XXXI Congresso de Iniciação Científica Unicamp 2023

Association between salivary metabolomic profile and cardiorespiratory fitness responsiveness to different aerobic training programs

Keywords: Cardiorespiratory fitness, Saliva, Metabolomics

Authors:
Matheus Alejandro Bolina Amaral (Scientific Initiation student), FEF – UNICAMP
Dr. Alex Castro (Co-supervisor), DQ - UFSCAR
Profa. Dra. Cláudia Regina Cavaglieri (Collaborator), FEF - UNICAMP
Profa. Dra. Mara Patrícia Traina Chacon-Mikahil (Supervisor), FEF - UNICAMP

Introduction

Cardiorespiratory fitness (CRF) reflects the ability of the cardiorespiratory system to supply oxygen and nutrient demands of exercising skeletal muscles [1]. High levels of CRF are associated with improved endurance performance [2], reduced risk of developing cardiovascular disease [3] and reduced rates of premature mortality from all causes [4].

Endurance training (ET) or high-intensity interval training (HIIT) programs are among the main strategies used to improve CRF [5,6]. However, there is wide variability in CRF responses (responsiveness) to standardized regular training programs, even for phenotypically similar individuals exposed to similar relative loads of exercise, which may vary from "low" to "high" responders, non-responders or even negative responders [7].

In this sense, for a better understanding of the determinant molecular changes of CRF responsiveness to training, the omics sciences have enabled the identification of biomarkers in different tissues and biological fluids [8]. Among the omics sciences, the metabolomic approaches more for the individual's phenotypic expression, because it enables the identification, quantification and characterization of numerous molecules of low molecular weight, called metabolites [9], which are the end product of chemical reactions in metabolic's pathways, bringing great attention the literature in studies of adaptive responses to exercise [9,10].

It is known in the literature that saliva is responsive to exercise, yet it is little explored, even though it is easy to collect and minimally invasive [11]. To the best of our knowledge, to date, no previous studies have investigated salivary biomarkers of CRF responsiveness through metabolomic approaches. To investigate associations between the baseline salivary metabolomic profile and CRF gains after standardized training programs can contribute to a better understanding of the determinant metabolic levels related to CRF responsiveness and identification of candidate biomarkers with practical applicability, enabling advances in personalized exercise medicine.

Therefore, the aim of this study was to investigate whether the pre-training saliva metabolic profile is associated with CRF responsiveness, in front of 8 weeks of ET and HIIT programs.

Method

The participants of this study were 80, young, sedentary and healthy men, free from obesity, dyslipidemia, hypertension, cardiovascular disease, and musculoskeletal disorders. Of these 80 individuals only 70 completed the intervention and were considered for further analysis. 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 State University of Campinas (Number: 2,717,688; CAAE: 52997216.8.0000.5404; April 2016). This study is part of an "Umbrella" project started in 2016, funded by FAPESP (process 2016/05741-7).

Prior to the intervention, baseline saliva samples were obtained at rest in the 12 h fasting state. After 72 h, body composition and mass were measured followed by a maximum effort test on a cycle ergometer designed to measure the maximum power output (MPO) that will be used as a measure of CRF. The MPO test will be repeated 48 hours after [12].

All participants were randomized in a 3:1 ratio between the HIIT, ET and control (CO) groups, approximately one week later, participants began the eight-week intervention protocol in their respective experimental group. Maximum exercise tests were repeated after 4 weeks (to adjust training loads if necessary) and 5 days after the last exercise session. The control group did not perform any regular exercise and was subjected to the same assessments as the other participants. MPO test results and metabolite concentrations were used as our primary and secondary results, respectively.

Saliva samples were collected between 7:00 am and 10:00 am, after a standardized meal followed by a 12-hour fast. Prior to saliva collection, participants rinsed their mouths with distilled water 3 times for 30 s and then remained at rest for 5 min. Afterwards, participants' saliva was collected sputtering in a 15 mL Sterile Falcon Type Tube until 2 mL was obtained. Then, these samples were centrifuged (1500 g) for 15 min and immediately stored in a -80 °C freezer.

