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Moderate-intensity continuous training has time-specific effects on the lipid metabolism of adolescents

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ABSTRACT

Background and Objectives: Moderate-intensity continuous training (MICT) is used to observe lipidomic effects in adults. However, the effects of MICT on lipid metabolism in adolescents remain unclear. Therefore, we aimed to longitudinally characterize the lipid profile in adolescents during different periods of 6-week MICT. **Methods:** Fifteen adolescents undertook bicycle training at 65% of maximal oxygen consumption. Plasma samples were collected at four time points (T0, T1, T2, and T3). Targeted lipidomics was assessed by ultra-performance liquid chromatography–tandem mass spectrometry to characterize the plasma lipid profiles of the participants to identify the lipids present at differing concentrations and changes in lipid species with time. **Results:** MICT affected the plasma lipid profiles of the adolescents. The concentrations of diglycerides, phosphatidylinositol, lysophosphatidic acid, lysophosphatidylcholine, and lysophosphatidylethanolamine were increased at T1, decreased at T2, and increased again at T3. Fatty acids (FAs) showed an opposite trend. Ether-linked alkylphosphatidylcholine and triglycerides were significantly increased and remained high. Sphingolipid concentrations initially decreased and then remained low. Therefore, a single bout of exercise had substantial effects on lipid metabolism, but by T3, fewer lipid species were present at significantly different concentrations and the magnitudes of the remaining differences were smaller than those at earlier times. Among all the changed lipids, only DG(14:1/18:1), HexCer(d18:1/22:1) and FA(22:0) showed no significant correlations with any other 51 lipids ($P < 0.05$). Glycerides and phospholipids showed positive correlations with each other ($P < 0.05$), but FAs were significantly negatively correlated with glycerides and phospholipids while positively with other FAs ($P < 0.05$). Pathway enrichment analysis showed that 50% of the metabolic pathways represented were related to lipid metabolism and lipid biosynthesis. **Conclusion**: MICT increases ether-linked alkylphosphatidylcholine and triglyceride concentrations. Diglyceride, phosphatidylinositol, and lysophosphatidylcholine concentrations initially rise and then decrease 6 weeks after MICT, but FA concentrations show an opposite trend. These changes might correlate with lipid metabolism or biosynthesis pathways.

Key words: moderate-intensity continuous training, adolescent, lipid profile

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INTRODUCTION

Regular exercise has a profound effect on human metabolism.[1,2] Previous studies have shown that regular exercise over a long time improves lipid metabolism, delays the adverse effects of aging, and reduces the effects of age-related diseases, including cardiovascular disease, diabetes, obesity, and cancer.[3–5] Although these beneficial effects of exercise are known to be achieved through the regulation of

metabolic homeostasis, its molecular mechanisms involved, especially in the lipid metabolism in adolescents, are still not completely understood.

The development of metabolomic technologies has provided a new approach to investigate the effects of exercise on homeostasis.[6] Such technologies have been widely used in competitive sports training,[7–9] sports nutrition research,[7] characterizing the effects of sport on chronic diseases,[10,11] and determining the metabolic response to exercise.[7,12] Lipidomics is a useful method of studying lipid metabolism under physiological and pathological conditions.[13,14] But to date, studies of the effects of exercise on lipid metabolism have mainly focused on the effects of acute or high-intensity exercise in adults or in individuals with specific diseases[7,9,11,15–21]. Nieman *et al.*[22] assessed the metabolite responses to long-distance and high-intensity running. They found alterations in the concentrations of 209 lipid metabolites, especially those of long- and medium-chain fatty acids (FAs), FA oxidation products, and ketone bodies. Manaf *et al.*[9] identified 68 metabolites with different concentrations at the end of a long-term, high-intensity exercise cycling test. Furthermore, they found that FAs and tryptophan play a central role in post-exercise fatigue. In addition, Gollasch *et al.*[23] measured the FA concentrations in healthy individuals undertaking standardized maximal treadmill exercise, and found an increase in epoxyoctadecenoic acid, dihydroxyeicosenotrienoic acid, and dihydroxyeicosenoic acid concentrations. Finally, a study of patients with sarcoidosis showed significant alterations in their lipid profiles following 3 weeks of exercise training, with significant reductions in their FA, triacylglycerol (TAG), and total cholesterol concentrations.[21] In this previous study, the samples were collected at different time points. Some samples were only collected twice before and after the exercise, and some samples were collected several times during the exercise. But few studies have considered metabolic profiling of exercise intensity in mediating physiological adaptations to training.

Furthermore, few studies have examined the lipid metabolic response of adolescents to exercise, especially the effects of classic physiological adaptations characteristic of moderate-intensity continuous training (MICT), such as increased aerobic capacity (VO_2·max). In contrast to untargeted lipidomics, targeted semi-quantitative methods provide a comprehensive coverage of more than 500 lipid species comprising phospholipids, glycerides, sphingolipids, and sterols.[24] There are sex differences in the pattern of substrate utilization and hormonal effects on exercise because estrogen and progesterone dominate in women and testosterone in men.[25–27] Compared to men, the hormonal levels of women are in rhythmic oscillation along the menstrual cycle. Therefore, in the present study, we only used male adolescents to investigate the effects of 6 weeks of MICT on lipid metabolism.

METHODS

Reagents

High-performance liquid chromatography (HPLC)-grade acetonitrile, methanol, and isopropanol (IPA) were purchased from Merck (99.9%; Merck KgaA, Darmstadt, Germany). HPLC-grade ammonium acetate (≤ 97%) and a lipid internal standard mixture (Avanti Splash Lipidomix™ lipid standards; Avanti Polar Lipids, Inc., Alabaster, AL, USA) containing d_7-phosphatidylcholine (15:0/18:1), d_7-phosphatidylethanolamine (15:0/18:1), d_7-phosphatidylglycerol (15:0/18:1), d_7-phosphatidylinositol (15:0/18:1), d_7-lysophosphatidylcholines (15:0/18:1), d_7-lysophosphatidylethanolamine (15:0/18:1), d_7-diacylglycerols (15:0/18:1), d_7-TAGs (15:0/18:1), d_9-sphingomyelins (18:1), d_7-cholesteryl esters (18:1), d_7-monoacylglycerols (18:1), and d_7-phosphatidic acids (15:0/18:1) were purchased from Sigma-Aldrich (USA).

Participants

Sixteen healthy male adolescents aged 12–14 years were enrolled. One participant dropped out and 15 participants finished the whole program. None of the participants had previously undergone systematic exercise training, and all of them were banned from drinking alcohol and consuming caffeine. The participants rested 3 days before the measurements and training sessions. They were also required to ensure that they had sufficient sleep during the training. The research was performed in accordance with the *Declaration of Helsinki*. This study was approved by the Ethics Committee of Beijing Sport University (Approval No. 2020139H). All the participants and their guardians provided informed written consent, in which all the experimental procedures and potential risks were explained. The participants were asked to maintain their previous daily activity and eating patterns during testing and training. Before the first training, anthropometric measures (height and weight) were recorded.

Exercise training and collection of clinical information and serum samples

The exercise protocol began with 15–20 min of warm-up activity, followed by training on a Monark Power bike (Monark 894E, Sweden) at 65% of maximal oxygen consumption (VO_2·max). After the training, 10–20 min of static stretching and relaxation exercises were performed. The exercise was performed three times a week for 6 weeks. The duration of the bouts of exercise training was 30 min during the first and second weeks, 45 min during the third and fourth weeks, and 60 min during the fifth and sixth weeks.

Blood samples were collected four times in different periods of MICT as follows: at baseline, immediately after the first bout of exercise, immediately after the last training, and 48 h after the exercise program (T0, T1, T2, and T3, respectively). Fasting blood samples (5 mL) were added to procoagulant-containing tubes, mixed, and centrifuged at 3000 r/min for 5 min at room temperature to obtain serum samples. Blood glucose levels were determined.

