Author(s): Sarin, Heikki; Ahtiainen, Juha; Hulmi, Juha; Ihalainen, Johanna; Walker, Simon; Schildt, Maria; Perola, Markus; Peltonen, Heikki

Title: Resistance Training Induces Antiatherogenic Effects on Metabolomic Pathways

Year: 2019

Version: Accepted version (Final draft)

Please cite the original version:

Sarin, H., Ahtiainen, J., Hulmi, J., Ihalainen, J., Walker, S., Schildt, M., Perola, M., & Peltonen, H. (2019). Resistance Training Induces Antiatherogenic Effects on Metabolomic Pathways. Medicine and Science in Sports and Exercise, 51(9), 1866-1875. https://doi.org/10.1249/mss.0000000000002003

Medicine & Science IN Sports & Exercise
The Official Journal of the American College of Sports Medicine
www.acsm-msse.org

. . . Published ahead of Print

Resistance Training Induces Antiatherogenic Effects on Metabolomic Pathways

Heikki V. Sarin[1,2], Juha P. Ahtiainen[3], Juha J. Hulmi[3,4], Johanna K. Ihalainen[3,5], Simon Walker[3], Maria Küüsmaa-Schildt[3], Markus Perola[1,2], and Heikki Peltonen[3]

[1]Genomics and Biomarkers Unit, Department of Health, National Institute for Health and Welfare, Helsinki, Finland; [2]Research Program for Clinical and Molecular Metabolism, Faculty of Medicine, University of Helsinki; Finland; [3]NeuroMuscular Research Center, Faculty of Sport and Health Sciences, University of Jyväskylä, Jyväskylä, Finland; [4]Department of Physiology, Faculty of Medicine, University of Helsinki, Finland; [5]Swedish Winter Sports Research Centre, Department of Health Sciences, Mid Sweden University, Östersund, Sweden

Accepted for Publication: 26 March 2019

Medicine & Science in Sports & Exercise, Publish Ahead of Print
DOI: 10.1249/MSS.0000000000002003

Resistance Training Induces Antiatherogenic Effects on Metabolomic Pathways

Heikki V. Sarin[1,2*], Juha P. Ahtiainen[3*], Juha J. Hulmi[3,4], Johanna K. Ihalainen[3,5], Simon Walker[3], Maria Küüsmaa-Schildt[3], Markus Perola[1,2], and Heikki Peltonen[3]

[1]Genomics and Biomarkers Unit, Department of Health, National Institute for Health and Welfare, Helsinki, Finland; [2]Research Program for Clinical and Molecular Metabolism, Faculty of Medicine, University of Helsinki; Finland; [3]NeuroMuscular Research Center, Faculty of Sport and Health Sciences, University of Jyväskylä, Jyväskylä, Finland; [4]Department of Physiology, Faculty of Medicine, University of Helsinki, Finland; [5]Swedish Winter Sports Research Centre, Department of Health Sciences, Mid Sweden University, Östersund, Sweden

*****These authors contributed equally to this work

Corresponding author: Heikki V. Sarin, M.Sc., B.Med.
Genomics and Biomarkers Unit, The Department of Public health solutions
National Institute for Health and Welfare (THL)
P.O. Box 30 (Mannerheimintie 166)

FIN - 00271 Helsinki

Finland

Email: heikki.sarin@thl.fi

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

ABSTRACT

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 years, body mass index (BMI) 28 ± 3 kg/m^2) and ii) a non-RT group (n=18, age 31 ± 4 years, BMI 27 ± 3 kg/m^2). Blood samples were collected at baseline (PRE), after 4 weeks (POST-4wk), and after 16 weeks of resistance training intervention (POST-16wk), as well as baseline and after the non-RT period (20–24 weeks). Nuclear magnetic resonance (NMR) -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 anti-atherogenic alterations in serum metabolome profile (FDR < 0.05) as reductions in non-HDL cholesterol (e.g., free cholesterol, remnant cholesterol, IDL cholesterols, LDL 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.

