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Effects of fat loss and low energy availability on the serum cardiometabolic profile of physique athletes

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Abstract

Low energy availability (LEA) is a health concern for athletes, although it may paradoxically lead to improved cardiometabolic health in the general population. We investigated the associations between LEA, body composition, and serum cardiometabolic profile in 23 physique athletes (DIET) and 21 controls (CONT) during a 5-month pre-competition diet (MID), followed by 1 week of increased energy availability (COMP) and a 5-month weight regain period (POST). Quantification of 250 serum metabolome variables was conducted by NMR spectroscopy, body composition by dual-energy x-ray absorptiometry, dietary intake by food diaries, and exercise levels by training logs. Body fat percentage decreased from 19.5 ± 7.0% to 8.3 ± 5.3% ($p < 0.001$) in DIET through increased exercise levels and decreased energy intake, while CONT maintained those constant. In MID, DIET had increased (FDR < 0.01) HDL cholesterol, HDL particle size and number, and decreased (FDR < 0.05) VLDL lipids, serum triglycerides, and low-grade inflammation (glycoprotein acetyls) compared to baseline and CONT. The changes were associated with reduced android fat mass (-78 ± 13%) and energy intake (-28 ± 10%). In COMP, most of the metabolic changes found in MID persisted, except for altered triglycerides in all lipoprotein classes. After weight regain in POST, serum metabolome, body composition, energy intake, and exercise levels had reverted to baseline levels. In conclusion, fat loss and LEA may have beneficial yet transient effects on the serum cardiometabolic profile of lean individuals. Especially the HDL lipidome and lipoprotein triglycerides offer potential novel biomarkers for detecting LEA in athletes.

KEYWORDS

cardiometabolic health, fat loss, lipoprotein lipidome, low energy availability in athletes, NMR metabolomics, physique sports, weight loss

J. J. Hulmi and M. Perola contributed equally.

1 | INTRODUCTION

Fat loss and energy restriction are effective ways to improve markers of lipid metabolism, inflammation, and insulin sensitivity in the general population,[1,2] but their effects on athletes' health have occasionally raised concerns. Low energy availability (LEA) caused by weight loss attempts, excessive training, or dietary restriction has been established as a risk factor for impaired bone health, reproductive function, and cardiovascular health in athletes.[3] It may compromise various metabolic functions already in the short term (days and weeks), resulting in decreased resting energy expenditure and disturbed glucose and lipid metabolism in the long-term (several months).[3–5] Indeed, the basic concept of LEA implies that after accounting for exercise energy expenditure, the athlete's daily energy intake is insufficient to maintain normal physiological functioning.[4] However, the independent effects of low body fat percentage, dietary energy restriction, and increased training volumes on the physiological manifestations of LEA remain unclear, despite their distinct contributions to the human metabolism. More research is also warranted about the effects of LEA on the serum cardiometabolic profile specifically, as the existing studies have mainly investigated markers of bone metabolism, fertility, and resting energy expenditure.[4,5]

In parallel to the cautionary observations about LEA in athletes, consuming an energy-restricted diet without malnutrition has emerged as a promising intervention for slowing down, and to some extent even reversing, biological aging processes related to metabolic health.[6,7] Large-scale energy restriction interventions have produced positive results also in previously healthy, nonobese individuals by decreasing their visceral and liver fat, increasing insulin sensitivity, and improving lipid profiles and markers of low-grade inflammation.[2,8,9] These findings emphasize the idea that the physiological effects of weight loss may vary substantially based on how it is achieved, especially in normal-weight individuals. The paradox of LEA being beneficial in some contexts and detrimental in others highlights the need for further prospective studies and a more detailed understanding on the matter.

Physique athletes are an ideal population for studying the various effects of LEA, because they voluntarily undergo rigorous fat loss phases prior to competitions, resulting in body fat percentages of below ~10% for men and ~15% for women.[10,11] The fat loss phase comprises of prolonged energy restriction, high-volume resistance training, and concurrent increases in aerobic exercise.[10] During the final week preceding the competition, athletes typically increase their energy and carbohydrate intakes and avoid fatiguing exercise to increase muscle fullness and improve visual appearance.[12] After the competition, athletes voluntarily regain their off-season body weight within approximately 5 months.[11] This design offers a unique possibility for investigating the metabolic differences of fat loss with and without ongoing LEA. We have previously shown that 21 weeks of pre-competition fat loss resulted in reduced markers of low-grade inflammation and anti-atherogenic changes in the serum lipid profile and transcriptomic markers of female physique athletes[13] The changes were strongly associated with android fat mass, suggesting a distinct role for body composition even in previously normal-weight athletes. However, as the serum cardiometabolic markers were measured only after the competition and not in the end of the energy restriction period, we were not able to investigate the possible separate effects of fat loss and LEA.

Therefore, the present study was set out to examine the cardiometabolic effects of fat loss with and without ongoing LEA in competing physique athletes, using the same nuclear magnetic resonance (NMR) assay as previously[13] for detailed information on serum biomarkers. For that aim, we investigated the physique athletes' serum cardiometabolic profile after (i) a 5-month fat loss period achieved by restricted energy intake and increased exercise levels, (ii) 1 week of alleviated LEA through increased energy intake and reduced exercise levels, and (iii) a 5-month weight regain period.

2 | MATERIALS AND METHODS

2.1 | Study design

The study is part of the second cohort under the Physique Study series conducted by the University of Jyväskylä during 2019–2020, of which the basic phenotype data has been published previously.[10] The design and methods were largely similar to our first Physique Study that was conducted exclusively in female athletes.[11] However, the present study included both male and female participants, and the study protocol consisted of four on-site test days (Figure 1) in contrast to three in the previous study: (i) at baseline (PRE), (ii) in the end of a pre-competition fat loss period (MID), (iii) 1 day after the competition (COMP), and (iv) after an extended period of weight regain (POST). Average duration of the pre-competition fat loss period (PRE–MID) was 20.4 ± 3.6 weeks. In each visit to the research center, the participants' body composition was measured and blood samples, food records, and training diaries were collected. Randomization and blinding were not possible from the participants' side, but the research staff handled participant information with identification numbers and without the group status.