Measurement of VO_2 · max of the participants

The participants were asked to complete VO_2·max and heart rate measurements. VO_2·max was measured using a metabolic cart (Cortex MetaMax 3B; Cortex, Germany). After a 5-min warm-up exercise, participants initially began with 1 min of unloaded cycling (0 W) at a cadence of 60 r/min. After this initial period, the work rate was increased by 25 W every 2 min until the participants reached volitional fatigue. The heart rate was measured continuously (Polar, Finland).

Targeted lipidomic analysis

Targeted lipidomics with the semi-quantitative approach was used in this study. Lipids were extracted from the plasma samples by simple protein precipitation using precooled IPA at 4℃.[28] Briefly, 95 μL of plasma or a pooled quality control sample was spiked with 5 μL of lipid internal standard mixture, and then 500 μL of precooled IPA was added. The mixtures were vortexed for 1 min, placed at −20℃ for 10 min, and then vortexed again for 10 min. After 2 h at 4℃, the samples were centrifuged at 10,300 × *g* for 10 min at 4℃, and the supernatants were transferred to a 96-well plate for ultra-high performance liquid chromatography–tandem mass spectrometry analysis.

A targeted lipidomic analysis was performed using the Waters iclass-Xevo TQ-S ultra-high performance liquid chromatography–tandem mass spectrometry system (Waters, MA, USA), which was equipped with an electron spray ionization (ESI) ion source. The mass spectrometer was operated using MassLynx v4.1 software (Waters, Milford, MA, USA). Lipid species were separated using a Waters Acquity UPLC BEH Amide column (1.7 μm, 2.1 mm × 100 mm) and then detected in the multiple reaction monitoring mode. The multiple reaction monitoring transitions are shown in Table S1. The dwell time was 3 ms for each lipid species. The source nitrogen gas temperature was set to 120℃ and the flow rate to 150 L/h. The desolvation gas temperature was 500℃, and the flow rate was 1000 L/h. The capillary voltage was set to 2.8 kV for the positive mode and 1.9 kV for the negative mode. The autosampler was operated at 4℃, and the column compartment was operated at 45℃ for the duration of the analysis. Solvent A was 95% acetonitrile containing 10 mmol/L ammonium acetate, and solvent B was 50% acetonitrile containing 10 mmol/L ammonium acetate. The mobile phase gradient was 0.1%–20.0% B for 2 min and then 20%–80% B for 3 min, followed by 3 min re-equilibration, with a flow rate of 0.6 mL/min. An injection volume of 1 μL was used. System blanks (95 μL distilled water, 5 μL lipid internal standard mixture, and 500 μL IPA) and process blanks (IPA) were initially injected three times each to check the instrument conditions. Quality control samples were injected three times before analysis of the biological samples, and this process was repeated for every 12 samples.

Enzymatic activity

The serum activities of citrate synthase (CS), succinate dehydrogenase (SDH), phosphofructokinase (PFK), and lactate dehydrogenase (LDH) before and after MICT were determined by enzyme-linked immunosorbent assay (ELISA) using the Synergy H1 Microplate Reader (Biotek, VT, USA) and the kits supplied by Jianglai Biological, following the manufacturers' instructions.

Statistical analyses

Statistical analyses of anthropometric characteristics, exercise indices, and enzymatic activities were performed using the paired *t*-test with IBM Statistical Package for the Social Sciences (SPSS) v20.0 (IBM Corp. Armonk, USA).

An analysis of the lipidomic data was performed using TargetLynx in MassLynx v4.1. Each peak was automatically integrated, but manually confirmed and adjusted if required. The retention time for the internal standards corresponding to each class of lipids was used to confirm the correct integration of peaks corresponding to lipids of the same class. The Wu Kong platform[29] (https://www.omicsolution.com/wkomics/main/) was used for lipidomic data analysis and visualization, along with analysis of variance, orthogonal partial least-squares discriminant analysis (OPLS-DA), heatmaps, enrichment analysis, and correlation analysis. The package Mfuzz in R 3.0.3 (The University of Auckland, Auckland, New Zealand), which is a soft clustering using a fuzzy C-means algorithm, was used to analyze the changes in lipid species concentrations over time. The number of clusters was set to four, and the fuzzification parameter m was set to 1.35. Correlation plots and a correlation network of lipid metabolites were constructed to analyze the interactions among lipid species during MICT using the Wu Kong platform and Cytoscape 3.1.1 (National Resource for Network Biology [NRNB], https://nrnb.org/),[30] respectively.

RESULTS

Basic characteristics of the participants

We studied 15 healthy male adolescents who were 12–14

years old (13.33 ± 0.81 years) and had a mean height of 164.94 ± 6.16 cm (Table 1). The weight of adolescents was increased after 6 weeks of MICT (Table 1). The VO_2·max was significantly increased by MICT ($P < 0.05$, Table 1), which indicated that MICT improved cardiopulmonary function. With regard to serum enzyme activities, CS, SDH, and PFK were significantly increased by 6 weeks of MICT (all $P < 0.05$, Table 1), but that of LDH was not affected (Table 1).

MICT significantly affects the plasma lipid profile

Fifty-eight samples were analyzed because one T2 sample and one T3 sample were insufficient to detect. We were able to successfully quantify 146 lipids, including phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylglycerol (PG), phosphatidylinositol (PI), phosphatidic acid (PA), lysophosphatidic acid (LPA), lysophosphatidylcholine (LPC), lysophosphatidylethanolamine (LPE), lysophosphatidylglycerol (LPG), and lysophosphatidylinositol (LPI). We also quantified two classes of ether-linked alkylphosphatidylcholine (PC-O and PC-P), diglycerides (DG), triglycerides (TAG), FA, and sphingolipids, including ceramide (Cer), ceramide-1-phosphate (Cer1P), hexosylceramide (HexCer), and sphingomyelin (SM). OPLS-DA was used to classify the samples according to the time points of MICT. Samples collected at different times were clearly separated, and in particular, T2 and T3 samples were significantly separated from the T0 samples (Figure 1A).

Analysis of variance/Kruskal–Wallis test was performed to identify the lipid metabolites that were present at different concentrations at the four time points, using a false discovery rate (FDR)-adjusted $P < 0.05$. The concentrations of 64 of the 146 lipid metabolites significantly changed during MICT. An FDR-adjusted $P < 0.05$ and a variable importance in projection > 1 in OPLS-DA were used to identify lipids with significant differences in concentrations during MICT. This method showed that the concentrations of 52 lipid metabolites significantly changed during MICT (Table S1). A heatmap of the differential lipid metabolites is shown in Figure 1B (FDR-adjusted $P < 0.05$ and variable importance in projection > 1). Hierarchical clustering trees revealed that lipid species in the same class showed similar changes. Taken together, these results indicated that specific changes in the lipid profile occurred during MICT, and 52 differential lipids were identified.

Lipid metabolism and synthesis pathways that are affected by MICT

We next performed an analysis using a specified Human Metabolome Database and Metabolomics Workbench, and showed the lipid subclasses identified in a pie chart (Figure 2A). After exercise, 22 glycerophosphocholines (PC-O, PC-P, and LPC), four TAGs, seven FAs and their conjugates, four PIs, five linoleic acids and their derivatives (DG[16:0/18:2], DG[18:1/18:2], DG[18:2/18:3], DG[18:2/18:2], and FA[18:3]), two LPAs, four LPEs, one SM, one DG (DG[14:1/18:1]), and two HexCers (HexCer[d18:1/22:0] and HexCer[d18:1/22:1]) were significantly affected.