Initially, heart rate (HR) was collected during 5 min of rest. Then the incremental test started with a warm-up of 3 min at 50 W followed by 25 $W.min^{-1}$ increments until voluntary exhaustion [13]. The test was conducted on a cycle ergometer with electromagnetic braking. HR control was continuously performed using a heart rate monitor and subjective perception of exertion was recorded at the final 15s of each test stage using the Borg scale of 6-20 [14].

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 maximum HR values achieved in incremental test [15]. 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 four times a week for the last four weeks. For the HIIT training group, participants trained at 50% HRr for 5 min, followed by 5 intervals of 4 min at 90% HRr (work phase) interspersed with 3 min at 50% HRr (recovery phase), three days a week, in the first 4 weeks of training, and at 60% HRr for 5 min, followed by 5 intervals of 4 min at 90% HRr interspersed by 3 min at 60% HRr, 4 days a week for the last four training weeks. Both training programs were designed to present the same total volume of exercise. For the CO group, they were asked to remain sedentary for 8 weeks, being reminded after 4 weeks. All training sessions were supervised and carried out in a controlled environment, to ensure that the target HR was achieved and to avoid alterations due to environmental changes.

Saliva samples were analyzed by metabolomics. Each spectrum was acquired using an Inova Agilent protons nuclear magnetic resonance (^{1}H NMR) spectrometer (Agilent Technologies Inc., Santa Clara, USA) equipped with a triple cold resonance probe operating at a resonance frequency of 599.89 MHz for hydrogen and a constant temperature of 298 K (25°C). A total of 256 free induction decays will be collected with 32-k data points over a spectrum width of 8000Hz. An acquisition time of 4 s and relaxation delay intervals of 1.5 s will be implemented between scans [9]. After the spectra were acquired, phase adjustment, baseline correction, water signal removal (4.6–5.1 ppm), spectral calibration and quantification were conducted following the parameters for profiling as defined in the Chenomx NMR Suite 8.31 software (Chenomx Inc., Edmonton, Canada) [16]. All spectra were processed with a line broadening (lb) of 0.5 Hz.

The associations between baseline metabolomic profile and CRF responsiveness were explored via three levels of evidence: (1) correlation between baseline metabolomic profile with MPO gains of at least with $(r) \geq |0.2|$; (2) metabolite contribution to significant pathways associated with MPO, identified by pathway enrichment analysis; and (3) metabolite retention in a multiple linear regression model using the stepwise method. The significance level adopted was 5% or a false discovery rate of 0.5.

Results and discussion

No significant correlations were observed between CRF gains and phenotype characteristics (age, body mass, body fat percentage, and body mass index (BMI)) for the ET and HIIT training

programs (P > 0.05 for all). 1H-NMR analysis resulted in 43 metabolites, which 15 metabolites were correlated with (r) ≥ |0.2| in both programs (Figure 1).