Keywords: resistance exercise; omics; Biomarkers; lipids; amino acids; cardiovascular disease

INTRODUCTION

Regular physical activity together with reduced adiposity is associated with beneficial modulation of biomarker profile, cardiometabolic health, and reduced risk of non-communicable 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, has 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 non-communicable 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 / non-responders) (14). However, it has been questioned whether this concept actually holds true or is it masked by the individual variation (e.g., age, training adaption, genetics) on how sensitive individuals are to different exercise regimens. Thus, it can be debated if it is more appropriate to call individuals high- 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 years, body mass index (BMI) 28 ± 3 kg/m^2) belonged to the resistance training (RT) group, and 18 non-training peers (age 31 ± 4 years, 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 Figure 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 gender 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 weeks. Measurements were performed at baseline (PRE), after 4 weeks (POST-4wk), and after 16 weeks (POST-16wk) of resistance training. The non-RT group was measured at baseline (PRE) and after (POST-control) 20-weeks (n = 8, (17)) or 24-weeks (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 Figure 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-week RT period with 4-day 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 were asked to maintain their pre-experimental 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 weeks of whole-body workouts twice-a-week. The participants performed 8–10 exercises within one workout, 2–3 sets for every exercise, and 10–15 repetitions in every set. Recovery time of two minutes was held constant between sets. Training loads were 50–80 % of one repetition maximum (1RM) increasing throughout this preparatory phase. Nine participants dropped out of the study after 4 weeks of the preparatory RT phase. The remaining participants (n = 59) were divided after the first 4-week 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 weeks. The specific resistance training programs consisted of 2–3 training sessions per week and were divided into three 4-week 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 non-linearly 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) = square root of (height (cm) x weight (kg)/3600) (19).

Muscle strength

Maximal strength was measured at baseline (PRE), after the 4-week preparatory RT period (POST-4wk) and after the 12-week RT period (POST-12wk) in the RT group or after the control period in the non-RT group (POST-control) (14–17). 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-day food diaries during the second block of the 12-week 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 minutes (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 pre-analytical 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., 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 standard deviation (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 (GLM) 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 (GEE), allows for deviations from normality. GEE 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 ≤ 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 (~2.8 %), 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 (false discovery rate, FDR < 0.05) cardiometabolically positive alteration of serum metabolome profile throughout the study period (Table 2; Figure 1). As expected, a time-dependent modulation of metabolites was observed where responses after 4 weeks (POST-4wk) and 16 weeks (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 anti-atherogenic 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 anti-atherogenic protective lipid changes in metabolome profile (Table 2; Figure 1). After the RT intervention, decreased (FDR < 0.05) levels of non-high-density lipoprotein (HDL) cholesterols (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 (Figure 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 (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 weeks of RT intervention (PRE- to POST-4wk). Similar trends throughout the study were observed in non-HDL cholesterols, 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 to 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 intervention (PRE- to POST-16wk) (Figure 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 (~20.6 %) and overall adiposity (~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 to low-responders (Figure 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) (Figure 3). In the end, increments in lean mass (LMI_{BSA}) explained most strongly these differences in HDL profile (Figure 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 (Figure 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 (Figure 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) (Figure 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 (Figure 3; see Supplemental Table 7, Supplemental Digital Content 2, Lean 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 (Figure 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 anti-atherogenic 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 (Figure 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 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 weeks of resistance training, while the remaining 12 weeks 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 weeks) in resistance training is sufficient in accomplishing favorable modulation of serum metabolome, especially lipid profile, and thus promoting anti-atherogenic effects and cardiometabolic health. Our findings on anti-atherogenic 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-week 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; Figure 1). This all suggests that resistance-training transmitted cardiometabolic positive effects could emerge mainly from enhanced body composition (e.g., lean mass ↑, subcutaneous and visceral fat mass ↓). Consequently, this hypothesis was supported by significant positive association between lean mass gains and HDL-related metabolite levels (Figure 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 unharmful 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 (Figure 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 (Figure 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 gender, 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 (MS). 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-week) period of resistance training leading to increased levels of lean mass and reduced overall adiposity also leads to anti-atherogenic 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.

ACKNOWLEDGEMENTS

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

REFERENCES:

1. Zierath JR, Wallberg-Henriksson H. Looking Ahead Perspective: Where Will the Future of Exercise Biology Take Us? *Cell Metab*. 2015;22(1):25–30.

2. Soininen P, Kangas AJ, Würtz P, et al. High-throughput serum NMR metabonomics for cost-effective holistic studies on systemic metabolism. *The Analyst*. 2009;134(9):1781–5.