Filtering for these lipid metabolites with a specified Human Metabolome Database identifier produced a set of 43 lipid metabolites. The lipids were then used to perform enrichment analysis. The top six enriched pathways were related to the metabolism of lipids and lipoproteins, metabolism, G-protein-coupled receptor signaling, signal transduction, glycerophospholipid metabolism, and phospholipid metabolism (Figure 2B). All of these are associated with lipid synthesis or metabolism and have been reported to be related to metabolic diseases, cardiovascular diseases, liver diseases, and cancer.[31–36]

Longitudinal changes in lipid species in response to MICT

To determine the effects of MICT on lipid species at the various time points, a soft clustering analysis was performed using the mean concentrations of lipid species from the 15 volunteers using the fuzzy C-means algorithm. The 52 lipid species identified were placed into four clusters representing distinct patterns of change (Figure 3). The lipid species in each cluster with probabilities > 0.8 are listed in Table S2. The concentrations of the lipid species in cluster 1 were lower at T1 and T2 than at T0, but were slightly increased at T3 than at T2, still lower than at T0; this cluster contained DG, Cer, and LPC. Cluster 2 contained DG, LPC, LPE, LPA, TAG, and PI species. The concentrations of the lipid species in this cluster were higher at T1 than at T0, low to the level at T0 at T2, and higher at T3 than at T0. Cluster 3 contained PC-O, PC-P, and LPE. The concentrations of these lipid species were higher at T1 compared to T0 and remained high during the whole training period. The changes in the lipid species placed in cluster 4 were opposite to those of the species in cluster 2. The concentrations of the lipid species in cluster 4 were lower at T1 than at T0, higher at T2 than at T1 and similar to the level at T0, and then slightly lower at T3 compared to that at T0; this cluster contained FAs. Therefore, only one bout of exercise had substantial effects on lipid metabolism, but the changes were less marked immediately after 6 weeks of MICT. At the group T3, there were fewer significantly affected lipid species and the fold differences in concentration were smaller than those after one bout of exercise.

Interactions between lipid species affected by MICT

A correlation analysis was used to analyze the relationships

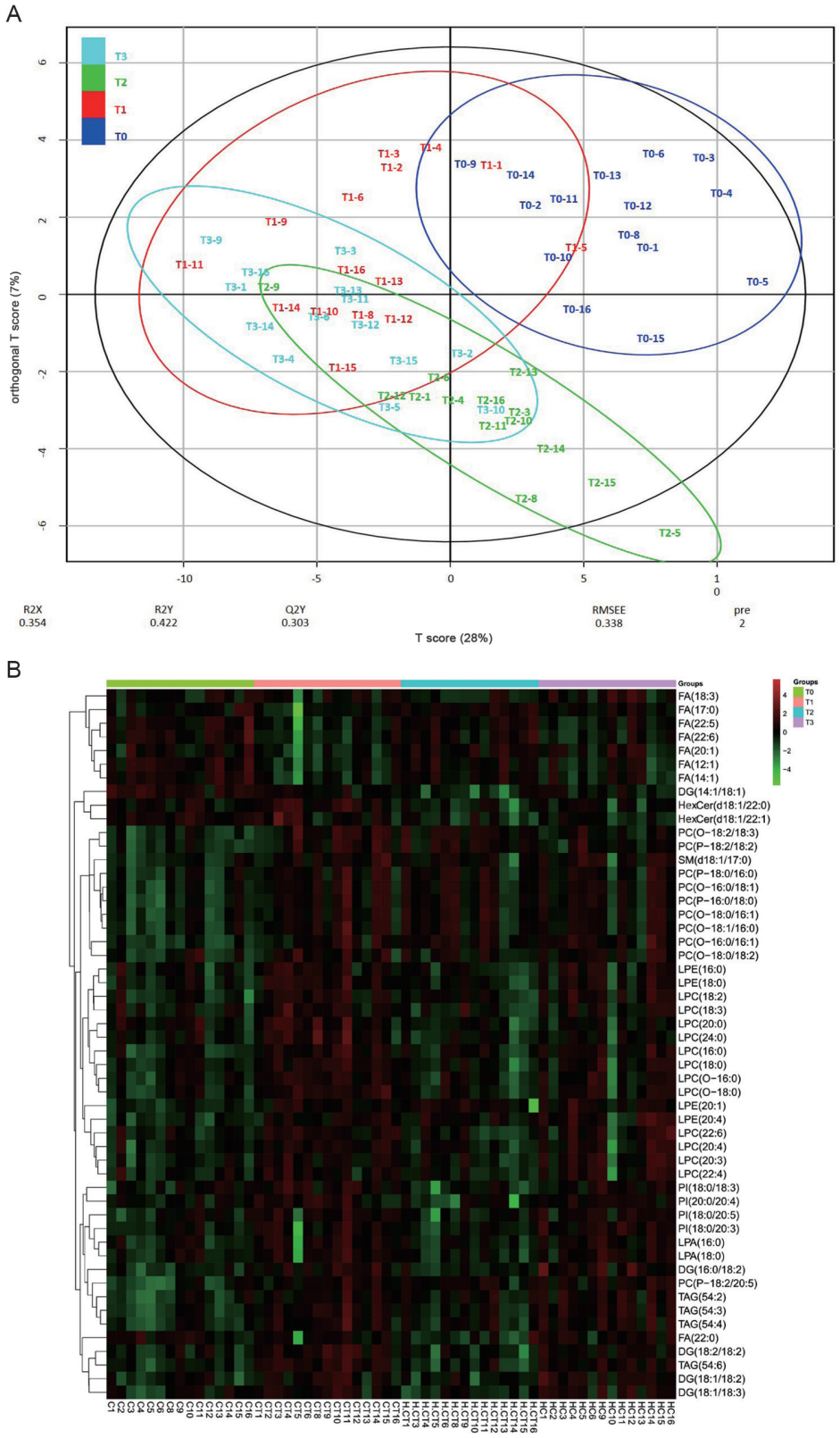

Figure 1: Human plasma lipidomics affected by exercise. (A) OPLS-DA score plot showing the lipid profile changed by MICT. (B) Heatmap of differential lipid metabolites (FDR-adjusted $P < 0.05$ and variable importance in projection > 1) significantly decreased at a 5% FDR-adjusted level and fold change lower than 0.83. Green represents lipid species that show no change. T0: time point T0; T1: time point T1; T2: time point T2; T3: time point T3. FA: free fatty acids; SM: sphingomyelin; HexCer: hexosylceramide; PC: phosphatidylcholine; LPC: lysophosphatidylcholine; LPE: lysophosphatidylethanolamine; LPA: lysophosphatidic acid; PI: phosphatidylinositol; DG: diglyceride; TAG: triglyceride; OPLS-DA: orthogonal partial least-squares discrimination analysis discriminant analysis; MICT: moderate-intensity continuous training; FDR: false discovery rate.

