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ASSOCIATION BETWEEN ACUTE METABOLIC RESPONSE AND CARDIORESPIRATORY FITNESS RESPONSIVENESS TO DIFFERENT AEROBIC TRAINING PROGRAMS

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Introduction

The cardiorespiratory fitness (CRF) reflects the ability of the cardiorespiratory system to supply the nutrient and oxygen demands of skeletal muscles during exercise [1]. The CRF can be measured directly by the maximal oxygen uptake ($\dot{V}O_2max$) or indirectly by the maximum power output (MPO) through with gradual increase in exercise intensity until exhaustion [2].

High levels of CRF have been associated with improved performance in endurance activities [2,3], reduced risk of developing cardiovascular disease [4], and all-cause mortality rates [5].

Endurance training (ET) or high-intensity interval training (HIIT) programs have been widely recommended for improving CRF [6,7,8].

However, the responsiveness of CRF to regular aerobic training is heterogeneous, even when phenotypically similar individuals are submitted to relative loads of physiologically equivalent exercises [9,10]. Typically, the CRF gains after regular standardized aerobic training programs is expected to range from 0 to 100% for VO_2max [11,12,13] or to 60% for MPO in sedentary adults [14,15].

Additionally, recent studies show that pre-training baseline metabolic levels are related to CRF responsiveness differently between ET and HIIT regimens [16,17].

It is well established that resting metabolic levels can be altered in response to an acute exercise session, depending on its duration and intensity of effort, and the extent of these metabolic responses is associated with the mechanisms that trigger physiological and cellular processes that lead to chronic adaptations with training [2].

Thus, in order to elucidate biomarkers and metabolic pathways associated with complex phenotypes, metabolomics is an exploratory possibility, which consists of the identification and quantification of numerous low molecular weight molecules, called metabolites, already reported in previous studies [18, 19].

Unlike other techniques belonging to the field of “omics” science, the metabolites observed by metabolomics can be more easily correlated to the individual's phenotype as they represent the final product of the interaction of a complex biomolecular chain and the cellular environment [18,19].

To investigating the association of acute metabolic responses with CRF responsiveness through metabolomics in chronic training has the potential to identify new biomarkers and advance Personalized Exercise Medicine.

Therefore, this study aimed to investigate whether serum metabolomic responses after an acute bout of exercise, at the beginning of

training, are associated with CRF responsiveness to ET and HIIT programs.

Methods

The study included 70 sedentary healthy men, not engaged in a regular physical exercise program involving more than 30 min of physical exercise per week, at moderate or vigorous intensity within the last 4 months. All subjects underwent a medical evaluation including a resting electrocardiogram and a detailed anamnesis to assess their health conditions.

Individuals were excluded from the study if they were: smokers, hypertensive patients (blood pressure > 140/90 mmHg), excessive obese [body mass index (BMI) > 33 $kg.m^{-2}$)], diabetics (fasting glucose > 7.0 mmol. L^{-1}), dyslipidemic (based on the use of medication); or presented heart disease, important metabolic disorders, and respiratory or musculoskeletal conditions limiting the practice of physical exercise. Additionally, individuals who attended training sessions less than 90% also were excluded.

All participants were informed of the risks and procedures related to the study, and signed an informed consent form approved by the Research Ethics Committee of the University of Campinas (Number: 2717688; CAAE: 52997216.8.0000.5404; April 2016).

After screening the participants, initially, an assessment of body composition was performed, followed by a cardiorespiratory assessment and re-test after 48 h [20]. Subsequently, the participants were randomly allocated, based on stratification of CRF levels in tertiles, to the groups: ET, HIIT and Control. Participants underwent the first exercise session (familiarization) or control session, and after 72 h a controlled experimental session of ET, HIIT or Control (40 min of rest) was conducted, depending on the assigned group.

The training programs were performed on cycle ergometers, 40 min per session, for 8 weeks. Training intensity was customized for each individual based on heart rate reserve (HRr) calculated as the difference between resting and maximal HR values [21].

For the ET group, participants trained at 70% HRr for 40 min, three times a week for the first four weeks, and at 75% HRr for 40 min, 4 times a week for the last four weeks. For the HIIT training group, participants train at 50% HRr for 5 min, followed by 5 4 min intervals at 90% HRr (work phase) interspersed with 3 min at 50% HRr (recovery phase) , three days a week, for the first 4 weeks of training, and at 60% HRr for 5 min, followed by 5 intervals of 4 min at 90% HRr interspersed with 3 min at 60% HRr, 4 days a week for the last four training weeks and the control group was instructed not to train during the 8 weeks.

The acute session of endurance training were composed of 40 min at 70% of resting heart rate(HRr), HIIT acute sessions were composed of 5 min at 50% of HRr followed by 5 sets of 4 min at 90% HRr, interposed by active pauses of 3 min at 50% HRr, while control sessions on participants remained 40 min sitting on the cycle ergometer.