3. Würtz P, Kangas AJ, Soininen P, Lawlor DA, Davey Smith G, Ala-Korpela M. Quantitative Serum Nuclear Magnetic Resonance Metabolomics in Large-Scale Epidemiology: A Primer on -Omic Technologies. *Am J Epidemiol*. 2017;186(9):1084–96.

4. Heaney LM, Deighton K, Suzuki T. Non-targeted metabolomics in sport and exercise science. *J Sports Sci*. 2017;1–9.

5. Lewis GD, Farrell L, Wood MJ, et al. Metabolic signatures of exercise in human plasma. *Sci Transl Med*. 2010;2(33):33ra37.

6. Strasser B, Schobersberger W. Evidence for Resistance Training as a Treatment Therapy in Obesity [Internet]. *J Obes*. 2011 [cited 2018 Sep 8];2011 available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2931407/. doi:10.1155/2011/482564.

7. Willis LH, Slentz CA, Bateman LA, et al. Effects of aerobic and/or resistance training on body mass and fat mass in overweight or obese adults. *J Appl Physiol*. 2012;113(12):1831–7.

8. W. Bryner R, H. Ullrich I, Sauers J, et al. Effects of Resistance vs. Aerobic Training Combined With an 800 Calorie Liquid Diet on Lean Body Mass and Resting Metabolic Rate. *J Am Coll Nutr*. 1999;18:115–21.

9. Hunter GR, Byrne NM, Sirikul B, et al. Resistance Training Conserves Fat-free Mass and Resting Energy Expenditure Following Weight Loss. *Obesity*. 2008;16(5):1045–51.

10. Lee DH, Keum N, Hu FB, et al. Predicted lean body mass, fat mass, and all cause and cause specific mortality in men: prospective US cohort study. *BMJ*. 2018;362:k2575.

11. Abramowitz MK, Hall CB, Amodu A, Sharma D, Androga L, Hawkins M. Muscle mass, BMI, and mortality among adults in the United States: A population-based cohort study. *PloS One*. 2018;13(4):e0194697.

12. Srikanthan P, Horwich TB, Tseng CH. Relation of Muscle Mass and Fat Mass to Cardiovascular Disease Mortality. *Am J Cardiol*. 2016;117(8):1355–60.

13. Ihalainen JK, Inglis A, Mäkinen T, et al. Strength Training Improves Metabolic Health Markers in Older Individual Regardless of Training Frequency [Internet]. *Front Physiol*. 2019 [cited 2019 Mar 13];10 available from: https://www.frontiersin.org/articles/10.3389/fphys.2019.00032/full. doi:10.3389/fphys.2019.00032.

14. Ahtiainen JP, Walker S, Peltonen H, et al. Heterogeneity in resistance training-induced muscle strength and mass responses in men and women of different ages [Internet]. *Age*. 2016 [cited 2018 Jun 21];38(1) available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5005877/. doi:10.1007/s11357-015-9870-1.

15. Hulmi JJ, Laakso M, Mero AA, Häkkinen K, Ahtiainen JP, Peltonen H. The effects of whey protein with or without carbohydrates on resistance training adaptations. *J Int Soc Sports Nutr*. 2015;12:48.

16. Ihalainen JK, Peltonen H, Paulsen G, et al. Inflammation status of healthy young men: initial and specific responses to resistance training. *Appl Physiol Nutr Metab Physiol Appl Nutr Metab*. 2018;43(3):252–8.

17. Walker S, Hulmi JJ, Wernbom M, et al. Variable resistance training promotes greater fatigue resistance but not hypertrophy versus constant resistance training. *Eur J Appl Physiol*. 2013;113(9):2233–44.

18. Küüsmaa M, Schumann M, Sedliak M, et al. Effects of morning versus evening combined strength and endurance training on physical performance, muscle hypertrophy, and serum hormone concentrations. *Appl Physiol Nutr Metab Physiol Appl Nutr Metab*. 2016;41(12):1285–94.

19. Mosteller RD. Simplified calculation of body-surface area. *N Engl J Med*. 1987;317(17):1098.

20. Ito K, Yoshida H, Yanai H, et al. Relevance of intermediate-density lipoprotein cholesterol to Framingham risk score of coronary heart disease in middle-aged men with increased non-HDL cholesterol. *Int J Cardiol*. 2013;168(4):3853–8.