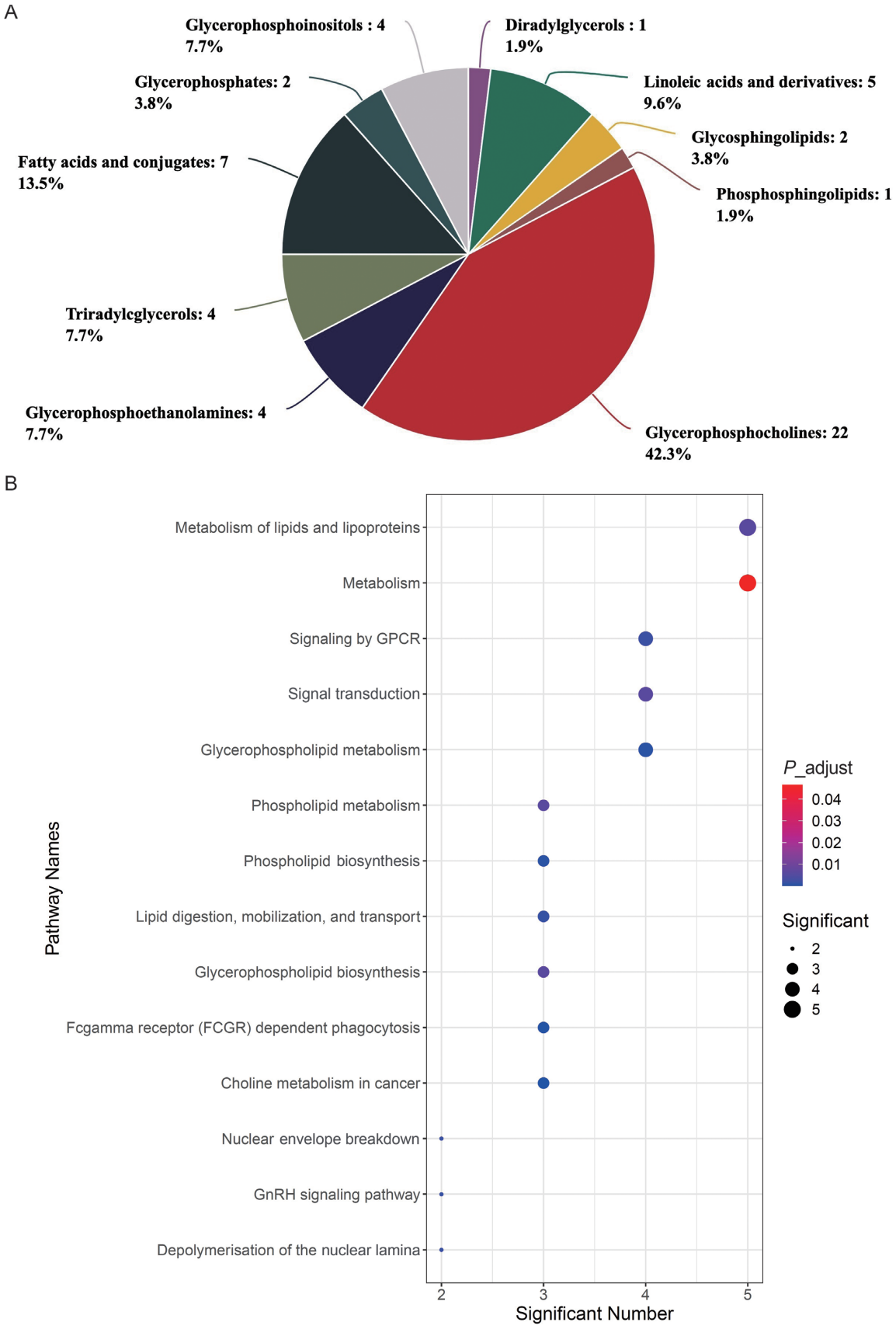

Figure 2: Subclass and pathway of lipid species affected by MICT. (A) Distribution of different lipid species. (B) Pathway analysis for different lipid metabolites. GPCR: G-protein-coupled receptor; MICT: moderate-intensity continuous training.

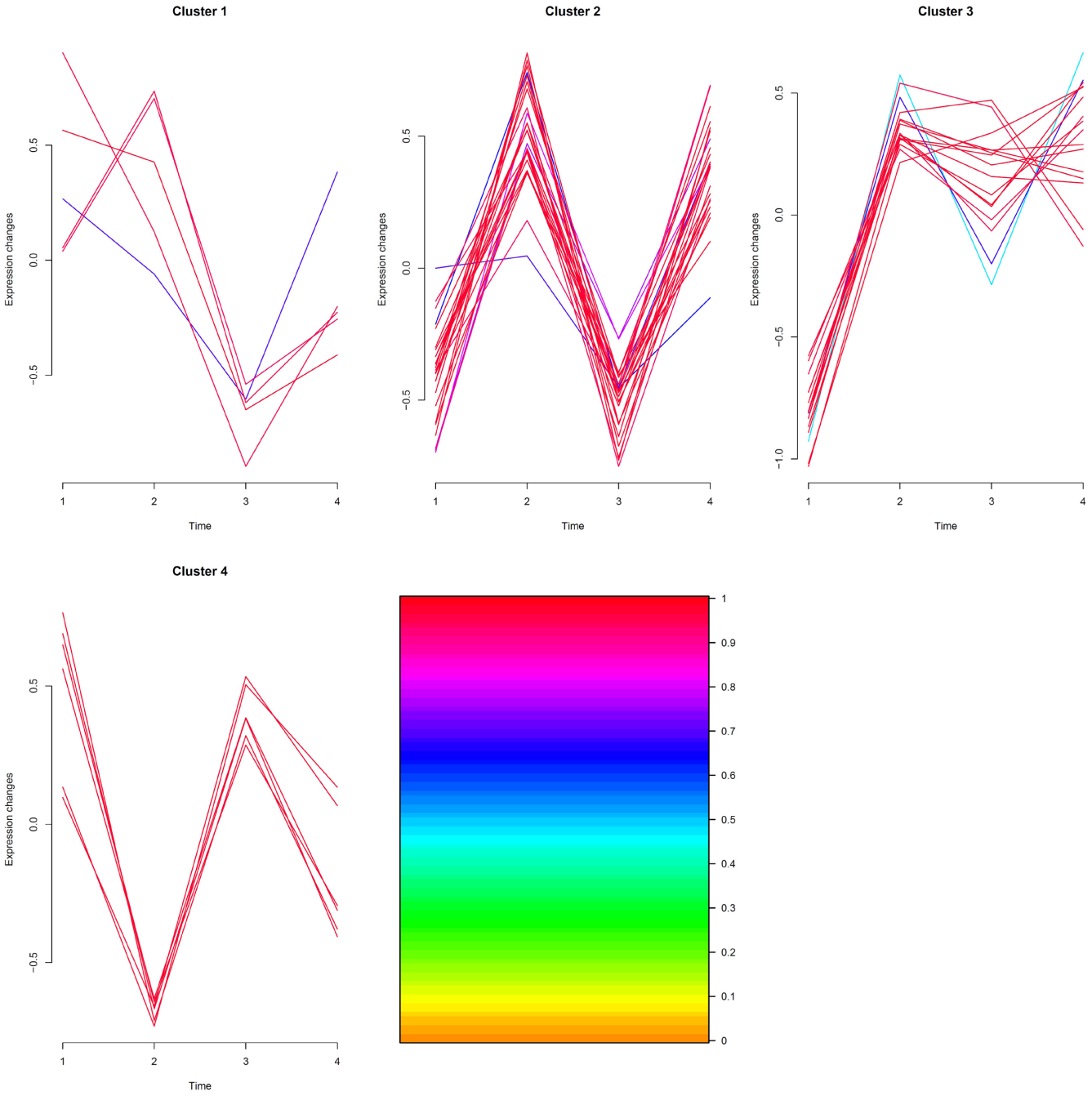

Figure 3: Temporal profiles of lipid species assigned to four clusters by fuzzy C-means clustering. The y axis is standardized; colors of the lines represent the membership level for each species.

between lipid metabolites during MICT (Figure 4). The Spearman correlation coefficients for each pair of lipid metabolites were calculated. Lipids of the same species were strongly positively correlated with the concentrations of other species representing most of the lipid classes, with the exception of FAs. Most FAs were negatively correlated with other lipid species. FA(12:1), FA(14:1), FA(20:1), FA(22:5), and FA(22:6) were negatively correlated with other lipid species, while there were no significant correlations with DG(14:1/18:1), HexCer(d18:1/22:1), and FA(22:0). FA(17:0) was only negatively correlated with DGs, LPCs, some LPEs, and LPAs. FA(18:3) was negatively correlated with some LPC species, LPEs, PC-O, and PC-P. However, FA(22:0) was positively correlated with other lipid species.