In these sessions, venous blood samples were collected pre (PreS) and immediately after the session (PostS). Participants consumed standardized meals 12 h (dinner) and 2 h (breakfast) before the venous blood collection to minimize the effects of dietary variations on the studied metabolic responses.

The intervention lasted 8 weeks. In the fourth week a new cardiorespiratory assessment was performed to adjust the intensity of the exercise training. At least 72 h after the end of the intervention, the assessments performed at the pre-training time (PreT) were performed again.

Only 61 participants completed the intervention (ET: n = 28; HIIT: n = 23; Control: n = 10). From the blood samples, serum was obtained, which was analyzed by metabolomics through Hydrogen Nuclear Magnetic Resonance (1H NMR) spectroscopy. The 1H NMR spectra were acquired at the National Biosciences Laboratory (LNBio-http://lnbio.cnpem.br/) using an Inova Agilent NMR spectrometer. The identification and quantification of metabolites was conducted using the Suite 7.6 Chenomx NMR software.

To identify the metabolites whose changes after the acute exercise session (PostS – PreS), prior to training, were associated with changes in CRF, only those metabolites supported by 3 levels of evidence from different approaches were considered. First, the metabolic changes observed after the

acute session performed PreT were correlated with the changes in CRF after training through Pearson's correlation. Afterwards, the metabolites that showed significant correlations were analyzed in a topology and enrichment analysis of pathways to access their most relevant metabolic pathways. Finally, the metabolites retained in the two previous steps were analyzed in a multiple linear regression model to identify the metabolites whose acute PreT changes are determinant for the changes in CRF after training. The significance criterion of this study was set at 1%.

Results and Discussion

No significant correlations were observed between the changes in CRF and the physical characteristics of the participants (age, body mass, percentage of body fat and BMI) for both the ET and HIIT programs, when analyzed PreT data and changes after training ($P > 0.01$ for all).

Of the 40 identified metabolites, 8 showed a significant correlation with CRF gains for the et group: 3-hydroxyisobutyrate ($r = 0.625$, $P < 0.001$), alanine ($r = 0.541$, $P < 0.001$), citrate ($r = 0.450$, $P = 0.005$), hypoxanthine ($r = 0.560$, $P < 0.001$), lactate ($r = 0.725$, $P < 0.001$), o-acetylcarnitine ($r = 0.732$, $P < 0.001$), pyruvate ($r = 0.669$, $P < 0.001$), succinate ($r = 0.676$, $P < 0.001$) (figure 1). For the HIIT group 9 metabolites demonstrated significant correlation with CRF gains: 2-hydroxyisovalerate ($r = 0.504$, $P = 0.003$), 3-hydroxyisobutyrate ($r = 0.697$, $P < 0.001$), alanine ($r = 0.613$, $P < 0.001$), hypoxanthine ($r = 0.604$, $P < 0.001$), lactate ($r = 0.799$, $P < 0.001$), o-acetylcarnitine ($r = 0.627$, $P < 0.001$), proline ($r = -0.591$, $P < 0.001$), pyruvate ($r = 0.791$, $P < 0.001$), succinate ($r = 0.777$, $P < 0.001$), (Figure 2).

From these metabolites for the ET group, 6 pathways were significantly enriched (Figure 3): citrate cycle (metabolites: citrate, pyruvate and succinate; $P < 0.001$), pyruvate metabolism (metabolites: lactate and pyruvate; $P = 0.005$), glycolysis and gluconeogenesis (metabolites: lactate and pyruvate; $P = 0.007$), dicarboxylate
and glyoxylate metabolism (metabolites: citrate and pyruvate; $P = 0.010$), degradation of valine, leucine and isoleucine (metabolite: 3-hydroxyisobutyrate; $P = 0.189$) and purine metabolism (metabolite: hypoxanthine; $P = 0.290$). For the HIIT group (Figure 4): citrate cycle (metabolites: pyruvate and succinate; $P = 0.004$), pyruvate metabolism (metabolites: lactate and pyruvate; $P = 0.005$), glycolysis and gluconeogenesis (metabolites: lactate and pyruvate; $P = 0.007$), arginine and proline metabolism (metabolites: pyruvate and proline; $P = 0.014$), degradation of valine, leucine and isoleucine (metabolite: 3-hydroxyisobutyrate; $P = 0.189$) and purine metabolism (metabolite: hypoxanthine; $P = 0.290$).

Finally, the metabolites retained in the linear regression supported by the 3 levels of evidence, divided by groups, which had a positive association with CRF gains, were: lactate ($\beta = 0.252$; $P = 0.038$) for ET; and succinate ($\beta = 0.224$; $P = 0.021$), for HIIT, with $r^2 = 0.803$ and $r^2 = 0.849$, respectively. There was no multicollinearity of measurements as observed VIF lower than 3.