21. Ridker PM, Rifai N, Cook NR, Bradwin G, Buring JE. Non–HDL Cholesterol, Apolipoproteins A-I and B100, Standard Lipid Measures, Lipid Ratios, and CRP as Risk Factors for Cardiovascular Disease in Women. *JAMA*. 2005;294(3):326–33.

22. Lu M, Lu Q, Zhang Y, Tian G. ApoB/apoA1 is an effective predictor of coronary heart disease risk in overweight and obesity. *J Biomed Res*. 2011;25(4):266–73.

23. Walldius G, Jungner I, Holme I, Aastveit AH, Kolar W, Steiner E. High apolipoprotein B, low apolipoprotein A-I, and improvement in the prediction of fatal myocardial infarction (AMORIS study): a prospective study. *Lancet Lond Engl*. 2001;358(9298):2026–33.

24. Wannamethee SG, Jefferis BJ, Lennon L, Papacosta O, Whincup PH, Hingorani AD. Serum Conjugated Linoleic Acid and Risk of Incident Heart Failure in Older Men: The British Regional Heart Study [Internet]. *J Am Heart Assoc Cardiovasc Cerebrovasc Dis*. 2018 [cited 2018 Sep 8];7(1) available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5778956/. doi:10.1161/JAHA.117.006653.

25. Lira FS, Yamashita AS, Uchida MC, et al. Low and moderate, rather than high intensity strength exercise induces benefit regarding plasma lipid profile. *Diabetol Metab Syndr*. 2010;2:31.

26. Sheikholeslami Vatani D, Ahmadi S, Ahmadi Dehrashid K, Gharibi F. Changes in cardiovascular risk factors and inflammatory markers of young, healthy, men after six weeks of moderate or high intensity resistance training. *J Sports Med Phys Fitness*. 2011;51(4):695–700.

27. Prabhakaran B, Dowling EA, Branch JD, Swain DP, Leutholtz BC. Effect of 14 weeks of resistance training on lipid profile and body fat percentage in premenopausal women. *Br J Sports Med*. 1999;33(3):190–5.

28. Mann S, Beedie C, Jimenez A. Differential Effects of Aerobic Exercise, Resistance Training and Combined Exercise Modalities on Cholesterol and the Lipid Profile: Review, Synthesis and Recommendations. *Sports Med Auckl Nz*. 2014;44(2):211–21.

29. Liu Y, Lee D-C, Li Y, et al. Associations of Resistance Exercise with Cardiovascular Disease Morbidity and Mortality. *Med Sci Sports Exerc*. 2019;51(3):499–508.

30. Katzmarzyk PT, Gagnon J, Leon AS, et al. Fitness, fatness, and estimated coronary heart disease risk: the HERITAGE Family Study. *Med Sci Sports Exerc*. 2001;33(4):585–90.

31. Nybo L, Sundstrup E, Jakobsen MD, et al. High-intensity training versus traditional exercise interventions for promoting health. *Med Sci Sports Exerc*. 2010;42(10):1951–8.

32. Kettunen J, Joensuu A, Hagnäs M, et al. Associations of increased physical performance and change in body composition with molecular pathways of heart disease and diabetes risk [Internet]. *Am J Physiol Endocrinol Metab*. 2018; doi:10.1152/ajpendo.00260.2018.

33. Stancáková A, Civelek M, Saleem NK, et al. Hyperglycemia and a common variant of GCKR are associated with the levels of eight amino acids in 9,369 Finnish men. *Diabetes*. 2012;61(7):1895–902.

34. Würtz P, Havulinna AS, Soininen P, et al. Metabolite profiling and cardiovascular event risk: a prospective study of 3 population-based cohorts. *Circulation*. 2015;131(9):774–85.

35. Kujala UM, Mäkinen V-P, Heinonen I, et al. Long-term leisure-time physical activity and serum metabolome. *Circulation*. 2013;127(3):340–8.

36. Bergström J, Fürst P, Hultman E. Free amino acids in muscle tissue and plasma during exercise in man. *Clin Physiol Oxf Engl*. 1985;5(2):155–60.

37. Umpierre D, Ribeiro PAB, Kramer CK, et al. Physical activity advice only or structured exercise training and association with HbA1c levels in type 2 diabetes: a systematic review and meta-analysis. *JAMA*. 2011;305(17):1790–9.