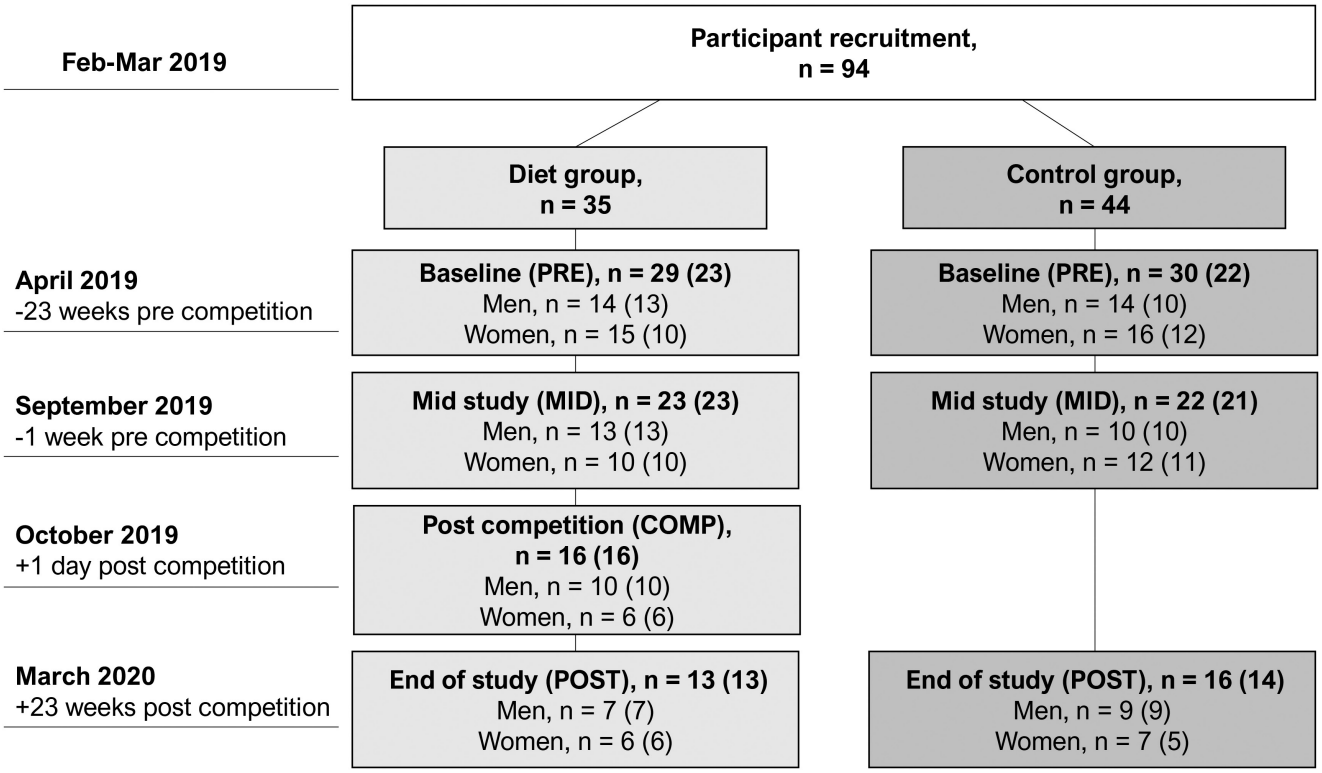

FIGURE 1 Flow chart of the study design. The number of all participants with serum metabolomics data is presented without brackets and the number of participants included in the statistical analyses is presented inside brackets at each timepoint. Participants with data from less than two time points were excluded from the analyses.

2.2 | Participants

Diet group (DIET) consisted of physique athletes (mean age 28 ± 5 years) who were preparing to compete in the Finnish National Championships in October 2019 and control group (CONT) of trained individuals (mean age 29 ± 5) whose age, sex, weight, height, and training experience were matched with the diet group. All participants were recruited via the Finnish Fitness Sports Association e-mail lists and social media pages, and a total of 94 individuals who claimed to meet the inclusion criteria were invited to fill out an online screening questionnaire. Healthy adults aged 20–40 years and with at least 2 years of resistance training experience were included in the study, while the exclusion criteria covered chronic diseases (e.g., hypertension and other cardiovascular diseases, diabetes, and hypothyroidism) and prescribed medication (excluding contraception), shift work, and self-disclosed use of anabolic steroids or other doping agents. Athletes had to be registered under the national doping control and testing organization that is regulated by the World Antidoping Agency (WADA) to participate. The Finnish National Championships had to be the athlete's first competition of the calendar year to reduce inter-individual variability in the pre-competition diet lengths. Participants in the control group aimed to maintain their current nutrition and training habits throughout the entire study period.

Of the 94 assessed individuals, 79 were invited to further health tests conducted by the study physician, resulting in 59 eligible participants (29 DIET and 30 CONT) in the baseline measurements (Figure 1). Measurements in the end of the fat loss period involved 45 participants (23 DIET and 22 CONT), after 14 participants had dropped out for personal reasons or changes in their training goals. One female competitor switched to the control group and hence did not have their serum sample taken in MID. The competition week measurements involved the 16 competitors that were able to arrive at the research site on the day after the competition. Due to the COVID-19 outbreak in spring 2020, all post weight regain measurements scheduled for April had to be canceled, which reduced the number of study completers to 29 (13 DIET and 16 CONT). The POST results of two female controls were excluded because they started preparing for a competition. Overall, participants with metabolomics data from at least two time points were included in the statistical analyses. This meant PRE and MID for all participants except for the one female control who only had data from PRE and POST due to switching group status.

Participants were informed about the study protocol, methods, and possible risks prior to beginning, and they signed a written informed consent in accordance with the Declaration of Helsinki. The study was approved by the Ethical Committee of the Central Finland Health Care District (19 U/2018), Finland, and was registered at ClinicalTrials.gov ID: NCT04392752.

2.3 | Serum samples

Participants were instructed to arrive at the measurements in a fasted state (~10 h without consuming food and liquids except for small amounts of water) and having avoided intense physical exercise and the use of caffeine, nicotine, and alcohol during the preceding 24 h. Measurements were taken at the same time of day at all time points (±1 h) to ensure that each laboratory visit had standardized conditions. Serum samples were analyzed with high-throughput NMR spectroscopy (Nightingale Health, Helsinki) for the absolute quantification of 250 biomarkers, including lipoprotein subclasses and relative lipoprotein lipid concentrations, amino acids, fatty acids, glycolysis-related metabolites, and inflammation markers (glycoprotein acetyls, validated biomarkers of systemic inflammation for clinical use[14]). A complete description of the NMR assay is available at https://research.nightingalehealth.com/biomarkers/. Of the 250 biomarkers, a total of 15 variables related to the relative lipoprotein lipid concentrations in chylomicrons and large (L-XXL) VLDL particles were excluded from the analyses because in 62 measurements, one or more of the respective lipid concentrations were below the limit where the platform offers highly accurate quantification (<0.01 mmol/L). Chylomicrons and large VLDL particles were grouped under the same category, because the NMR method is unable to distinguish them.

2.4 | Body composition

After the blood draws, participants' body composition parameters were assessed with dual-energy x-ray absorptiometry (DXA) (Lunar Prodigy Advance, GE Medical Systems-Lunar, Madison WI USA). The DXA protocol has been described in detail previously by Isola et al.[10] and Hulmi et al.[11] The DXA analysis (enCORE 2005, version 9.30 and Advance 12.30) provided estimates of body weight, fat-free mass (FFM), total fat mass, and fat distribution among body parts, including android fat, which was measured from the region between pelvis and lower ribs.

2.5 | Nutritional intake and exercise

Due to the observational nature of the study, all participants followed their nutritional regimens and exercise programs independently, without guidance from the research staff. The diet group reported their nutritional intake throughout the study period with a nutrition log and informed the research staff whenever their intakes were adjusted.[10] During the competition week (MID-COMP), nutritional intake was reported for each day. The competitors received detailed instructions from their coaches for pre-competition nutrition and supplementation, which were utilized for dietary data analysis. Participants in the control group were instructed to maintain their current off-season dietary habits, and they delivered a food diary (3 + 1 days) three times during the study (PRE, MID, POST). Nutritional data received from the participants was analyzed using the AivoDiet dietary analysis software (Flow Team Oy, Oulu, Finland). Energy availability (EA) was calculated as earlier (energy intake–exercise energy expenditure)/FFM.[15] Regarding the definition of LEA in our cohort, we concluded not to apply a universal cut-off value. Although the cut-off point of 30 kcal/kg FFM/day is widely used for females, it may not apply to all individuals and may be different for males.[3] Instead, we evaluated that the magnitude of changes in body composition, energy intake, exercise levels, and hormone profiles[10,11] between a healthy off-season status and the final weeks of competition dieting led the physique competitors to a state of LEA,[4] regardless of the absolute EA value they ended up with.