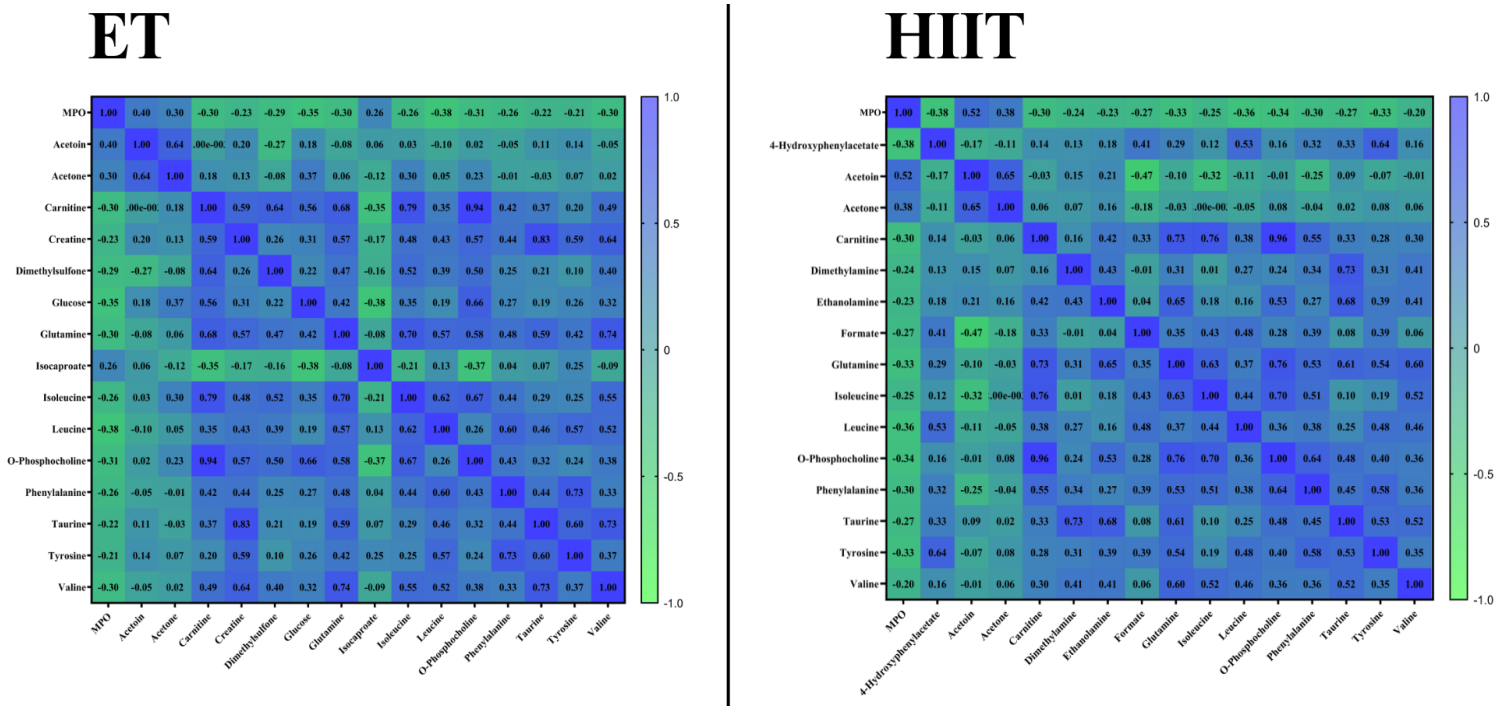

Figure 1: Heatmap of the overall correlations between baseline metabolic levels with MPO gains for metabolites that presented r ≥ 0.2 | in ET and HIIT programs.

Thus, 6 pathways were most enriched by these metabolites with p adjusted by false discovery rate of 0.1 and Impact value calculated from pathway topology analysis > 0 (Figure 2).