38. Strasser B, Siebert U, Schobersberger W. Resistance training in the treatment of the metabolic syndrome: a systematic review and meta-analysis of the effect of resistance training on metabolic clustering in patients with abnormal glucose metabolism. *Sports Med Auckl NZ*. 2010;40(5):397–415.

39. Couillard C, Després J-P, Lamarche B, et al. Effects of Endurance Exercise Training on Plasma HDL Cholesterol Levels Depend on Levels of Triglycerides: Evidence From Men of the Health, Risk Factors, Exercise Training and Genetics (HERITAGE) Family Study. *Arterioscler Thromb Vasc Biol*. 2001;21(7):1226–32.

40. Kettunen J, Demirkan A, Würtz P, et al. Genome-wide study for circulating metabolites identifies 62 loci and reveals novel systemic effects of LPA. *Nat Commun*. 2016;7:11122.

FIGURE LEGENDS

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-week resistance training intervention (n=59) on metabolome profile. The depicted polar plot is derived from metabolite raw-values where outliers based on 4 standard deviation (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., HDLs ↑, LDLs ↓, VLDL ↓, IDLs ↓, triglycerides ↓, overall serum cholesterol ↓); although, the statistical analysis revealed significant difference in only some of the measured metabolites (Table 2).

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 ($kg \cdot m^2$) 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 low- and high responders. For plotting, 155 health related biomarkers were selected to demonstrate the overall differences in metabolome profile between low- and high-responders in response to 16-week resistance training intervention. Panel A depicts the changes in high-responders (n=15) whereas Panel B demonstrates the changes in metabolome profile in low-responders following the intervention (n=14) (PRE – POST-16wk). Depicted polar plots are derived from metabolite raw-values where outliers based on 4 standard deviation (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 high-responders. Particularly, low- and high-responders showed differences in HDL metabolites as high-responders depicted increased levels of HDL metabolites as opposed to low-responders in which HDL metabolites had a tendency to decrease.

Figure 4. Most significant metabolite changes between high- and low-responders 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 low-responders (n=14) based on highest and lowest quartile of lean mass index change. Panel A

boxplots show overall trends where high-responders depict greater increase in HDL-metabolite concentration compared to low-responders. High-responders also depict lower levels of HDL-metabolites at the baseline. LR = Low-responder. HR = High-responder. PRE = Baseline. POST-16wk = After 16 weeks of intervention. 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 = ~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 non-resistance training groups (n=18). Boxplots and line plots are derived from 4 standard deviations (SD) quality-controlled data.

SUPPLEMENTAL DIGITAL CONTENT (SDC)