Diet standardization was not possible since physique athletes strive to follow their own best practices to achieve their desired level of conditioning. However, nutritional data accuracy is expected to be high, as physique athletes are generally highly adherent to their diets and experienced in weighing out their portions with a food scale.[16]

Participants provided their training programs throughout the study period and reported any changes that were made to them to the research staff.[10] The training programs included the number and type of resistance and endurance exercise sessions per week, the duration and intensity of the endurance exercise sessions, and the exercises and numbers of sets and repetitions used in resistance exercise sessions. All participants practiced resistance training at the gym, and the diet group's pre-competition endurance training protocols consisted mainly of cycling, running, swimming, walking, or using an elliptical trainer or a stair climber. Based on the reported training volume and intensity, the average weekly training load was determined for each participant in METh units according to the Ainsworth et al. classification.[17]

2.6 | Statistical analyses

Histograms and scatter plots were used to visually inspect the normality of the variable distributions prior to analyses. Metabolomics data was log2-transformed and pareto scaled using the VIIME analytics platform[18] to reduce skewness and the relative importance of large values. To understand the magnitude of changes in metabolite values between timepoints, we calculated mean changes, standard deviations, and effect sizes (Cohen's d) within groups using Microsoft Excel (version 2310) (Table S1). The rest of the statistical analyses were performed with R software (version 4.0.3, http://r-project.org).

We analyzed within-group changes in serum metabolite values, body composition, energy and macronutrient intakes, and exercise levels by comparing timepoints MID, COMP, and POST to baseline using Generalized Estimating Equations (GEE) models with linear link function and independent correlation structure. All metabolomics analyses included age and sex as covariates, and *p*-values were adjusted using the Benjamini-Hochberg method (false discovery rate, FDR). After analyzing the groups separately, we repeated the GEE models for the entire dataset, this time including a Group x Time interaction term in the model for a preliminary understanding of group differences (Table S2).

To further narrow down the most significant metabolic changes, the diet and control groups were compared to each other by using ANOVA tests that only included the metabolome variables that had been statistically significant in the primary within-group analyses. Specifically, the ANOVA models compared ΔMID-PRE and ΔCOMP-PRE changes in the diet group's log-transformed metabolite values to the control group's ΔMID-PRE changes with baseline value, age, and sex as covariates (ΔMID/COMP-PRE ~ Group + Baseline[metabolite] + Age + Sex).

To examine the associations of body composition, energy intake, and total weekly exercise volume with the serum metabolome, additional within-group analyses were conducted in the diet group by adjusting the base model with either android fat mass (g), energy intake (kcal/kg/d), or total weekly training volume (METh/week) as covariates (Tables S3–S5). After the main analyses, correlations between the diet group's HDL and VLDL subclass lipid concentrations in MID and COMP were analyzed with the R corrplot package using Spearman correlations.

Finally, a sensitivity analysis was conducted to ensure that the main differences between PRE-MID and PRE-COMP results were not due to the different numbers of participants. The sensitivity analysis included only the 16 participants with complete metabolomics data from PRE, MID, and COMP. The results of the analysis are presented in Table S6.

3 | RESULTS

3.1 | Fat loss period (PRE-MID)

3.1.1 | Changes in body composition, nutrition, and exercise

In the present subcohort of Isola et al.,[10] the physique competitors lost 13 ± 5% ($p < 0.001$) of their initial body mass while maintaining FFM ($p > 0.05$ vs. PRE) (Table 1). Body fat percentage decreased from 19.5 ± 7.0% to 8.3 ± 5.3% ($p < 0.001$), and android fat mass decreased by 78 ± 13% ($p < 0.001$). No changes occurred in any of the control group's body composition parameters ($p > 0.05$ vs. PRE) (Table 1). Energy intake decreased in the diet group by 28 ± 10% ($p < 0.001$) mainly due to decreased carbohydrate and fat intakes ($p < 0.01$) (Table 2). Resistance training

TABLE 1 DXA body composition measures for physique competitors (Diet) and non-competing controls (Control) at baseline (PRE), in the end of the fat loss period (MID), and in the end of the recovery period (POST).

	PRE	MID	POST
Body mass (kg)			
Diet	81.7 ± 14.9	71.1 ± 11.5$^{\dagger, ***}$	79.8 ± 16.9
Control	74.0 ± 12.5	75.8 ± 13.1	78.4 ± 15.9
Total fat mass (kg)			
Diet	15.6 ± 5.6	5.7 ± 3.4$^{\dagger, ***}$	16.0 ± 5.9
Control	14.1 ± 5.6	15.6 ± 5.9	14.9 ± 7.1
Android fat mass (g)			
Diet	1338 ± 624	303 ± 265$^{\dagger, ***}$	1354 ± 667
Control	1131 ± 644	1268 ± 671	1250 ± 940
Fat-free mass (kg)			
Diet	66.1 ± 14.7	65.4 ± 12.6	63.8 ± 14.7
Control	60.0 ± 12.3	60.2 ± 13.0	63.5 ± 11.9
Body fat percentage (%)			
Diet	19.5 ± 7.0	8.3 ± 5.3$^{\dagger, ***}$	20.2 ± 6.4
Control	19.2 ± 7.3	20.9 ± 7.4	18.5 ± 6.4
BMI (kg/m^2)			
Diet	26.6 ± 3.6	23.1 ± 2.4$^{\dagger, ***}$	26.5 ± 3.4
Control	24.9 ± 2.2	25.5 ± 2.1	25.8 ± 3.0

Note: Values are presented as mean ± standard deviation. Within-group differences between time points were analyzed with Generalized Estimation Equations.

***$p < 0.001$; compared to PRE;

$^{\dagger}p < 0.05$ time × group interaction.

TABLE 2 Dietary intake and exercise levels for physique competitors (Diet) and non-competing controls (Control) at baseline (PRE), in the end of the fat loss period (MID), during the competition week (COMP), and in the end of the fat regain period (POST). Complete data on nutritional intake were available for $n = 20/22$ at PRE (Diet/Control), $n = 19/19$ at MID, $n = 14$ at COMP, and $n = 10/15$ at POST. For exercise, the corresponding numbers were $n = 20/19$ at PRE, $n = 20/17$ at MID, $n = 13$ at COMP, and $n = 12/14$ at POST.

	PRE	MID	COMP	POST
Energy intake (kcal/kg)				
Diet	38.0 ± 5.6	26.5 ± 4.6†, ***	34.4 ± 5.9*	37.9 ± 6.0
Control	34.5 ± 5.0	33.3 ± 6.1		32.0 ± 7.0
Protein intake (g/kg)				
Diet	2.8 ± 0.5	2.6 ± 0.5	2.3 ± 0.5***	2.6 ± 0.5
Control	2.6 ± 0.5	2.4 ± 0.5		2.5 ± 0.6
Carbohydrate intake (g/kg)				
Diet	4.2 ± 0.9	2.3 ± 0.8†, ***	4.1 ± 1.4	4.2 ± 1.1
Control	3.4 ± 0.8	3.4 ± 1.1		3.2 ± 1.3
Fat intake (g/kg)				
Diet	1.0 ± 0.3	0.7 ± 0.2†, ***	0.9 ± 0.2	1.0 ± 0.2
Control	1.0 ± 0.4	1.0 ± 0.3		0.9 ± 0.3
Resistance exercise (METh/wk)				
Diet	22 ± 4	22 ± 7	14 ± 7**	21 ± 5
Control	19 ± 8	18 ± 8		20 ± 7
Endurance exercise (METh/wk)				
Diet	6 ± 9	23 ± 16†, ***	7 ± 12	5 ± 10
Control	10 ± 15	10 ± 13		10 ± 13
Total exercise (METh/wk)				
Diet	28 ± 10	44 ± 17†, ***	21 ± 17	26 ± 11
Control	29 ± 15	28 ± 13		30 ± 15

Note: Values are presented as mean ± standard deviation. Within-group differences between time points were analyzed with Generalized Estimation Equations.