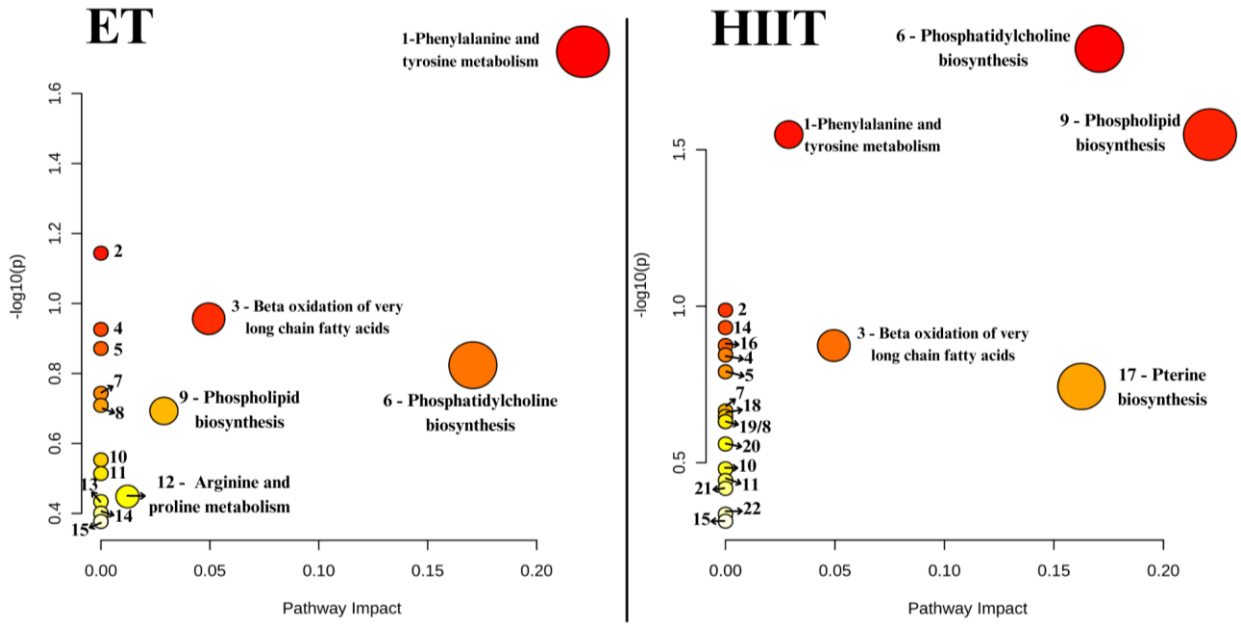

Figure 2: Pathway enrichment analysis of ET and HIIT group metabolites; 1 phenylalanine and tyrosine metabolism; 2 valine, leucine and isoleucine degradation; 3 beta oxidation of very long chain fatty acids; 4 catecholamine biosynthesis; 5 carnitine synthesis; 6 phosphatidylcholine biosynthesis; 7 oxidation of branched chain fatty acids; 8 mitochondrial beta-oxidation of long chain saturated fatty acids; 9 phospholipid biosynthesis; 10 sphingolipid metabolism; 11 fatty acid metabolism; 12 arginine and proline metabolism; 13 glycine and serine metabolism; 14 tyrosine metabolism; 15 bile acid biosynthesis; 16 phosphatidylethanolamine biosynthesis; 17 pterine biosynthesis; 18 androstenedione metabolism; 19 folate metabolism; 20 androgen and estrogen metabolism; 21 steroid biosynthesis; 22 tryptophan metabolism.

Finally, the metabolites retained by the multiple linear regression model using the stepwise method were glutamine and acetone (Table 1).

Table 1. Results of the linear regression model with stepwise selection for changes at the MPO in response to training.

Model	β		B (95% CL)	t-value	p	r^2 Change	r^2 model	VIF
Control	Ref	---	--- ---	---	---	---	---	---
ET	0.997	56.418	(42.890 ; 69.947)	8.354	<0.001	0.096	0.080	2.341
HIIT	0.955	53.005	(39.749 ; 66.261)	8.010	<0.001	0.491	0.572	2.339
Glutamine	-0.195	-5.446	(-9.886 ; -1.005)	0.017	0.017	0.037	0.604	1.039
Acetone	0.192	5.365	(0.916 ; 9.814)	0.019	0.019	0.035	0.635	1.043

MPO: Maximum power output; ET: Endurance training; HIIT: High-intensity interval training; β: standardized coefficient; B: unstandardized coefficient; VIF: Variation inflation factor.

This study investigated whether the salivary metabolomic profile pre-training is associated with CRF responsiveness to ET and HIIT programs. The main findings show that CRF gains are associated with positive correlation of the fatty acids beta oxidation, Krebs cycle, oxidative phosphorylation and phospholipids biosynthesis processes in both programs.

