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Non-targeted sportomics analyses by mass spectrometry to understand exercise-induced metabolic stress in soccer players

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ABSTRACT

We have been using "-Omics" sciences with classic laboratory analyses to understand the systemic metabolic and signaling changes induced by sport and exercise. We called this approach Sportomics. Our samples were collected *in situ* either during competitions or training to mimic the genuine challenges and conditions faced during sports. Non-targeted analysis (NTA) has opened the door to a new era of high-throughput exercise-induced metabolic research. In the present study, 30 male semi-professional soccer players were observed for two subsequent days. Both blood and urine samples were collected immediately pre-match and after matches. The most up-regulated prominent molecules were fatty acyls, carboxylic acids and derivatives, steroids and steroid derivatives. The most down-regulated molecules were fatty acyls, carboxylic acids and derivatives, as well as benzene and substituted derivatives. After metabolite identification and determining which metabolites were up- or down-regulated, we took the metabolites and grouped them into classes to examine the metabolic pathways involved with purine metabolism and to investigate hyperammonemia. To follow-up on our findings in urine, we used point-of-care instrument analysis to measure capillary blood metabolites. Glucose significantly increased by 35%, whereas urate increased by 16% and uremia by 17% without any changes in creatinemia. In the present study, we showed that hypoxanthine and related metabolites were up-regulated in urine after a soccer match, which suggested that AMP deamination was increased. In this study, we demonstrated several results through urine non-target mass spectrometry (NTMS) to understand exercise-induced changes during a soccer match using a Sportomics approach. These data together demonstrated that during a soccer game, there was an increase in ATP use provided by ADP synthesis via myokinase. Our data may show that the use of urinary metabolomics can be a less invasive way to follow the metabolism of athletes during exercise. We demonstrated that the use of NTMS may be useful for future studies that aim to design holistic interventions for improving athletic performance.

1. Introduction

Recently, we assembled a new concept in metabolic studies and exercise science called Sportomics through the use of "-Omics" sciences with classic laboratory analyses to understand the systemic

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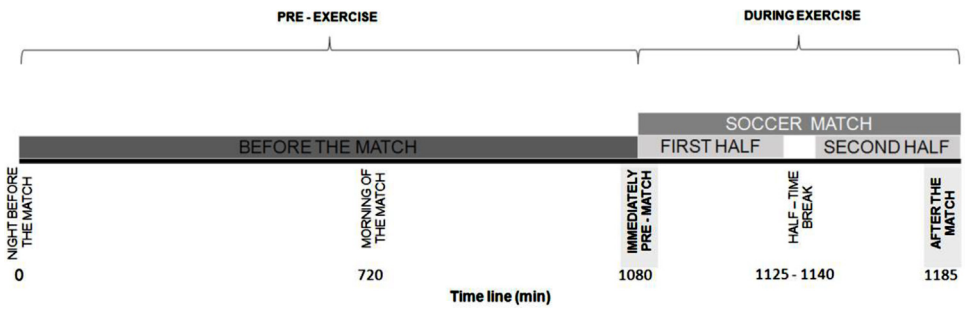

Fig. 1. Experiment design. Urine samples from young male semi-professional soccer players collected at two different time points, namely pre-match and post-match, were tested.

metabolic and signaling changes induced by participation in sport. Using this approach, the samples were collected *in situ* either during competitions or training to mimic the genuine challenges and conditions faced during participation in sports [3].

We believe that this approach can offer interdisciplinary connections among fields to reduce the barriers between sport trainers and scientists, including translational science, to improve athletic performance significantly. We propose that in-field metabolic analyses are better for understanding, supporting and training elite athletes.

As with any "-Omics" science, Sportomics requires analytical techniques with great computational capability to manage large amounts of data. The use of non-targeted analysis by mass spectrometry (NTMS) is considered the "gold medal" champion approach to holistically understand an *in vivo* appraisal of proteomic and metabolomic changes induced by exercise. NTA opened the gate to a new era of high-throughput exercise-induced metabolic research by delivering a better understanding of the integration of biological processes and extending our knowledge of exercise to systems biology [5,10,1].