*$p < 0.05$ compared to PRE; **$p < 0.01$ compared to PRE; ***$p < 0.001$ compared to PRE;

†$p < 0.05$ time × group interaction.

volumes were maintained ($p > 0.05$ vs. PRE) and endurance training significantly increased ($p < 0.001$), as many of the competitors added consistent endurance training protocols to their training routines (Table 2). Overall, the competition preparation resulted in EA of 21 ± 6 kcal/ kg FFM (Isola et al. manuscript in preparation). Participants in the control group maintained their baseline dietary intakes and exercise levels ($p > 0.05$ vs. PRE).

3.1.2 | Serum cardiometabolic profile

In the end of the fat loss period, a total of 108 serum metabolite concentrations and ratios were significantly different from baseline (FDR < 0.05)—exact beta values, standard errors, and FDR-adjusted *p*-values for the within-group results are reported in Table S1. The most distinct changes occurred in the HDL lipidome: HDL cholesterol, particle number, phospholipids, and total lipid concentration were all increased together with apoA-1 concentration ($\beta \geq 0.4$, FDR < 0.05) (Figure 2A). Lipid composition changes were particularly pronounced in the largest HDL subclasses (Figure 3), and increased particle mean diameter ($\beta = 0.23 \pm 0.05$, FDR < 0.001) indicated a general shift towards a larger HDL particle profile. Most of the changes in L–XL HDL lipid concentrations and ratios were also significantly different from the control group when adjusted for baseline values (FDR < 0.05) (Figure 2A). In accordance with these anti-atherogenic changes, the concentration of inflammation markers (glycoprotein acetyls) decreased ($\beta = -0.48 \pm 0.12$, FDR < 0.001) (Figure 4A).

Among the apoB-containing lipoproteins, the most notable changes occurred in large VLDL particles and chylomicrons. VLDL triglycerides and particle total lipid concentration decreased together with particle mean diameter ($\beta < -0.25 \pm 0.04$, FDR < 0.05) (Figure 2B). In the largest subclasses (L–XXL), particle number and cholesterol and phospholipid concentrations decreased (FDR < 0.001). These changes were significantly different from the control group (Figure 2B) and negatively correlated with the increased HDL lipid concentrations in Spearman correlation analysis ($p < 0.05$) (Figure 5). In spite of the changes in VLDL lipid composition, LDL cholesterol concentration (FDR = 0.96) and particle size (FDR = 0.41) remained unchanged.

In addition to VLDL triglyceride concentration, serum total triglyceride concentration decreased in the diet group

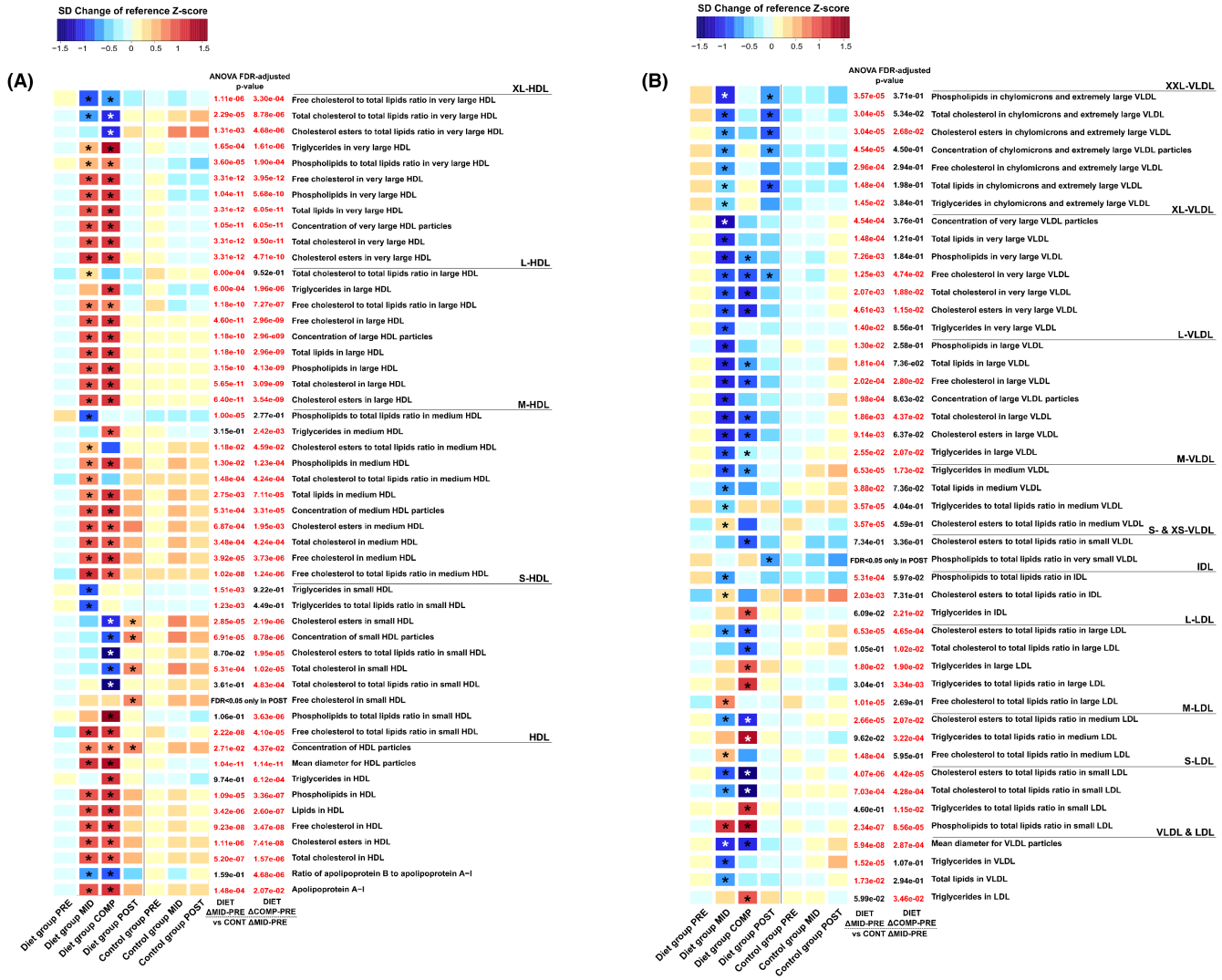

FIGURE 2 Heatmap of the HDL lipid concentrations and ratios that were significantly changed in the diet group (DIET) in the end of the fat loss period (MID), in the end of the competition week (COMP), or in the end of the weight regain period (POST) compared to baseline (PRE). Metabolite values and color key are represented as standard deviation (SD) change from reference *Z*-score. Calculated baseline *Z*-score values (PRE) from both diet and control group were pooled together and set as the reference level to which each individual group/timepoint-level was compared. Statistically significant (FDR <0.05) within-group changes are marked in the figure with asterisks (*). The red color in the right-hand side columns indicates significant differences to the control group (CONT). Between-group analyses were conducted in two separate baseline-adjusted ANOVA models: (i) DIET ΔMID-PRE changes in metabolite values vs CONT ΔMID-PRE and ii) DIET ΔCOMP-PRE versus CONT ΔMID-PRE. The between-group analyses only included metabolite values that were significant in DIET PRE-MID or PRE-COMP within-group analyses. (B) Heatmap of the lipid concentrations and ratios in ApoB-containing lipoproteins that were significantly changed in the diet group (DIET) in the end of the fat loss period (MID), in the end of the competition week (COMP), or in the end of the weight regain period (POST) compared to baseline (PRE). Metabolite values and color key are represented as standard deviation (SD) change from reference Z-score. Calculated baseline Z-score values (PRE) from both diet and control group were pooled together and set as the reference level to which each individual group/timepoint-level was compared. Statistically significant (FDR < 0.05) within-group changes are marked in the figure with asterisks (*). The red color in the right-hand side columns indicates significant differences to the control group (CONT). Between-group analyses were conducted in two separate baseline-adjusted ANOVA models: (i) DIET ΔMID-PRE changes in metabolite values versus CONT ΔMID-PRE and (ii) DIET ΔCOMP-PRE versus CONT ΔMID-PRE. The between-group analyses only included metabolite values that were significant in DIET PRE-MID or PRE-COMP within-group analyses.