MICT affects the lipid metabolism associated with SDH and PFK

We measured the activities of CS, SDH, PFK, and LDH (Table 1) and found that CS, SDH, and PFK activities were

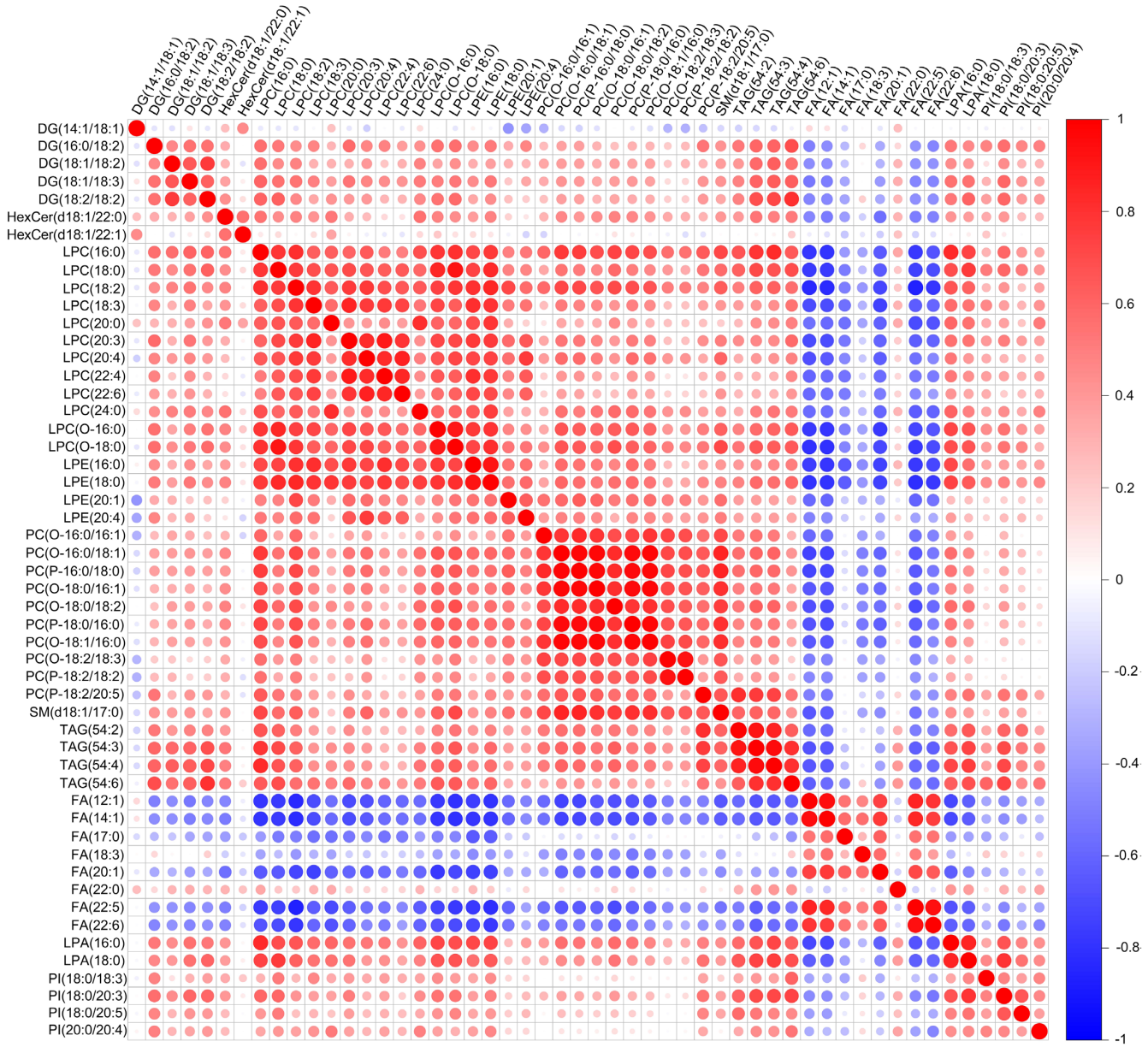

Figure 4: Correlation of lipid species with significantly changed after exercise. Lipid pairs with correlation coefficients > 0.5 or < −0.5 and an FDR-adjusted *P* < 0.05 were considered to be closely correlated. Red and blue represent positive and negative correlations, respectively. The depth of each color and the size of circle represent the closeness of the correlation, with a darker color and larger circle indicating a stronger correlation. FA: free fatty acids; SM: sphingomyelin; HexCer: hexosylceramide; PC: phosphatidylcholine; LPC: lysophosphatidylcholine; LPE: lysophosphatidylethanolamine; LPA: lysophosphatidic acid; PI: phosphatidylinositol; DG: diglyceride; TAG: triglyceride; FDR: false discovery rate.

significantly higher after 6 weeks of MICT compared to those before MICT (paired *t*-test, all $P < 0.05$, Table 1). A correlation analysis was performed to examine whether the changes in the concentrations of lipid species were related to the changes in metabolic enzyme activities (Figure 5). DG(18:2/18:2) and TAG(54:6) were positively correlated with CS (both $P < 0.05$), but there were no other significant correlations with CS. Most glycerolipids (DG[16:0/18/2], DG[18:1/18:2], DG[18:2/18:2], TAG[54:2], TAG[54:3], TAG[54:4], and TAG[54:6]) and some phospholipids (LPC[16:0], LPC[20:3], LPC[O-16:0], LPC[O-18:0], LPE[20:1], PC[P-18:2/20:5], PI[18:0/20:3], and PI[18:0/20:5]) were significantly positively correlated with SDH activity. However, DG(14:1/18:1) and HexCer(d18:1/22:1) were significantly negatively correlated with SDH activity. The correlations between lipid species and PFK activity were similar to those with SDH activity. Exceptions were for those of DG(18:1/18:3) and LPC(18:0), which were significantly positively correlated with PFK activity, and for those of FA(12:1) and

Table 1: Characteristics of volunteers

Characteristic	Before 6-week MICT (group T0)	After 6-week MICT (group T3)	*t*	*P*-value
Age (years)	13.33 ± 0.81	—	—	—
Height (cm)	164.94 ± 6.16	—	—	—
Weight (kg)	61.62 ± 18.13	64.62 ± 17.42	−7.117	< 0.001
BMI (kg/m^2)	22.38 ± 5.18	23.19 ± 4.82	−4.861	< 0.001
Muscle (kg)	26.69 ± 5.63	26.83 ± 5.14	−0.535	0.602
Glu (mmol/L)	5.05 ± 0.57	5.02 ± 0.32	0.206	0.840
CS (mL/kg/min)	40.74 ± 28.57	60.64 ± 23.08	−3.030	0.010
SDH (beat/min)	8.97 ± 6.25	18.59 ± 4.66	−6.917	< 0.001
PFK (m)	26.96 ± 24.80	72.94 ± 11.99	−7.991	< 0.001
LDH (mmol/L)	3.21 ± 1.75	3.79 ± 0.84	−1.356	0.198
VO_2·max (mL/kg/min)	41.07 ± 6.40	46.3 ± 7.98	−6.025	< 0.001

Data are shown as mean ± standard deviation. BMI: body Mass Index; Glu: glucose; CS: citrate synthase; SDH: succinic dehydrogenas; PFK: phosphofructokinase; LDH: lactate dehydrogenase; MICT: moderate-intensity continuous training.