The metabolites retained by the three levels of evidence were glutamine and acetone with a negative and positive association with the gains in CRF respectively. glutamine is a non-essential amino acid, which can be resynthesized in the body into glutamic acid, what is associated with the release of an ammonia molecule, which supplies the synthesis of 4-aminobutanoate (GABA), which gives rise to succinate semialdehyde and finally through the enzyme SLC25A10 form succinate inside the mitochondria [17]. Therefore, lower baseline glutamine levels in the salivary metabolomic profile can indicate a positive regulation of glutamine influx and degradation into the mitochondria, with greater production of this intermediary of the citric acid cycle and oxidative phosphorylation, two fundamental processes for the improvement of CRF. Additionally, the metabolism of phenylalanine and tyrosine has associations with the endogenous production of thyroid hormones which can lead to mitochondrial biogenesis and density [18]. Furthermore, the metabolism of arginine and proline has association with the production of nitric oxide and creatine, a vasodilator and one of the energy suppliers of the cellular respiration processes and the proline is a osmoprotectant who responses a stress stimulus like the exercise and as well has the endogenous production by glutamic acid which can lead to a processes associated with the cellular respiration and oxidative phosphorylation processes in the mitochondria [19].

Also, acetone is a product of lipid degradation, and it is well established that the use of lipids as an energy substrate occurs in a predominance of aerobic metabolism and in moments with lower glycemic levels like in fasting [20]. Consequently, higher baseline acetone levels in the salivary metabolomic profile, may indicate an individual with greater capacity to efficiently use the degradation of lipids as an energy source, what can be supported for the most enrichment metabolics pathways (beta oxidation of very long chain fatty acids, phosphatidylcholine biosynthesis, phospholipid biosynthesis, pterine biosynthesis) and for the metabolites who enrich that pathways (carnitine, ethanolamine and phosphorylcholine), with negative correlation, what can indicate the influx and degradation of this molecules to supply energetics demands, which can predict the capacity of the individual to maintain the exercise in a predominance of aerobic metabolism, even with greater demands of effort, increasing the possible duration of the exercise with less oxidative and general metabolic stress, characteristics that is observed in aerobically trained individuals [21].

Conclusions

These results suggest that for young and sedentary men, CRF responsiveness to ET and HIIT programs is linked to the positive correlation of the fatty acids beta oxidation, Krebs cycle, oxidative phosphorylation and phospholipids biosynthesis processes in both training programs.

Acknowledgement

We express our gratitude to all participants of this study and the foment of FAPESP, CNPq, PIBIC-CNPq. We thank the Nuclear Magnetic Resonance (NMR) Facility at Brazilian Biosciences National Laboratory (LNBio), for the use of the NMR spectrometer (600MHz, Varian Inova), FAEPEX-Unicamp and the laboratory of exercise physiology (FISEX).

Bibliography

[1] FLOREZ H, COBBS JR, GREGOSKI MJ. Importance of Optimizing Cardiorespiratory Fitness in Early Adulthood and Through Midlife. JAMA Netw Open. 2023 Feb 1;6(2):e233637.

[3] RIEBE, D. et al. ACSM's Guidelines for Exercise Testing and Prescription. Tenth Edition. Wolters Kluwer, 2018.

[4] MEHTA, A. et al. Running away from cardiovascular disease at the right speed: The impact of aerobic physical activity and cardiorespiratory fitness on cardiovascular disease risk and associated subclinical phenotypes. Prog Cardiovasc Dis. 2020 Nov-Dec;63(6):762-774.

[5] GORNY AW, YAP J, NEO JW, CHOW WE, YEO KK, Tan CS, Müller-Riemenschneider F. Cardiorespiratory fitness, body mass index, cardiovascular disease, and mortality in young men: A cohort study. Front Public Health. 2023 Feb 15;11:1076065.

[6] ABOUZEID N, ELNAGGAR M, FATHALLAH H, AMIRA M. Eight Weeks of High-Intensity Interval Training Using Elevation Mask May Improve Cardiorespiratory Fitness, Pulmonary Functions, and Hematological Variables in University Athletes. Int J Environ Res Public Health. 2023 Feb 17;20(4):3533.