($\beta = -0.47 \pm 0.19$, FDR = 0.03), and several changes occurred in the serum fatty acid composition (Figure 4A). Ratios of omega-3 fatty acids, docosahexaenoic acid (DHA), linoleic acid, and saturated fatty acids to total serum fatty acids increased (FDR <0.05), and the corresponding ratio for monounsaturated fatty acids decreased (FDR <0.05). Significant increases were also observed in the absolute concentrations of DHA and omega-3 fatty acids ($\beta > 0.50$,

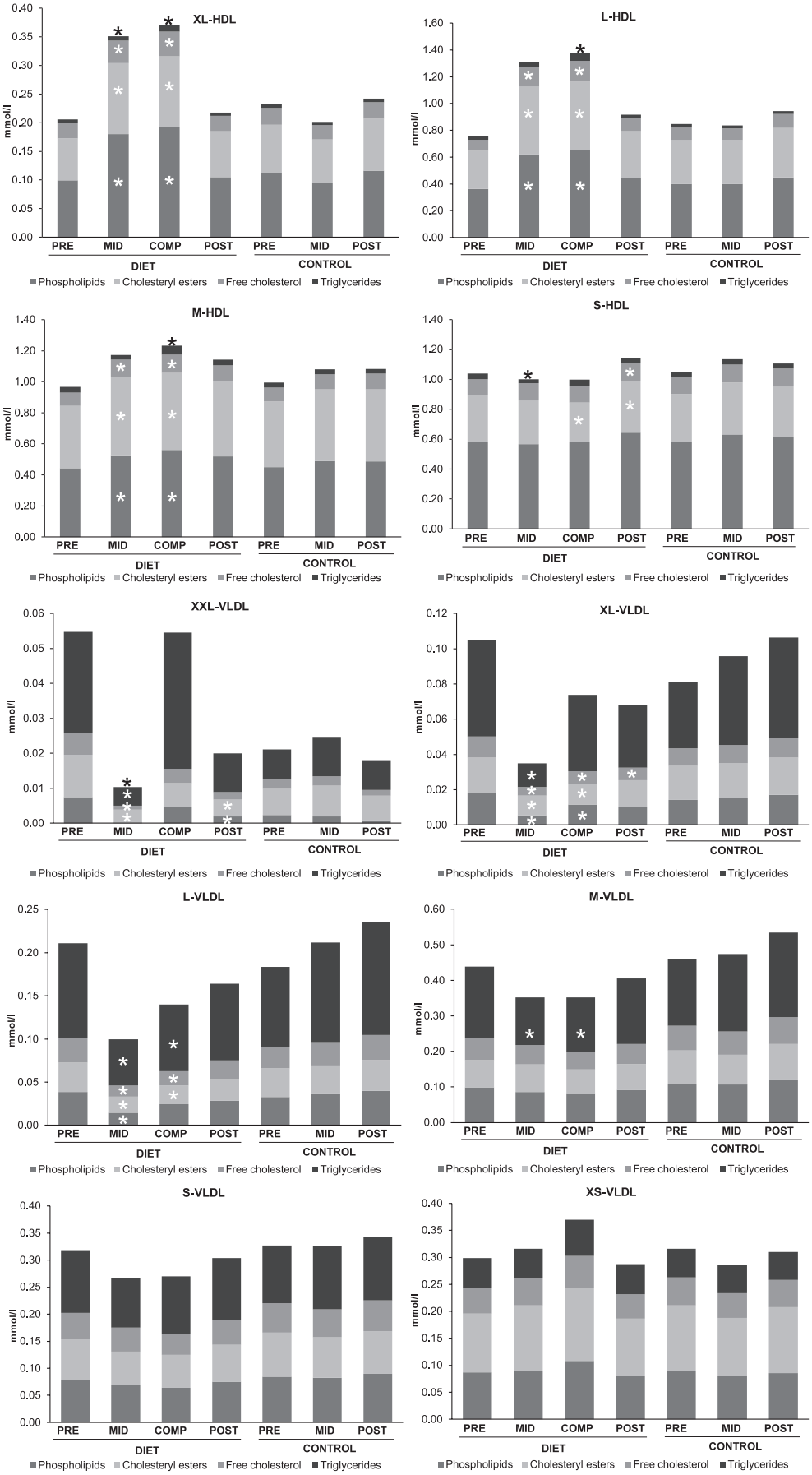

FIGURE 3 Mean lipid concentrations (mmol/L) in HDL and VLDL subclasses in the diet (DIET) and control (CONT) groups at each time point (PRE, MID, COMP, POST) throughout the study. Statistically significant (FDR <0.05) within-group changes in lipid concentrations compared to PRE are marked with asterisks (*). During the fat loss period (PRE-MID) of DIET, total cholesterol and phospholipids increased in HDL subclasses and triglycerides decreased especially in large VLDL particles. During the competition week (PRE-COMP), triglycerides increased in HDL subclasses and reverted back to baseline in large VLDL particles.