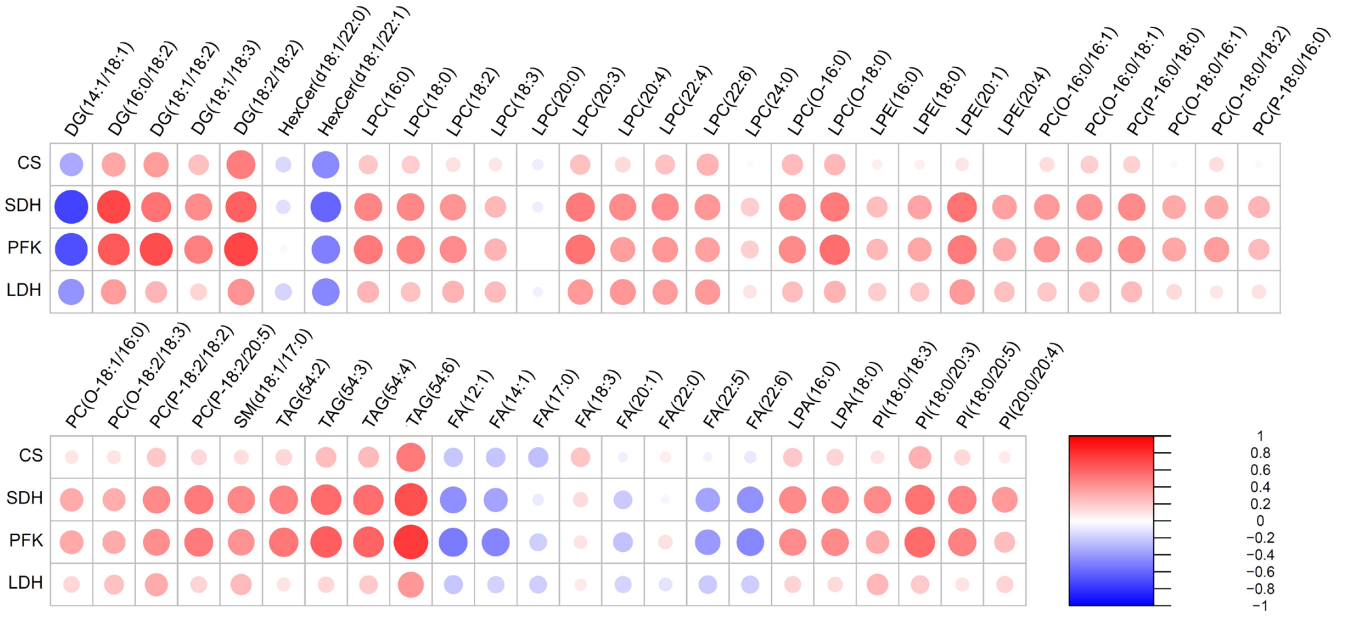

Figure 5: Correlation of different lipid species and metabolic enzymes. A correlation coefficient > 0.5 or < −0.5 and an FDR-adjusted *P* < 0.05 indicate a significant positive correlation (red circles) or a significant negative correlation (blue circles), and as the circle increases and the color grows deep, the correlation coefficient increases. FA: free fatty acids; SM: sphingomyelin; HexCer: hexosylceramide; Cer: ceramide; PC: phosphatidylcholine; LPC: lysophosphatidylcholine; LPE: lysophosphatidylethanolamine; LPA: lysophosphatidic acid; PI: phosphatidylinositol; DG: diglyceride; TAG: triglyceride; FDR: false discovery rate; CS: citrate synthase; SDH: succinate dehydrogenase; PFK: phosphofructokinase; LDH: lactate dehydrogenase.

FA(14:1), which were significantly negatively correlated with PFK activity. However, none of the lipid species were significantly correlated with LDH activity. These results suggest that SDH and PFK are involved in the same metabolic pathway and have similar effects on lipid metabolism.

DISCUSSION

Our study demonstrates that MICT plays an important role in male adolescents' aerobic capacity development. After 6 weeks of MICT, VO_2·max and related enzymes (CS and SDH) were significantly increased. This means the MICT significantly improves aerobic capacity and is an effective exercise mode for adolescents. Table 1 shows that glucose was not significantly changed in healthy adolescents, but serum lipid profile significantly changed with exercise. This means that lipid metabolites are more susceptible to external stimuli. To the best of our knowledge, little is known regarding how MICT is related to modulation of the lipidome and physiological adaptions in adolescents. Therefore, we performed this study to investigate the effects of MICT on the lipidome of adolescents. The major finding of our study was that the lipid profile showed different trends during different periods of MICT intervention. Compared to the lipid

levels at T0, phospholipid and glyceride concentrations were increased, while the content of FAs was decreased at T1, which means a bout of exercise could significantly disturb the lipid metabolism. At time point T3, the trend in lipid species was similar to that at T1. Comparing the lipid contents at T2 and T1, the levels of PC-O, PC-P, and TAG species were significantly decreased, while DG, LPC, LPE, and PI species remained at higher levels and the levels of FAs were increased.

Endurance exercise can effectively improve participants' aerobic capacity and the activity of the main energy supply system. Correlates with an increased VO_2·max, the activities of CS and SDH, the two key enzymes of aerobic metabolism, were significantly increased after 6 weeks of MICT (Table 1). These results show that MICT induced physiological adaptations characteristic of an increased aerobic energy metabolism in adolescents.

Physiological response and adaptation of energy metabolism to MICT also contained lipids metabolism in this study. Compared to the baseline, the acute metabolic response of lipids to a single 30-min bout of cycle ergometer training showed that six plasma FAs (FA[12:1], FA[14:1], FA[17:0], FA[20:1], FA[22:5], FA[22:6]) were significantly decreased, while glycerides and phospholipids were increased (at T1 vs. at T0). During exercise, fats and carbohydrates are oxidized simultaneously, but their relative contribution is associated with exercise duration and intensity. Glucose utilization is greater during high-intensity exercise, while FAs' oxidation increases during moderate-intensity exercise.[37] Therefore, in this study, FAs' β-oxidation may be the important energy supply mode during one bout of moderate-intensity (65% VO_2·max) exercise. With sufficient oxygen supply, FAs are completely oxidized to CO_2 and H_2O through β-oxidation and release a large amount of energy in the form of adenosine triphosphate (ATP) at the same time. Moreover, FAs are located in the center of lipid metabolism and can interconvert with other lipids with acyl chains (Figure 6). FAs are activated by fatty acyl CoA synthetases to produce long-chain acyl coenzyme A, which is esterified to glyceraldehyde 3-phosphate. LPA species are generated in this reaction and then acylated to form PA species. PA can be converted into cytidine diphosphate diacylglycerol (CDP-DG), which is a substrate for the synthesis of certain glycerophospholipids and cardiolipins, or can be dephosphorylated by phosphatidate phosphohydrolase (PAP) to form DG, which serve as precursor molecules for the synthesis of TAG, as well as PC, PE, PG, and PI (Figure 6). Previous reports showed a single bout of exercise induced the storage of FAs as neutral TAG[38] and stimulated the expression of multiple genes related to TAG synthesis and LD assembly and mobilization in skeletal muscle.[39] *LPIN1* gene is upregulated immediately post-exercise,[8] which is the most important member of the lipin family of proteins known to regulate triglyceride and phospholipid metabolism.[40] It can be seen that the TAG and phospholipids' biosynthesis pathways are activated after one bout of exercise. So, the content of FAs decreased, but those of TAG and phospholipids increased at T1.

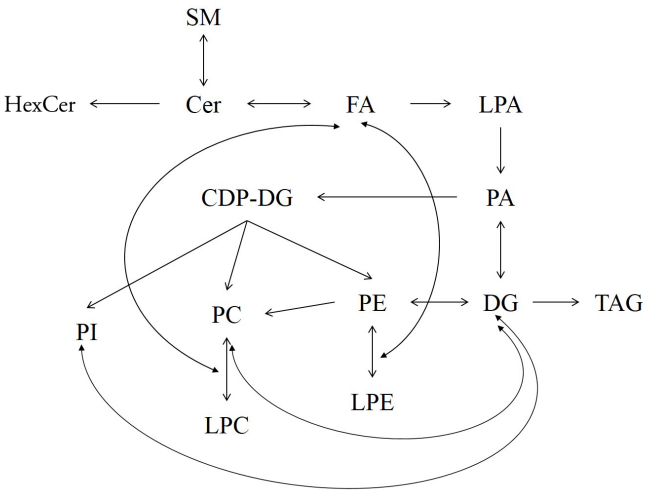

Figure 6: Schematic illustration of lipids' conversion. SM: sphingomyelin; Cer: ceramide; FA: free fatty acids; PA: phosphatidic acid; LPA: lysophosphatidic acid; CDP-DG: cytidine diphosphate diacylglycerol; PC: phosphatidylcholine; LPC: lysophosphatidylcholine; PE: phosphatidylethanolamine; LPE: lysophosphatidylethanolamine; PI: phosphatidylinositol; DG: diglyceride; TAG: triglyceride; HexCer: hexosylceramide.