Supplementary data, SDC 1 (Supplementary Figure 1.).pdf

Supplementary data, SDC 2 (Supplementary Tables 1-8).xlsx

Figure 1

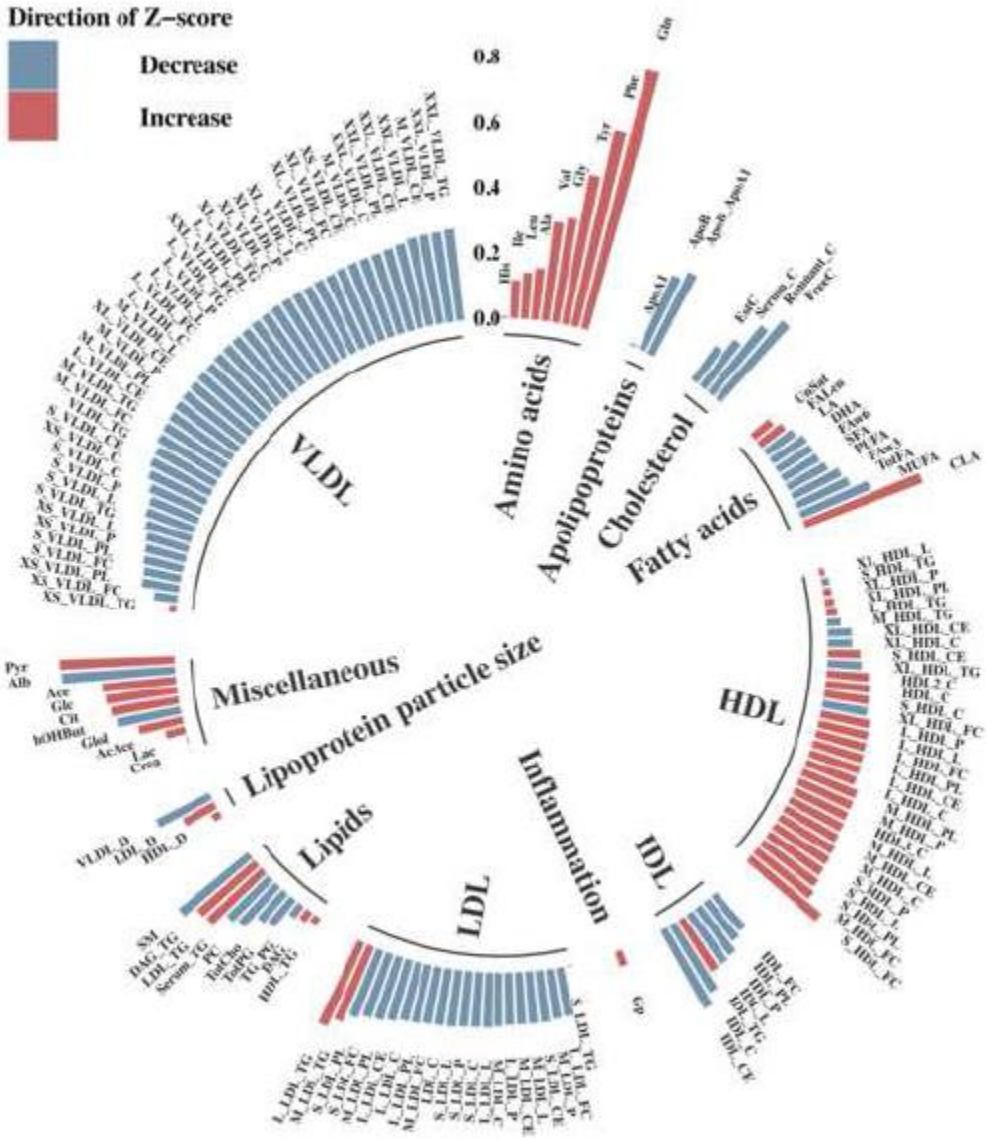

Figure 2

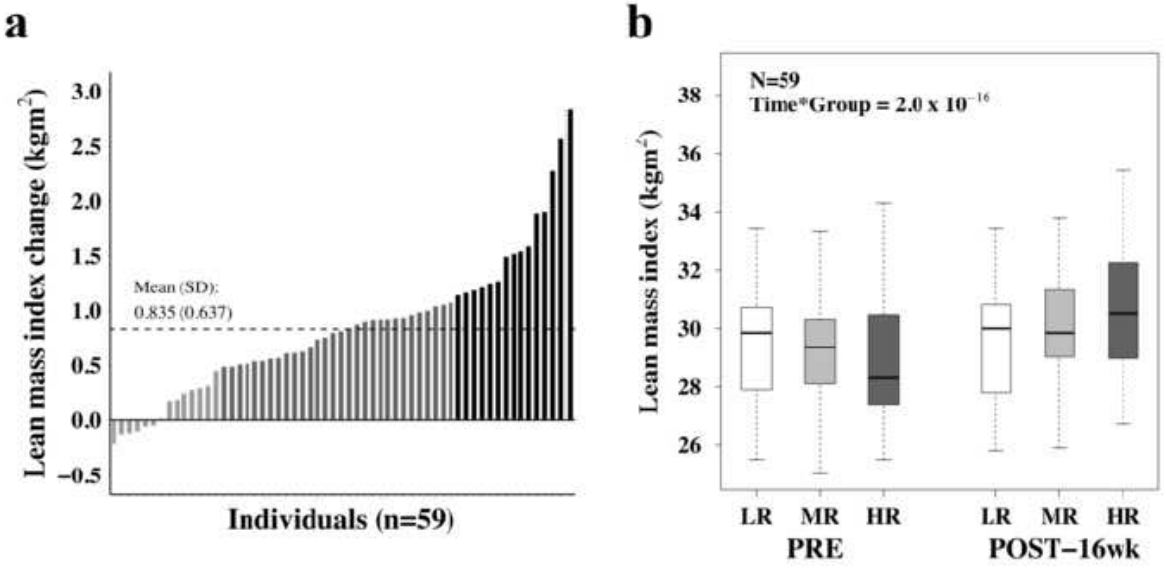

Figure 3

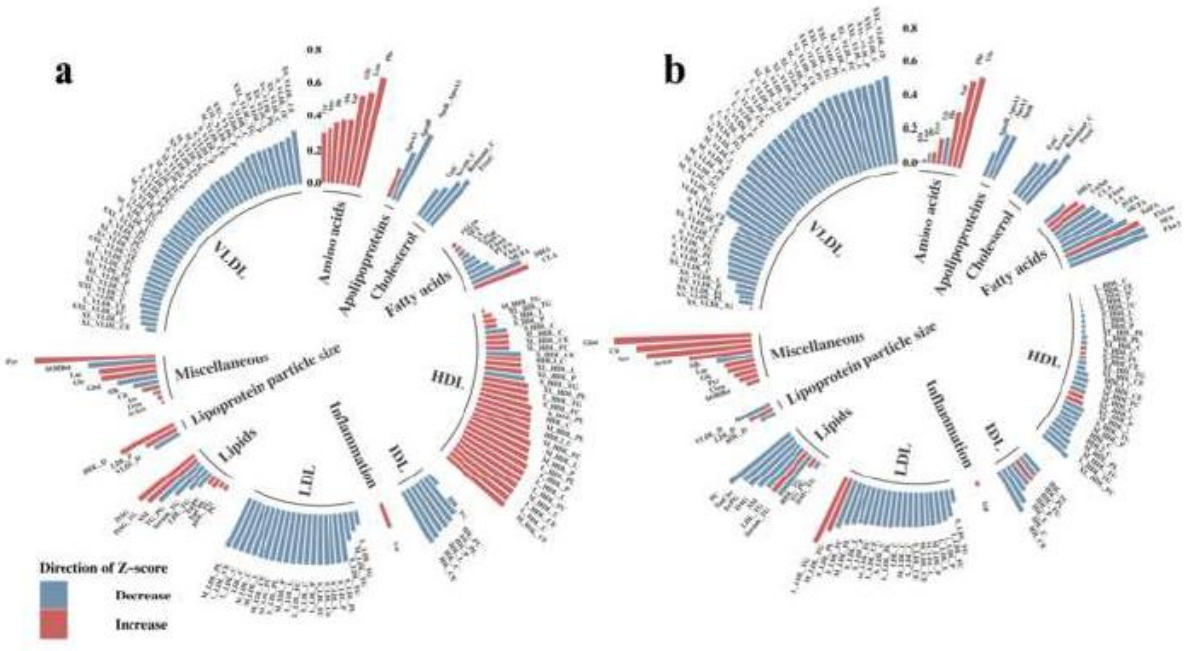

Figure 4

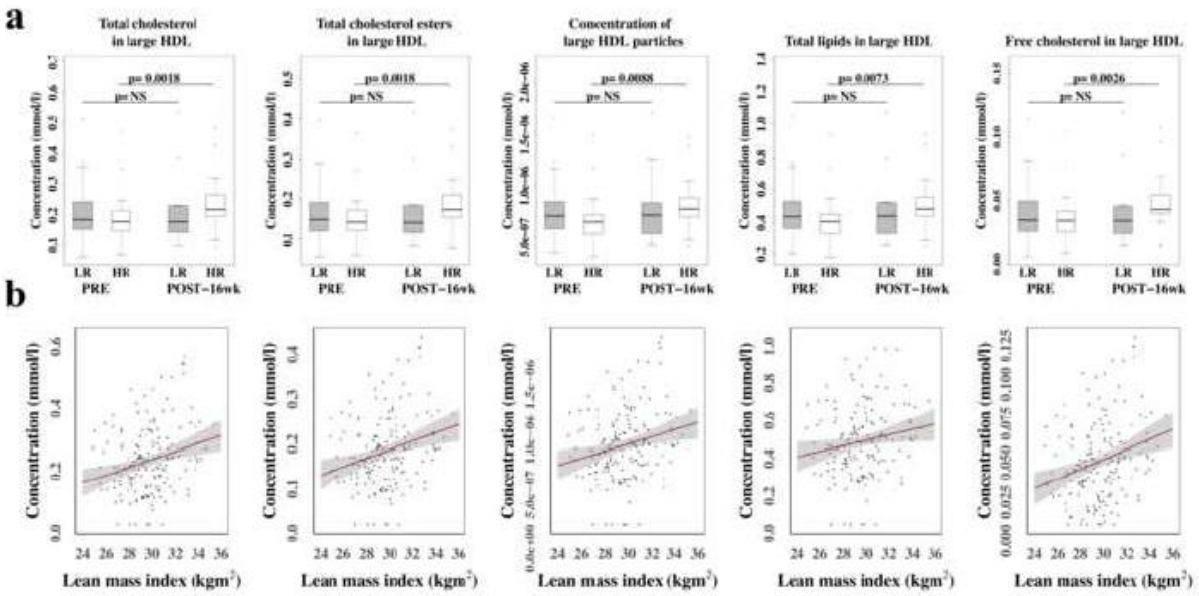

Table 1. Mean (SD) changes in physiological characteristics of the resistance training (RT) (from Baseline to post-4-weeks, n = 68; from Baseline to post-intervention, n = 59) and non-RT (n = 18) groups during the study.

		Baseline	**post-4-weeks**	**Post-intervention**	**Change[a] from baseline to post-intervention (95% CI)**	***P* value**
Body mass (kg)	RT	82.4 (10.5)	82.6 (10.1)	83.2 (10.5)	0.44 (-0.07, 0.95)	0.560
	non-RT	80.0 (11.7)		80.9 (11.5)	0.75 (-0.18, 1.67)	
Lean mass normalized to body surface area ($kg \cdot m^2$)	RT	29.4 (2.1)	29.7 (2.1) *	30.1 (2.1) *	0.83 (0.65, 1.00)	< 0.001