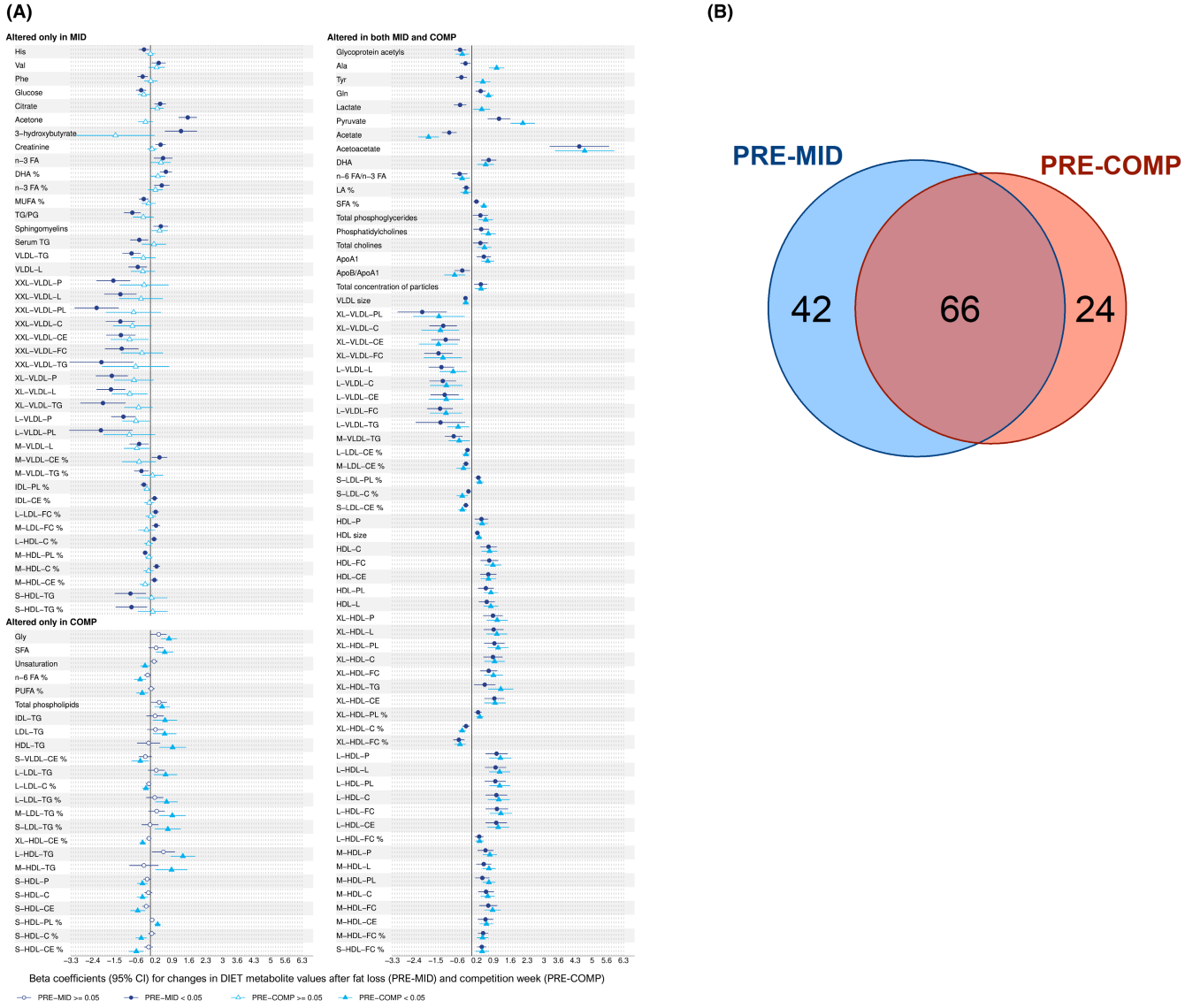

FIGURE 4 Changes in the diet group's (DIET) serum metabolite values that were statistically significant (FDR <0.05) (i) only after the fat loss period (MID), ii) only after the competition week (COMP), or iii) both in MID and in COMP versus baseline (PRE). Within-group changes were analyzed with Generalized Estimation Equations with age and sex as covariates. Metabolite values are represented as beta coefficients for PRE-MID and PRE-COMP comparisons, and lines display 95% confidence interval (CI). Ala, alanine; C, total cholesterol; CE, cholesterol esters; DHA, docosahexaenoic acid; FA, fatty acids; FC, free cholesterol; Gln, glutamine; Gly, glycine; His, histidine; L, total lipids; LA, linoleic acid; MUFA, monounsaturated fatty acids; *n*-3 FA, omega-3 polyunsaturated fatty acids; *n*-6 FA, omega-6 polyunsaturated fatty acids; P, total concentration of lipoprotein particles; Phe, phenylalanine; PL, phospholipids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids; TG, triglycerides; TG/PG, ratio of triglycerides to phosphoglycerides; Tyr, tyrosine; Unsaturation, degree of unsaturation; Val, valine; %, ratio to total fatty acids or to total lipids. (B) Venn diagram displaying the number of metabolites that were altered (i) only after the fat loss period (MID) (blue), (ii) only after the competition week (COMP) (red), or (iii) at both MID and COMP (joint area) compared to baseline (PRE) in the diet group.

FDR < 0.05), which decreased the ratio of omega-6 to omega-3 fatty acids ($\beta = -0.50 \pm 0.16$, FDR = 0.005). Of the measured phospholipid classes, concentrations of sphingomyelins and phosphatidylcholines increased (FDR < 0.05).

Of the energy metabolism markers, concentrations of glucose, lactate, and acetate decreased ($\beta < -0.35$, FDR < 0.001), while ketone bodies (acetone, acetoacetate, and 3-hydroxybutyrate [$\beta > 1.25$, FDR < 0.001]), creatinine, citrate, and pyruvate increased ($\beta \geq 0.40$, FDR < 0.05) (Figure 4A). Of the amino acids, concentrations of alanine, histidine, and aromatic amino acids (phenylalanine and tyrosine) decreased ($\beta < -0.25$, FDR < 0.05), while glutamine increased ($\beta > 0.35$, FDR < 0.001) and branched-chain amino acids remained unaltered (FDR > 0.05).

In the control group, only five metabolite values were different from baseline in MID: acetate, acetoacetate, pyruvate, glutamine, and the ratio of free cholesterol to total lipids in XS-VLDL (FDR < 0.05).

3.1.3 | Covariate analyses (diet group)

The significant reduction of android fat mass explained a vast majority of the changes observed during the fat loss period. In the android-fat-adjusted analysis, only 21 statistically significant changes remained, with no significant pattern changes in VLDL and HDL lipids (Table S3). Body-weight-adjusted daily energy intake (kcal/kg/d) had a similar explanatory power for changes in the HDL subclasses, yet it did not fully explain the lipid reductions in large VLDL particles (Table S4). Of the covariates examined (android fat mass, energy intake, exercise levels), total weekly training volume (METh/week) was the weakest predictor of changes in the serum cardiometabolic profile at all time points (Table S5).

3.2 | Competition week (PRE-COMP)

3.2.1 | Nutrition and exercise

During the competition week, the physique competitors increased their carbohydrate and fat intakes and decreased their weekly training volumes towards baseline levels (Table 2), which resulted in EA of 34 ± 6 kcal/kg FFM (Isola et al. manuscript in preparation). Training volume of the week consisted of similar endurance exercise volumes and lower resistance exercise volumes than at baseline (Table 2).

3.2.2 | Serum cardiometabolic profile

After 1 week of increased energy availability in the diet group, 42 metabolite values reverted to baseline levels, and 24 new changes were detected compared to MID (Figure 4B). The most evident differences to PRE-MID results were observed among HDL and VLDL triglycerides (Figure 3): HDL total triglycerides increased ($\beta = 0.92 \pm 0.28$, FDR = 3.95×10^{-3}) as a result of triglyceride increases in XL-, L-, and M-sized particles ($\beta > 0.85$, FDR < 0.05) (Figure 2A), and VLDL total triglycerides, VLDL total lipids, and L–XXL-VLDL particle number were no longer different from PRE (FDR > 0.05) (Figure 2B). These changes in HDL and VLDL particles were positively correlated with each other ($p < 0.05$) in Spearman correlation analysis (Figure 5) and significantly different from the control group (Figure 2A,B). Notably, most of the increases in lipoprotein triglycerides were unique to PRE-COMP results and not significant in PRE-MID nor in PRE-POST results (FDR > 0.05) (Figure 2) (Table S2).

Otherwise, the changes in the HDL lipidome were largely similar to PRE-MID, as HDL cholesterol, HDL total lipids, particle mean diameter, particle number, and serum apoA-1 concentration maintained elevated concentrations compared to PRE ($\beta > 0.30$, FDR < 0.001) (Figure 2A). The decrease in glycoprotein acetyls from baseline was also sustained ($\beta = -0.39 \pm 0.13$, FDR = 0.01) (Figure 4A). All the main results in lipoprotein composition changes were replicated in a sensitivity analysis that only included participants with complete metabolomics data from PRE, MID, and COMP measurements (Table S6).

Some of the changes in markers of energy metabolism that occurred during the fat loss period were no longer significant in COMP: the concentrations of glucose, 3-hydroxybutyrate, acetone, citrate, phenylalanine, tyrosine, histidine, and creatinine reverted back to baseline levels (FDR > 0.05 vs PRE) (Figure 4A). However, both alanine and lactate increased significantly compared to baseline (FDR < 0.001) after having decreased in MID (Figure 4A).

3.3 | Weight regain period (PRE-POST)

3.3.1 | Body composition, nutrition, and exercise

The diet group regained virtually all of the lost body weight and fat mass ($p > 0.05$ vs. PRE) during a prolonged period of recovery from competition dieting (Table 1). At the end of this phase, training volumes and energy and macronutrient intakes were also no longer different from baseline ($p > 0.05$) (Table 2). Participants in the control group maintained their baseline body compositions, dietary intakes, and training volumes constant throughout the entire study period (PRE-POST) (Tables 1 and 2).