In this study, we obtained several results through urine NTMS to understand exercise-induced changes during a soccer match using a Sportomics approach. We also described changes such as purine and pyrimidine metabolism, which can be utilized to recognize exercise-induced hyperammonemia. Additionally, we conducted this investigation to comprehend the metabolic changes that occur in athletes undergoing exercise from a practical perspective.

2. Methods

2.1. Subjects

Thirty male semi-professional soccer players (18-20 years old) from a team affiliated with the Confederação Brasileira de Futebol (CBF, Brazilian Soccer Confederation) participated in this study as volunteers. The players were healthy and did not have detectable diseases. They were evaluated clinically twice per year. The subjects were informed previously about the study, and written informed consent was obtained from each one. All of the procedures were performed according to the ethical standards of the Ethics Committee for Human Research at the Federal University of the State of Rio de Janeiro (117/2007, renewed in 2011) and met the requirements for regulating research on human subjects (Health National Council, Brazil, 1996). The subjects were tested during two subsequent days (n = 30).

2.2. Experimental Design, Sample Collection and Preparation

Five different urinary samples were collected the night before the match (0 min), in the fasting state on the morning of the match (720 min), immediately prior to the match (1080 min), at the half-time break (1125 min) and after the match (1185 min). We compared two time points, including the pre-match and post-match times (Fig. 1).

After collection, urine was used as a biological matrix, and the samples were immediately transferred to a dry ice cooler and were transported to the Laboratory of Protein Biochemistry (LBP) located at the Federal University of the State of Rio de Janeiro (UNIRIO). Later, the samples were stored in an ultra-low temperature freezer (−80 °C) until they were prepared for ultra-pressure liquid chromatography followed by alternating low- and high-energy multiplexed MS/MS (UPLC-MS^E) injections. Mass spectrometry analyses were further performed at a starting volume of 700 μL of raw urine samples that were thawed to room temperature. Raw urine samples were then centrifuged at 10,000 × *g* for 30 minutes at 4 °C, and the supernatants were perfused through a dialysis membrane with a 3,000 Da molecular weight cutoff (MWCO) (Amicon, Merck Millipore, Germany). The filtrate was desalted using a solid-phase hydrophilic-lipophilic-balanced extraction cartridge (Oasis® HLB, Waters Corporation, USA). The samples were concentrated using a SpeedVac Plus (Model: SC110A, ThermoSavant, USA) and were reconstituted in solvent solution containing 3% acetonitrile and 0.1% formic acid in Milli-Q pure water. Finally, the samples were transferred to a UPLC auto-sampler vial (Waters Corporation, USA).

2.3. Point-of-care analysis

For comparison, blood was collected from seven athletes immediately prior to the match (1080 min) and after the match (1185 min) for measurement of glucose, urea, urate and creatinine. The venous blood was collected and immediately centrifuged for 10 min at 3,000 × *g* to separate the plasma. The samples were stored at −20 °C. Each plasma sample of the players was thawed and pipetted into General Chemistry 13 and MetLyte 8 discs to be analyzed by Piccolo Xpress®.

2.4. UPLC-MS^E method

UPLC-MS^E data were acquired in an ultra-high-performance liquid chromatography system (Acquity UPLC I-Class, Waters, USA) coupled to an ESI (+) Qq-*oa*TOF mass spectrometer (Xevo G2-S Q-Tof, Waters, UK). A total of 10 μL of each sample was injected, and the separation was performed on an ACQUITY UPLC CSH C18 Column, 130 Å, 1.7 μm, 2.1 mm × 50 mm conditioned at 40 °C. The mobile phases were 0.1% formic acid (pump A) and 0.1% formic acid in acetonitrile (pump B), and the flow rate was 900 μL min^{-1}. The gradient method was programmed to achieve maximum separation performance as follows: initial condition 3% B (pump B), 2.37 min 35% B, 4.37 min 85% B, 5.37 min 85% B, and 6.37 min 3% B with a total run time of 8.37 min and a calculated percent B/column volume (cV) factor of 2.6%B/cV. The sample tray temperature was