METABOLOMICS RESPONSES IN SALIVA AFTER ACUTE SESSIONS OF HIGH-INTENSITY INTERVAL TRAINING AND CONTINUOUS ENDURANCE TRAINING

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Abstract
Introduction: The aim of this study was to investigate the metabolomics responses in saliva after acute sessions of high-intensity interval training (HIIT) and continuous endurance training (ET). Methods: Nine young untrained men (18 to 30 years old), were underwent to three 40-min acute sessions: HIIT [5 x 4 min 90% of reserve heart rate (HRr) interspersed with 3 min at 50% FCr] and ET (70% FCr) and control session (CO) in a randomized cross-over experimental design. Saliva samples were collected before (pre) and after (post) sessions and were analyzed by H1 NMR spectroscopy to identify discriminant salivary metabolites of metabolic responses between acute sessions of HIIT, ET and CO. Multivariate statistical analyses were applied, such as: Principal Component Analysis (PCA) to identify segregation in metabolic profile (set of metabolites) between acute training sessions for pre and post moments; Partial Least Squares Discriminant Analyzes (PLS-DA) for identification of the metabolites that best explain the total variances in the salivary metabolome; and metabolic pathway analysis by over-representation and pathway topology. The significance criterion (a) was set at 5% (P < 0.05). Results: The discriminant metabolites (VIP Score > 1) were: 2-hydroxybutyrate, pyruvate, acetone, alanine, lactate, valine, acetate, propylene glycol. The most enriched metabolic pathways by these set of metabolites were: pyruvate metabolism; propanoate metabolism; taurine and hypotaurine metabolism, and glycolysis or gluconeogenesis. Conclusion: Saliva can be considered a sensitive and robust biofluidic alternative for metabolic analysis of acute responses to HIIT and ET.

Key words: HIIT, Aerobic training, Metabolomics, Saliva

Introduction
Physical training promotes health and improvement benefits by causing alterations in cellular biochemical processes, reflecting remarkable changes in the systemic metabolic profile. Metabolomics has been identified as a sensitive and robust method for metabolic profile analysis in biological samples such as blood, muscle tissue and saliva. The metabolic profile expressed in saliva is little explored as a potential biomarker of responses to physical exercise.

Results and Discussion
Nine healthy young men (21 ± 3 years old) performed three randomized controlled 40-min trials: HIIT (5 min warm up + 5 sets of 4 min-90% HRr interspersed 3 min-50% HRr), ET (70% HRr), and control session (seated rest). Pre and Post sessions were collected saliva samples, which were analyzed by Protons Nuclear Magnetic Resonance (H1 NMR) spectroscopy (metabolomics). Multivariate statistical analyses were conducted in MetaboAnlyst 4.0 software, such as: PCA to identify segregation in metabolic profile (set of metabolites) between acute training sessions for pre and post moments; PLS-DA for identification of the metabolites that best explain the total variances (VIP score > 1) in the salivary metabolome; and metabolic pathway analysis by over-representation and pathway topology. There was no segregation between metabolome of HIIT, ET and CO in the Pre session. However, a significant segregation (permutation test: P = 0.027) was observed between HIIT, ET and CO in the Post session. The discriminant metabolites and their enriched metabolic pathways (in parentheses) were: 2-hydroxybutyrate, acetone and valine (propanoate metabolism), alanine and acetate (taurine and hypotaurine metabolism), piruvate (pyruvate metabolism and glycolysis or gluconeogenesis), lactate (propanoate metabolism and glycolysis or gluconeogenesis), and propylene glycol (pyruvate metabolism) (Figure 1).

Figure 1: A: Score plot of partial least square discriminant analysis (Pre) (PLS-DA); B: PLSDA (Post); C: VIP scores (Post); D: Pathway Impact (Post).

Legenda: 1- Pyruvate metabolism; 2-Propanoate metabolism; 3-Taurine and hypotaurine metabolism; 4-Glycolysis or Gluconeogenesis. 1 A e B: analysis carried out with 53 metabolites. Permutation test p=0.027.

Conclusions
Saliva can be considered a sensitive and robust biofluidic alternative for metabolic analysis of acute responses to HIIT and ET.

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