	non-RT	30.2 (1.7)		30.0 (1.9)	-0.23 (-0.55, 0.10)	
Fat mass normalized to body surface area ($kg \cdot m^2$)	RT	9.4 (3.7)	9.1 (3.6) *	8.9 (3.5) *	-0.69 (-0.93, -0.46)	0.001
	non-RT	8.1 (3.1)		8.3 (3.1)	0.18 (-0.25, 0.61)	
Android fat	RT	2.1 (1.0)	2.0 (1.0)	2.0 (1.0) *	-0.20 (-0.26, -	0.001

mass (kg)			*		0.13)	
	non-RT	1.7 (0.9)		1.8 (0.91)	0.04 (-0.08, 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, 0.62)	< 0.001
	non-RT	2.6 (0.4)		2.1 (0.4) *	0.05 (-0.09, 0.20)	

CI, confidence intervals; 1RM, one repetition maximum; * Statistically significant change from the baseline, Bonferroni adjusted; # Statistically significant difference between the groups at baseline; [a] Corresponding baseline values as covariate; *P* value represents between-group comparisons of changes.

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

Metabolite	Estimate	Standard error
Cholesterol esters to total lipids ratio in IDL	-1.100	0.262
Total cholesterol to total lipids ratio in IDL	-1.050	0.217
Cholesterol esters to total lipids ratio in large LDL	-0.799	0.228
Total cholesterol to total lipids ratio in large LDL	-0.676	0.223
Remnant cholesterol	-0.097	0.031
Free cholesterol	-0.069	0.019
Apolipoprotein B	-0.046	0.015
Total cholesterol in IDL	-0.044	0.015
Cholesterol esters in IDL	-0.037	0.011
Apolipoprotein B / Apolipoprotein A1 -ratio	-0.035	0.011
Sphingomyelins	-0.018	0.006
Cholesterol esters in very small VLDL	-0.010	0.004
Tyrosine	0.003	0.001
Phenylalanine	0.005	0.001

Glutamine	0.047	0.008
Conjugated linoleic fatty acids	0.053	0.015
Phospholipids to total lipids ratio in small VLDL	0.284	0.092
Triglycerides to total lipids ratio in medium LDL	0.538	0.139
Triglycerides to total lipids ratio in large LDL	0.600	0.149
Triglycerides to total lipids ratio in IDL	0.777	0.181
Phospholipids to total lipids ratio in very large HDL	1.710	0.495

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