particle distributions and clinically they are measured by different methods, thus making comparisons difficult.

Ultimately, we conclude that a short-term (4 to 16 wk) period of resistance training leading to increased levels of lean mass and reduced overall adiposity also leads to antiatherogenic modulation of serum metabolome in healthy young men. Our study also suggested that the change in lean mass could be used as a predictor of metabolome profile, especially regarding HDL subpopulations. Furthermore, individuals with the poorest baseline body composition and metabolome profile benefit the most from initiating resistance training in terms of positive cardiometabolic health effects.

The study was funded by The Academy of Finland grant 269517 (M. P.), NovoNordisk Foundation grant NNF16OC0020866 (M. P.), Tekes-National Technology Agency of Finland with University of Jyväskylä (Decision 70007/13) (H. P.), Finnish Ministry of Education and Culture (2006) (M. K.-S.), Juho Vainion Foundation (H. V. S.), and Orion Pharma Foundation (H. V. S.). The authors of this article declare no competing interests and that the results of the study are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation. The authors also state that results of the present study do not constitute endorsement by ACSM.

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