After 6 weeks of MICT, the body attains physiological adaptation, and the complex metabolic characteristics in the body reach a new stable state and a new metabolic homeostasis is formed. The homeostasis of FAs is achieved by lipolysis in the adipose tissue, intestine, and liver. To delineate FAs' turnover and metabolism in response to chronic training, we assessed the lipid metabolism at time point T2. Compared to T1, the levels of eight FAs increased, DG, LPC, LPE, PI, and TAG (54:6) decreased, while the contents of PC-O, PC-P and TAG (54:2), TAG (54:3), and TAG (54:4) did not change. Given that TAGs, medium- and long-chain FAs were the main substrates for energy, these results indicate that a dependence on oxidation of plasma FAs as a source of energy was decreased during exercise after 6 weeks of MICT. Previous studies have reported that exercise training induced extra FAs' utilization, possibly coming from intramuscular TAG stores instead of plasma stores during exercise.[41] This greater use of fat is related to mitochondrial quantitative and qualitative adaptations, which increase skeletal muscle mitochondrial volume density and intrinsic mitochondrial FA oxidation associated with increment of VO_2·max. For pediatric studies which must also comply with additional ethical considerations, we could not provide the FAs oxidation of muscle fibers in this study. In addition, the above results also showed that PC-O and

PC-P were stable during moderate-intensity exercise. PC-O and PC-P are important constituents in the construction of biological membrane, which affect membrane fluidity and membrane fusion.[42] This observation may indicate that moderate-intensity stress does not affect the stability of biological membranes.

To study the biological events that occurred during the later stages of MICT, blood samples were collected 48 h after 6 weeks of training. As shown in Figure 3, the levels of four FAs (FA[12:1], FA[14:1], FA[22:5], FA[22:6]) were decreased and the levels of TAGs and phospholipids (LPE, PC-O, PC-P, and PI) were increased at T3 compared to T0. TAGs are an efficient and inert form of FAs for storage and transport. They are unable to traverse cell membranes. Consequently, the transport of TAGs into or out of cells requires either their hydrolytic breakdown into FAs and glycerol or their specialized vesicle-mediated transport across cell membranes. Previous studies have reported that endurance training induced a higher maximal fat oxidation rate due to expressing high levels of adipose triglyceride lipase and hormone-sensitive lipase in muscle fibers.[43] In our study, the changes in FAs mean that the lipolysis and the release of FAs into plasma were decreased at rest time due to 6 weeks of MICT. It is well accepted that a reduced FA mobilization from adipose stores leads to significantly increased glucose tolerance and improved insulin sensitivity.[44–47] At the same time, circulating plasma FAs were utilized by muscles to replenish their fat stores. Therefore, these results imply that chronic aerobic training can decrease FAs' flux from TAGs, which presents a beneficial metabolic phenotype in adolescents. Wang *et al.*[48] reported that augmented levels of PC biosynthesis are related to an increase in the number of circulating high-density lipoproteins (HDLs) in glucokinase maturity-onset diabetes in the young. Given the HDLs' positive contribution to maintaining the health of vascular endothelial cells, the increased PCs we saw in plasma after 6 weeks of MICT likely induced improved fitness through the lipid metabolic adaptation of HDLs in adolescents. LPE is a minor component of the cell membrane[49] and has been recently reported to be involved in anti-inflammatory effects on macrophages' polarization induced by lipopolysaccharide.[50] In our results, increased level of LPE in the plasma may be reporting anti-inflammatory effects of MICT. These findings have important implications for the role of MICT in maintaining overall health and improving physical fitness.

CONCLUSION

MICT at 65% VO_2·max increases PC-O, PC-P, and TAG species. DG, lysophospholipids, and PI species initially rise and then decrease after 6 weeks of MICT, while FA species show an opposite trend. This study suggests that these changes in FAs, phospholipids, and glycerides are caused by the adaptation of energy metabolism to MICT. This is the first study to use targeted lipidomics to examine changes in lipid profiles in adolescents undergoing MICT. Our findings may have important implications for adolescents' future development, reasonable energy metabolism, and biosynthesis.

Supplementary Materials

Supplementary materials mentioned in this article are online available at the journal's official site only.

Availability of Data and Materials

The data of this article would be shared on making reasonable request to the corresponding author.

Author Contributions

Zhang H, Liu J, Cui M, Zhang T, Mi J, and Zhao L designed the study. Liu J, Chen L, and Zhao L were responsible for moderate-intensity continuous training, sample collection, anthropometric data collection, exercise index measurement, and enzymatic activity analysis. Zhang H and Chai H performed lipidomics data collection and data analysis. Zhang H, Guan H, and Cui M wrote and revised the manuscript. All the authors approved the final version of the manuscript for publication. The manuscript has been read and approved by all the authors, and each author believes that the manuscript represents honest work.

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Ethics Approval and Consent to Participate

The experimental procedures and potential risks were fully explained to the participants and their guardians before the study, and all subjects provided written informed consent. The experimental protocol was approved by the Scientific Ethics Committee of Beijing Sport University (Approval No. 2020139H) and was carried out in accordance with the Declaration of Helsinki.

Conflict of Interest

The authors declare no conflict of interest.