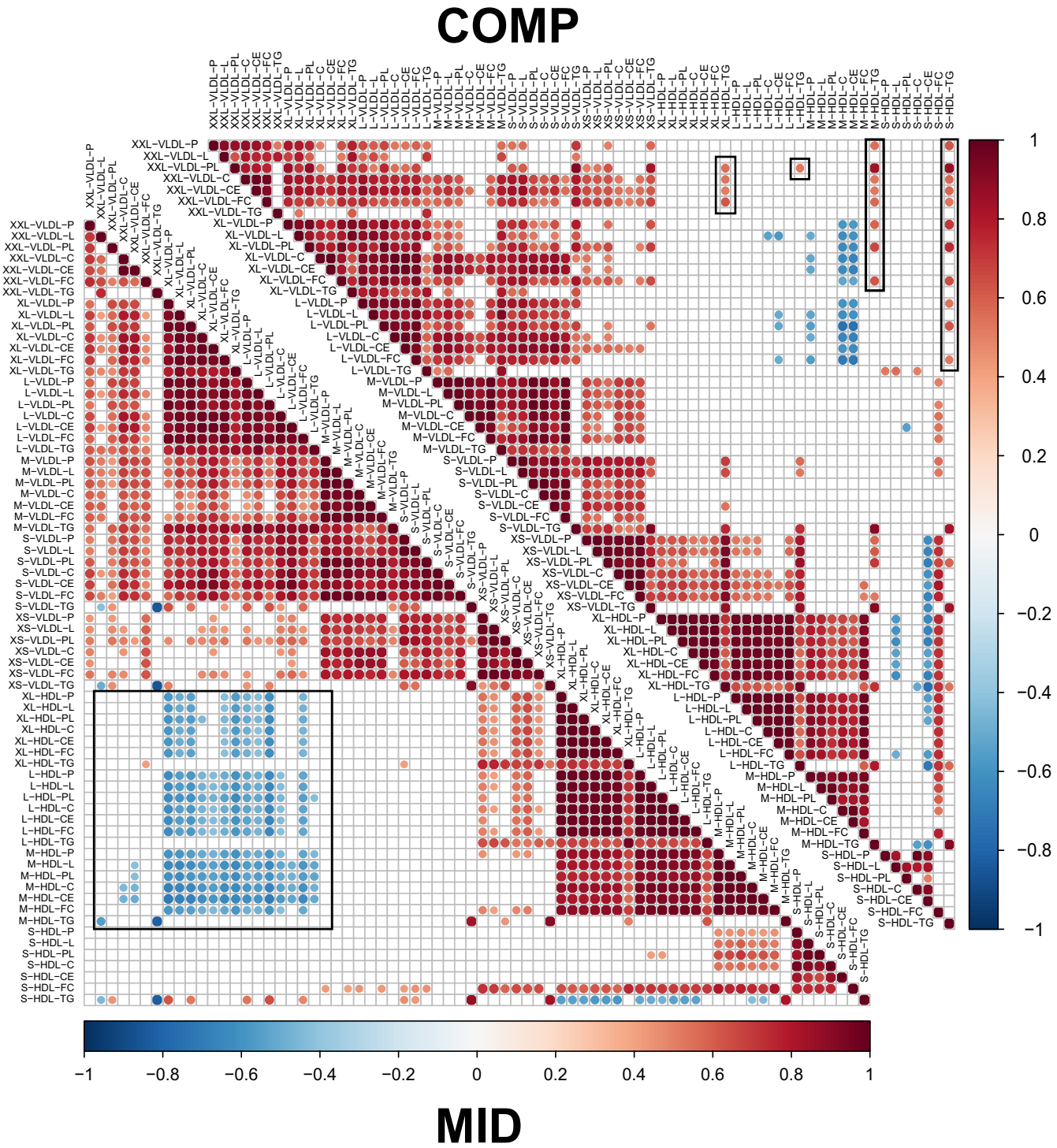

FIGURE 5 Spearman correlations between VLDL and HDL subclass lipid concentrations in the diet group after the fat loss period (MID) and after the competition week (COMP). Colored circles represent statistically significant ($p < 0.05$) correlations. The black squares highlight possible interactions in lipid exchange between HDL and VLDL particles: at MID, the negative correlations between lipids in large HDL particles and lipids in L–XL VLDL particles suggest increased lipid transfer from VLDL to HDL. At COMP, these correlations were no longer observed, but instead the increased S-, M- and XL-HDL triglycerides were positively correlated with L–XXL VLDL cholesterol, phospholipids, and particle number.

3.3.2 | Serum cardiometabolic profile

After re-establishing baseline body composition, nutrition, and exercise levels, the diet group had 19 serum metabolite values that were significantly different from baseline (FDR < 0.05). The particle number and concentrations of total lipids, cholesterol, and phospholipids in XXL-VLDL remained lower (FDR < 0.05) (Figures 2B and 3) and HDL particle number (FDR = 0.04) higher than before the diet (Figure 2A). In addition to the aforementioned changes that were preserved from the fat loss period, a couple of new changes occurred in HDL particles, as the number and cholesterol concentration of S-HDL particles were increased compared to baseline (FDR < 0.05) (Figure 2A).

In the control group, five metabolite values were different from baseline at POST: pyruvate, acetoacetate, acetate, glutamine, and the mean diameter for LDL particles (FDR < 0.05).

4 | DISCUSSION

The present study is among the most comprehensive longitudinal serum metabolome assessments in physique athletes, and its design enables the rare comparison between the effects of a low body fat percentage with and without an ongoing state of LEA. Our results indicate that fat loss via energy restriction and increased exercise training in previously lean individuals leads to increased number, size, cholesterol and phospholipid concentrations of HDL particles and decreased size and triglyceride concentration of VLDL particles. These changes were accompanied by a decrease in markers of low-grade inflammation (glycoprotein acetyls). Most of the anti-atherogenic alterations in the serum cardiometabolic profile were preserved even after a week of increased energy availability prior to the competition, excluding serum triglycerides, which increased to baseline level or beyond in all lipoprotein classes. The results are in line with our previous study,[13] where a group of 25 healthy female physique athletes experienced similar changes in their HDL subclass composition, serum triglycerides, and markers of low-grade inflammation as a result of a 21-week pre-competition fat loss period.

Clinical trials indicate that increased number and cholesterol concentration especially in large HDL particles are inversely associated with cardiovascular risk.[19] In addition, phospholipids have been shown to improve the capability of HDL particles to remove and transport cholesterol from peripheral tissues due to their amphiphilic properties.[20] The presently observed correlations between HDL and VLDL lipids suggest that the changes in the physique athletes' HDL subclass composition were at least partly a consequence of increased lipid transfer between the different lipoprotein classes. Increased particle number, cholesterol and phospholipid concentrations of large HDL particles were correlated with opposite changes in large VLDL particles, possibly due to the reduced dietary intake and/or increased exercise accelerating VLDL particle lipolysis and concomitant lipid transfer to HDL particles.[21,22] A similar link between the metabolism of triglyceride-rich particles and HDL was also proposed in an NMR-spectroscopy study in lactating women,[23] where both diet- and exercise-induced weight loss conditions were found to have favorable effects on the size and phospholipid concentration of HDL particles. Furthermore, our previous study found decreased level of lipoprotein lipase inhibitors in the end of the competitors' fat loss period,[13] suggesting that enhanced VLDL lipolysis could have contributed to the increased HDL lipid concentrations. The proposed dynamics between the HDL and VLDL lipoprotein particles occurred without significant changes in LDL particles in both of our cohorts.[13]

Similarly to our previous study, HDL-TG were temporarily increased from baseline during the competition week. The present study enabled comparing these changes to the end-of-diet LEA state for the first time, as in the previous study serum samples were taken only after and not directly before the competition week. A new finding from the present study is that the changes in HDL-TG do not seem to reflect long-term changes in fat mass but rather the short-term increase in energy availability after an extended period of LEA. The increase in HDL-TG stood out as an exception to the anti-atherogenic changes in the previous study,[13] since triglycerides generally impair the structural stability of the HDL particle and its ability to clear cholesterol from peripheral tissues in the reverse cholesterol transport process.[24] The presently observed positive correlations between HDL-TG and VLDL subclass lipids in COMP but not in MID suggest that the acutely increased energy availability induced another transient increase in lipid transfer between HDL and triglyceride-rich lipoprotein particles, this time the latter acting as triglyceride donors to HDL.[24] Overall, our results indicate that triglyceride concentrations in HDL and VLDL subclasses are particularly responsive to changes in energy availability.