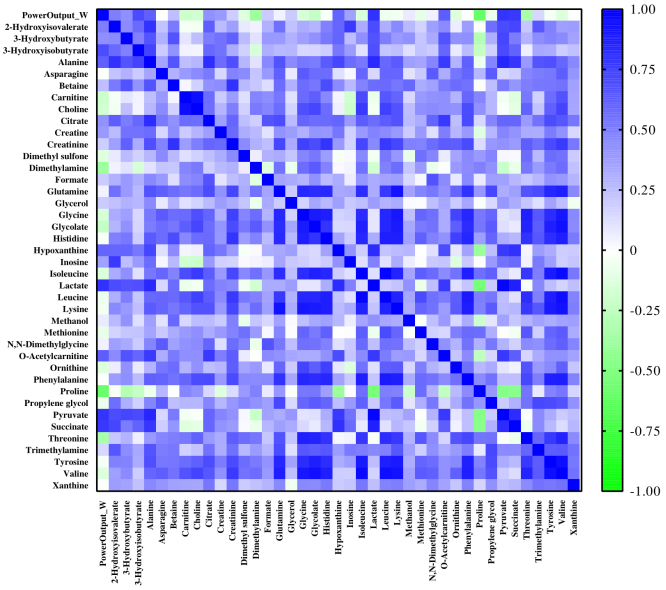

Figure 1. Heat map with overall correlations between CRF gains and metabolic chances after bout of exercise for ET.

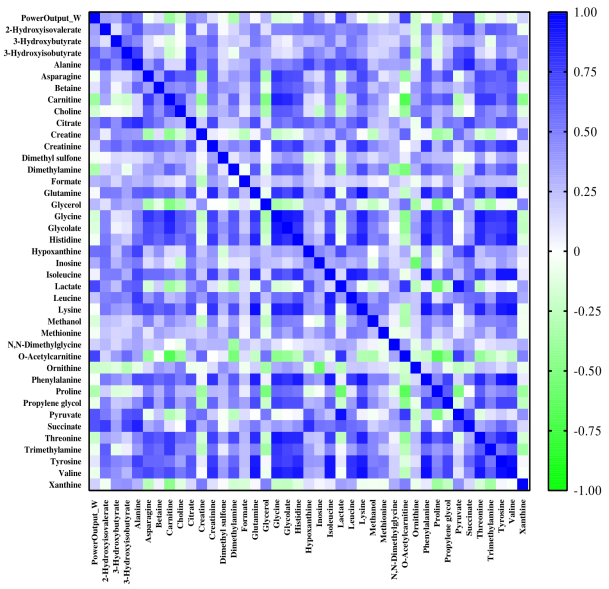

Figure 2. Heat map with overall correlations between CRF gains and metabolic chances after bout of exercise for HIIT.

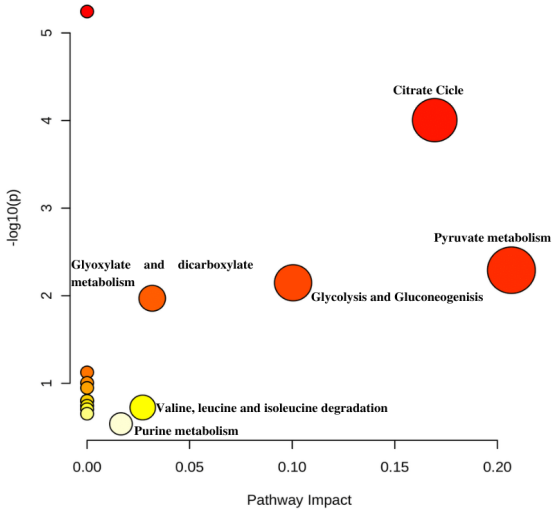

Figure 3.Pathway enrichment analysis for the ET group.

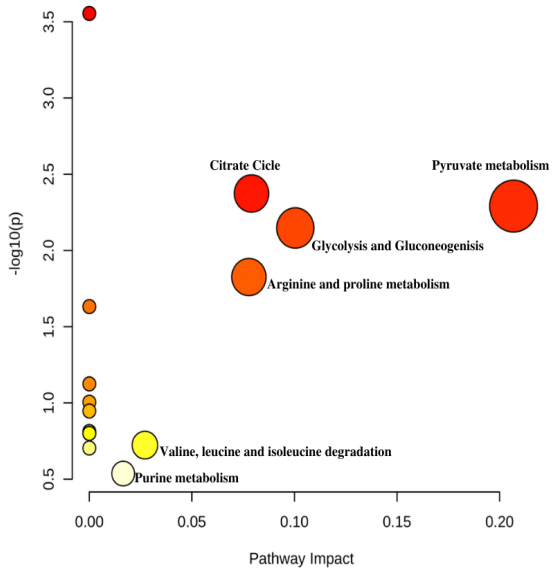

Figure 4. Pathway enrichment analysis for the HIIT group.

Conclusions

For sedentary young men, the metabolic changes in an acute bout of exercise, at the beginning of training are associated with responsiveness of the CRF through a positive regulation in the carbohydrate metabolism in both ET and HIIT programs, with additional evidence for a negative regulation in amino acid metabolism for HIIT.

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