REFERENCES

1. Booth FW, Roberts CK, Laye MJ. Lack of exercise is a major cause of chronic diseases. Compr Physiol 2012;2:1143–211.
2. Lavie CJ, Ozemek C, Carbone S, Katzmarzyk PT, Blair SN. Sedentary behavior, exercise, and cardiovascular health. Circ Res 2019;124:799–815.
3. Sattelmair JR, Pertman JH, Forman DE. Effects of physical activity on cardiovascular and noncardiovascular outcomes in older adults. Clin Geriatr Med 2009;25:677–702.
4. Green JS, Crouse SF. Endurance training, cardiovascular function and the aged. Sports Med 1993;16:331–41.
5. Gao J, Lu Y, Gokulnath P, Vulugundam G, Li G, Li J, *et al*. Benefits of physical activity on cardiometabolic diseases in obese children and adolescents. J Transl Intern Med 2022;10:236–45.
6. Li C, Jiang W, Xu Y. Omics and bioinformatics: time for new data analysis approaches? OMICS 2017;21:749.
7. Messier F, Laurence LM, Carole S, Anne-Fleur G, Mohamed T, Emma R, *et al*. The impact of moderate altitude on exercise metabolism in recreational sportsmen: a nuclear magnetic resonance metabolomic approach. Appl Physiol Nutr Me 2017;42:1135–41.
8. Mukherjee K, Edgett BA, Burrows HW, Castro C, Griffin JL, Schwertani AG, *et al*. Whole blood transcriptomics and urinary metabolomics to define adaptive biochemical pathways of high-intensity exercise in 50-60 year old masters athletes. PLoS One 2014;9:e92031.
9. Manaf FA, Nathan L, Peiffer JJ, Maker GL, Boyce MC, Fairchild TJ, *et al*. Characterizing the plasma metabolome during and following a maximal exercise cycling test. J Appl Phys 2018;125:1193–203.
10. Kuehnbaum NL, Gillen JB, Kormendi A, Lam KP, DiBattista A, Gibala MJ, *et al*. Multiplexed separations for biomarker discovery in metabolomics: elucidating adaptive responses to exercise training. Electrophoresis 2015;36:2226–36.
11. Nayor M, Shah RV, Miller PE, Blodgett JB, Tanguay M, Pico AR, *et al*. Metabolic architecture of acute exercise response in middle-aged adults in the community. Circulation 2020;142:1905–24.
12. Shi R, Zhang J, Fang B, Tian X, Wu J. Runners' metabolomic changes following marathon. Nutr Metab 2020;17:19.
13. Postle AD. Phospholipid lipidomics in health and disease. Eur J Lipid Sci Tech 2009;111:2–13.
14. Liu L, Huwatibieke B, Lu X, Li D, Dong H, Han J, *et al*. TCDD-inducible poly (ADP-ribose) polymerase promotes adipogenesis of both brown and white preadipocytes. J Transl Intern Med 2022;10:246–54.
15. Morville T, Sahl RE, Moritz T, Helge JW, Clemmensen C. Plasma metabolome profiling of resistance exercise and endurance exercise in humans. Cell Rep 2020;33:108554.
16. Contrepois K, Wu S, Moneghetti KJ, Hornburg D, Ahadi S, Tsai MS, *et al*. Molecular choreography of acute exercise. Cell 2020;181:1112–30 e1116.
17. Nemkov T, Skinner SC, Nader E, Stefanoni D, D'Alessandro A. Acute cycling exercise induces changes in red blood cell deformability and membrane lipid remodeling. Int J Mol Sci 2021;22:896.
18. Karl JP, Margolis LM, Murphy NE, Carrigan CT, Castellani JW, Madslien EH, *et al*. Military training elicits marked increases in plasma metabolomic signatures of energy metabolism, lipolysis, fatty acid oxidation, and ketogenesis. Physiol Rep 2017;5:e13407.
19. Varga TV, Ali A, Herrera JAR, Ahonen LL, Mattila IM, Al-Sari NH, *et al*. Lipidomic profiles, lipid trajectories and clinical biomarkers in female elite endurance athletes. Sci Rep 2020;10:2349.
20. Wang Y, Shen L, Xu D. Aerobic exercise reduces triglycerides by targeting apolipoprotein C3 in patients with coronary heart disease. Clin Cardiol 2019;42:56–61.
21. Jastrzebski D, Toczylowska B, Zieminska E, Zebrowska A, Kostorz-Nosal S, Swietochowska E, *et al*. The effects of exercise training on lipid profile in patients with sarcoidosis. Sci Rep 2021;11:5551.
22. Nieman DC, Sha W, Pappan KL. IL-6 linkage to exercise-induced shifts in lipid-related metabolites: a metabolomics-based analysis. J Proteome Res 2017;16:970–7.
23. Gollasch B, Wu G, Dogan I, Rothe M, Gollasch M, Luft FC. Maximal exercise and erythrocyte epoxy fatty acids: a lipidomics study. Physiol Rep 2019;7:e14275.
24. Goh EXY, Guan XL. Targeted lipidomics of drosophila melanogaster during development. In mass spectrometry-based lipidomics: methods and protocols. Edited by Hsu F-F. New York, NY: Springer US 2021:187–213.
25. Tarnopolsky MA. Gender differences in substrate metabolism during endurance exercise. Can J Appl Physiol 2000;25:312–27.
26. Hirsch KR, Smith-Ryan AE, Blue MNM, Mock MG, Trexler ET. Influence of segmental body composition and adiposity hormones on resting metabolic rate and substrate utilization in overweight and obese adults. J Endocrinol Invest 2017;40:635–43.
27. Boisseau N, Isacco L. Substrate metabolism during exercise: sexual dimorphism and women's specificities. Eur J Sport Sci 2022;22:672–83.
28. Sarafian MH, Gaudin M, Lewis MR, Martin FP, Holmes E, Nicholson JK, *et al*. Objective set of criteria for optimization of sample preparation procedures for ultra-high throughput untargeted blood plasma lipid profiling by ultra performance liquid chromatography-mass spectrometry. Anal Chem 2014;86:5766–74.
29. Wang S, Li W, Hu L, Cheng J, Yang H, Liu Y. NAguideR: performing and prioritizing missing value imputations for consistent bottom-up proteomic analyses. Nucleic Acids Res 2020;48:e83.
30. Yan M, Cai WB, Hua T, Cheng Q, Ai D, Jiang HF, *et al*. Lipidomics reveals the dynamics of lipid profile altered by omega-3 polyunsaturated fatty acid supplementation in healthy people. Clin Exp Pharmacol Physiol 2020;47:1134–44.
31. Sonkar K, Ayyappan V, Tressler CM, Adelaja O, Cai R, Cheng M, *et al*. Focus on the glycerophosphocholine pathway in choline phospholipid metabolism of cancer. NMR Biomed 2019;32:e4112.
32. Wang J, Ma J, Nie H, Zhang XJ, Zhang P, She ZG, *et al*. Hepatic regulator of G protein signaling 5 ameliorates nonalcoholic fatty liver disease by suppressing transforming growth factor beta-activated kinase 1-c-Jun-N-terminal kinase/p38 signaling. Hepatology 2021;73:104–25.
33. Yuan Z, Yang L, Zhang X, Ji P, Hua Y, Wei Y. Mechanism of Huang-lian-Jie-du decoction and its effective fraction in alleviating acute ulcerative colitis in mice: regulating arachidonic acid metabolism and glycerophospholipid metabolism. J Ethnopharmacol 2020;259:112872.
34. Zeng C, Wen B, Hou G, Lei L, Mei Z, Jia X, *et al*. Lipidomics profiling reveals the role of glycerophospholipid metabolism in psoriasis. Gigascience 2017;6:1–11.
35. Zeman M, Macasek J, Burda M, Tvrzicka E, Vecka M, Krechler T, *et al*. Chronic pancreatitis and the composition of plasma phosphatidylcholine fatty acids. Prostaglandins Leukot Essent Fatty Acids 2016;108:38-44.
36. Wang B, Tontonoz P. Phospholipid remodeling in physiology and disease. Annu Rev Physiol 2019;81:165–88.

37. Chycki J, Zajac A, Michalczyk M, Maszczyk A, Langfort J. Hormonal and metabolic substrate status in response to exercise in men of different phenotype. Endocr Connect 2019;8:814–21.
38. Schenk S, Horowitz JF. Acute exercise increases triglyceride synthesis in skeletal muscle and prevents fatty acid-induced insulin resistance. J Clin Invest 2007;117:1690–8.
39. Koves TR, Sparks LM, Kovalik JP, Mosedale M, Arumugam R, DeBalsi KL, *et al*. PPARgamma coactivator-1alpha contributes to exercise-induced regulation of intramuscular lipid droplet programming in mice and humans. J Lipid Res 2013;54:522–34.
40. Csaki LS, Reue K. Lipins: multifunctional lipid metabolism proteins. Annu Rev Nutr 2010;30:257–72.
41. Romijn JA, Coyle EF, Sidossis LS, Gastaldelli A, Horowitz JF, Endert E, *et al*. Regulation of endogenous fat and carbohydrate metabolism in relation to exercise intensity and duration. Am J Physiol Endocrinol Metab 1993;265:E380–E391.
42. Dean JM, Lodhi IJ. Structural and functional roles of ether lipids. Protein Cell 2017;9:196–206.
43. Shaw CS, Swinton C, Morales-Scholz MG, McRae N, Erftemeyer T, Aldous A, *et al*. Impact of exercise training status on the fiber type-specific abundance of proteins regulating intramuscular lipid metabolism. J Appl Physiol 2020;128:379–89.
44. Haemmerle G, Lass A, Zimmermann R, Gorkiewicz G, Meyer C, Rozman J, *et al*. Defective lipolysis and altered energy metabolism in mice lacking adipose triglyceride lipase. Science 2006;312:734–7.
45. Hoy AJ, Bruce CR, Turpin SM, Morris AJ, Febbraio MA, Watt MJ. Adipose triglyceride lipase-null mice are resistant to high-fat diet–induced insulin resistance despite reduced energy expenditure and ectopic lipid accumulation. Endocrinology 2011;152:48–58.
46. Xia B, Cai GH, Yang H, Wang SP, Mitchell GA, Wu JW. Adipose tissue deficiency of hormone-sensitive lipase causes fatty liver in mice. PLoS Genet 2017;13:e1007110.
47. Yuan F, Zhang Q, Dong H, Xiang X, Zhang W, Zhang Y, *et al*. Effects of des-acyl ghrelin on insulin sensitivity and macrophage polarization in adipose tissue. J Transl Intern Med 2021;9:84–97.
48. Wang X, Lam SM, Cao M, Wang T, Wang Z, Yu M, *et al*. Localized increases in CEPT1 and ATGL elevate plasmalogen phosphatidylcholines in HDLs contributing to atheroprotective lipid profiles in hyperglycemic GCK-MODY. Redox Biol 2021;40:101855.
49. Makide K, Kitamura H, Sato Y, Okutani M, Aoki J. Emerging lysophospholipid mediators, lysophosphatidylserine, lysophosphatidylthreonine, lysophosphatidylethanolamine and lysophosphatidylglycerol. Prostaglandins Other Lipid Mediat 2009;89:135–9.
50. Park SJ, Im DS. 2-arachidonyl-lysophosphatidylethanolamine induces anti-inflammatory effects on macrophages and in carrageenan-induced paw edema. Int J Mol Sci 2021;22:4865.

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