Previous studies have established increased abdominal fat mass as a potent cardiometabolic risk factor, regardless of BMI class.[25] The accumulation of fat in visceral sites disturbs lipid and glucose metabolism and elicits low-grade inflammation, and weight loss can be effective at improving these conditions.[26] Even in nonobese individuals, visceral fat mass reduction through prolonged energy restriction has produced positive outcomes in lipoprotein lipids and other cardiovascular risk markers.[8,9] The results

from our present and previous cohort[13,27] support this evidence, as reduced visceral (android) fat was strongly associated with the beneficial metabolic changes in both studies. Yet, sports nutrition experts do not recommend weight-loss dieting to athletes unconditionally because of the potential negative effects of extended LEA to health and athletic performance.[3] LEA may disrupt the endocrine and immune systems,[4,5,28,29] although the current evidence on cardiometabolic markers specifically appears relatively scattered. For example, it draws from studies in amenorrhoeic female athletes and patients with anorexia nervosa, where poor nutritional status and low concentrations of estrogen have been associated with increased total and LDL cholesterol, serum triglycerides, and inflammation markers under high-stress conditions.[30] These findings are not supported by the present results that instead suggest that the possible LEA-induced impairments in lipid metabolism can be mitigated with a proper approach to fat loss, that is, losing weight at a moderate pace, paying attention to the nutritional quality of the diet, and taking a recovery period after extended time in LEA. Taken together, our findings suggest that the metabolic changes during dieting (i) are not exclusively attributable to decreased energy intake but also depend on body fat levels and (ii) there is no apparent lower limit for android fat mass in terms of improving markers of cardiometabolic health. However, as was also found in our previous cohort,[13,27] reducing total and android fat mass did not lead to any long-lasting metabolic improvements after weight regain, indicating that temporary fat loss diets may not yield net cardiometabolic benefits for lean individuals.

In contrast to the changes in body composition and energy intake, increased endurance training did not display clear statistical associations with the serum metabolome. This contradicts the evidence that endurance training can impact the serum lipoprotein profile positively even in the absence of weight loss. For example, intervention studies have found that regular exercise induces a shift towards smaller VLDL particle sizes and larger HDL particle sizes[31] and lowers the ApoB/ApoA1 ratio,[32] although the magnitude of these effects may vary depending on the duration and intensity of exercise and the individual's basal training status.[31,32] Athletes have also been reported to have increased HDL particle size and cholesterol concentration together with a lower serum triglyceride concentration compared to sedentary controls.[33,34] Therefore, it seems likely that the increase in the physique competitors' endurance exercise training volumes also contributed to the anti-atherogenic changes in the present study, even though the associations could not be demonstrated due to small sample size, measurement accuracy, or other factors.

The metabolic adaptations commonly attributed to LEA include decreased resting energy expenditure and altered concentrations of thyroid and appetite-regulating hormones and cortisol.[5,11,35] Signs of metabolic adaptation were also reported in the current cohort by Isola et al.,[10] as the physique competitors' resting energy expenditure, heart rate, and concentrations of leptin and thyroid hormones were decreased in the end of the fat loss period. Although these adaptations commonly occur during weight loss,[36] the debate is still ongoing whether metabolic adaptation is merely a transient reflection of negative energy balance or a more persistent state predisposing to future weight gain.[37] Based on the present results, it seems that at least some of the changes are quickly reverted to baseline once EA is improved without restoring fat mass, including lipoprotein triglycerides, glucose, ketone bodies (3-hydroxybutyrate and acetone), amino acids, and creatinine. However, our results also suggest that some underlying metabolic changes do persist until fat mass is regained, as the physique competitors' HDL subclass composition and low-grade inflammation markers (glycoprotein acetyls) remained altered from baseline after the competition week. More research is still needed to better understand the distinct roles of energy intake and body composition in metabolic changes related to weight loss.

The current study has several strengths but also some limitations. Together with the previous Physique Study cohort,[11,13] it represents one of the largest and most comprehensive assessments of the physiological consequences of competing in physique sports. Only a few prospective weight loss studies have been conducted in this population apart from case studies, despite the growing interest in LEA and its effects on athletes. The study results are applicable to competitive athletes from various disciplines besides physique athletes, as well as to active individuals pursuing a leaner body composition. Utilizing an NMR metabolomics assay for serum biomarker analysis allowed us to investigate the serum cardiometabolic profiles in more detail than would have been possible in routine clinical lipid panels. On the other hand, the study had a limited sample size especially after the weight regain period due to the COVID-19 outbreak in spring 2020. The number of male and female participants did not allow us to analyze their metabolomes separately but instead sex was used as a covariate in pooled analyses. In addition, our results include unavoidable noise from the non-standardized diet and exercise routines. Future studies should include more participants to enable comparisons between the sexes and to study the effects of weight cycling, that is, weight loss and subsequent weight regain. We also encourage further investigations on the biomarkers of LEA utilizing high-throughput omics methods that allow for more mechanistic understanding of our results.

To conclude, intense fat loss and LEA over 5 months resulted in anti-atherogenic alterations in HDL and VLDL

lipid profiles and markers of low-grade inflammation in previously lean and healthy physique athletes. One week of increased EA with no major changes in fat mass (Isola et al. manuscript in preparation) did not significantly alter the HDL lipidome or low-grade inflammation but increased triglycerides in all lipoprotein classes to baseline or beyond. Therefore, HDL subclass composition and low-grade inflammation may be closely linked to the underlying changes in total and android fat mass, while lipoprotein triglycerides are more susceptible to short-term changes in EA. The changes in the serum metabolome were no longer observable after an extended period of increased EA and weight regain, suggesting that transient periods of LEA have a net neutral effect on athletes' serum cardiometabolic profile.

4.1 | Perspectives

LEA can impair the endocrine function of athletes,[4,5,38,39] yet chronic energy restriction and maintaining relatively low levels of body fat have concurrently been promoted as potential strategies against age-related metabolic diseases.[2,6,7] Our results suggest that fat loss, especially abdominal fat loss, through energy restriction and increased exercise training volumes may promote similar anti-atherogenic modulations in the lipoprotein profile of previously lean athletes as in the general population.[8,9] However, as was also demonstrated in our previous cohort,[13] these changes are largely dissipated when body composition, energy intake, and exercise levels revert back to baseline, meaning that temporary periods of energy restriction and fat loss may not yield long-lasting benefits to lean individuals. These findings are of interest to athletes, coaches, and individuals pursuing the fit body ideal and add prospective evidence to the currently scarce literature on LEA and the serum cardiometabolic profile. Additionally, they bring new data on the HDL lipidome and lipoprotein triglycerides as potential sources for novel biomarkers related to body composition changes and LEA in athletes.

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CONFLICT OF INTEREST STATEMENT

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DATA AVAILABILITY STATEMENT

The data that supports the findings of this study are available in the results and the supplementary material of this article.

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SUPPORTING INFORMATION

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