[7] MURIAS JM. et al. Time course and mechanisms of adaptations in cardiorespiratory fitness with endurance training in older and young men. J Appl Physiol (1985). 2010 Mar;108(3):621-7

[8] BOUCHARD, C. et al. Adverse metabolic response to regular exercise: is it a rare or common occurrence? PLoS One, v. 7, n. 5, p. e37887, 2012. ISSN 1932-6203.

[9] CASTRO, A., DUFT, R. G., SILVA, L. M., FERREIRA, M. L. V., ANDRADE, A. L. L., BERNARDES, C. F., et al. (2021). Understanding the Relationship between Intrinsic Cardiorespiratory Fitness and Serum and Skeletal Muscle Metabolomics Profile. J. Proteome Res. 20, 2397–2409

[10] KHORAMIPOUR K, SANDBAKK Ø, KESHTELI AH, GAEINI AA, WISHART DS, CHAMARI K. Metabolomics in Exercise and Sports: A Systematic Review. Sports Med. 2022 Mar;52(3):547-583.

[11] NTOVAS P, LOUMPRINIS N, MANIATAKOS P, MARGARITIDI L, RAHIOTIS C. The Effects of Physical Exercise on Saliva Composition: A Comprehensive Review. Dent J (Basel). 2022 Jan 5;10(1):7.

[12] DIDERIKSEN, K.; MIKKELSEN, U. R. Reproducibility of incremental maximal cycle ergometer tests in healthy recreationally active subjects. Clin Physiol Funct Imaging, Sep 2015.

[13] BUCHFUHRER, M. J. et al. Optimizing the exercise protocol for cardiopulmonary assessment. J Appl Physiol Respir Environ Exerc Physiol, v. 55, n. 5, p. 1558-64, Nov 1983.

[14]BORG, G.; LINDERHO, H. Perceived exertion and pulse rate during graded exercise in various age groups. Acta Medica Scandinavica v. 181, n. S472, p. 194-206, 1967.

[15] LOUNANA, J. et al. Relationship between %HRmax, %HR reserve, %VO2max, and %VO2 reserve in elite cyclists. Med Sci Sports Exerc, v. 39, n. 2, p. 350-7, Feb 2007.

[16] KOSTIDIS S, ADDIE RD, MORREAU H, MAYBORODA OA, GIERA M. Quantitative NMR analysis of intra- and extracelular metabolism of mammalian cells: A tutorial. Anal Chim Acta. 2017; 980:1–24.

[17] Zhou X, Paredes JA, Krishnan S, Curbo S, Karlsson A. The mitochondrial carrier SLC25A10 regulates cancer cell growth. Oncotarget. 2015 Apr 20;6(11):9271-83.

[18] Cioffi F, Senese R, Lanni A, Goglia F. Thyroid hormones and mitochondria: with a brief look at derivatives and analogues. Mol Cell Endocrinol. 2013 Oct 15;379(1-2):51-61.

[19] Moreira JBN, Wohlwend M, Fenk S, Åmellem I, Flatberg A, Kraljevic J, Marinovic J, Ljubkovic M, Bjørkøy G, Wisløff U. Exercise Reveals Proline Dehydrogenase as a Potential Target in Heart Failure. Prog Cardiovasc Dis. 2019 Mar-Apr;62(2):193-202.

[20]Kim MJ, Hong SH, Cho W, Park DH, Lee EB, Song Y, Choe YS, Lee JH, Jang Y, Lee W, Jeon JY. Breath Acetone Measurement-Based Prediction of Exercise-Induced Energy and Substrate Expenditure. Sensors (Basel). 2020 Dec 1;20(23):6878.

[21] Martins HA, Barbosa JG, Seffrin A, Vivan L, Souza VRDA, De Lira CAB, Weiss K, Knechtle B, Andrade MS. Sex Differences in Maximal Oxygen Uptake Adjusted for Skeletal Muscle Mass in Amateur Endurance Athletes: A Cross Sectional Study. Healthcare (Basel). 2023 May 22;11(10):1502.