Data Descriptor

Pilot Study of the Metabolomic Profile of an Athlete after Short-Term Physical Activity

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Abstract: A comprehensive analysis of indicators of the state of the body between training and recovery allows a comprehensive evaluation of various aspects of health, athletic performance, and recovery. In this pilot study, an assessment of the metabolomic profile of athletes was performed, and the immunological reaction of the athlete’s body to food before exercise and 48 h after exercise was studied. As a result, 15 amino acids and 3 hormones were identified, the plasma levels of which differed between the training and recovery states. In addition, immunological reactions or hyperactivity to food allergens were assessed using an enzyme immunoassay. It is likely that for the athletes in the study sample, 48 h is not enough time for the complete recovery of the body.

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1. Summary

The role of sports in society has become more important over the years; however, rest and recovery are equally important components of an exercise program because they provide the body time to recover and strengthen between workouts.

Exercise, particularly intense exercise, causes damage to the muscles, which are restored during the rest period; however, in addition to physical changes, changes occur at the molecular level [1,2]. During meals, metabolic pathways are enriched with new metabolites, and intense exercise changes the rate of metabolic reactions [1]. In a trained athlete, during maximum physical exertion, oxygen consumption at the level of the whole organism increases by 20 times [3]. This corresponds to an energy expenditure of ~100 kJ/min during maximum exercise, which corresponds to the utilization of 6 g of glucose per minute. The fact that there is only ~4 g of free glucose in the human body [4] demonstrates that this increase in energy expenditure poses a problem to the exerciser’s metabolism.

The multiplicity of changes in metabolic reactions in the working muscles is even greater. During the transition from rest to exercise, the rate of adenosine triphosphate (ATP) hydrolysis, particularly by force-generating muscle fiber heads, can increase by more than 100 times [5]. Considering that muscle fiber contains only ~10 mM ATP [6] and
that a dramatic drop in ATP concentration will cause rigor mortis, ATP synthesis reactions
must immediately increase their speed in order for ATP synthesis to match ATP hydrolysis
in a fraction of a second.

Physical activity also affects hormone levels, which are important because many hor-
mones are classified as metabolites. The most characteristic change in physical activity is
an increase in the level of catecholamines [7,8], which causes an increase in heart rate and
contractility of the heart and regulates metabolism and blood flow. Finally, resistance ex-
ercise increases muscle protein synthesis for several days after exercise [9] and increases
muscle protein breakdown [10]. Although proteins are not classified as metabolites, their
constituent amino acids can enter the bloodstream when catabolic processes are activated.

In summary, these studies demonstrate that the three main branches of metabolism,
namely, energy metabolism, anabolism, and catabolism, change profoundly in response
to a series of exercises.

A triathlon is a multi-sport race, consisting of a continuous successive passage of
three stages by its participants, namely, swimming, cycling, and running, each of which
comes from an independent cyclic sport. Modern triathlons make high demands on the
motor abilities and functional capabilities of the athlete’s body. To achieve a high level of
performance, it is necessary to perform a large training load in terms of volume and inten-
sity, as a triathlon includes three cyclic types and is considered an endurance sport.

Metabolomics is an “omics” approach that allows supplementing genomic and pro-
teomic results with data on the semi-quantitative and quantitative content of metabolites
in the body. Variations in metabolic traits are rapidly identified through the precise quan-
tification of “biomarker metabolites” that are reliably specific to certain pathological con-
ditions [11]. In addition, metabolomics allows for the determination of the metabolic pro-
files of professional athletes in order to identify biomarkers associated with their perfor-
ance, response to fatigue, and, possibly, their respective sports disorders. [12].

The purpose of this work was to determine the effectiveness of the process of recov-
ery of athletes after exercise, for this, we investigated the metabolite profile of triathletes
(n = 11) participating in an endurance experiment (functional load testing and running on
a track to failure at a comfortable temperature). The study involved male triathletes who
had the following anthropometric characteristics: age 29 ± 3.5 years; weight 72 ± 6.3 kg;
growth 17 ± 3.8 sm; and BMI 23 ± 1.4 kg/m². The study participants performed stress test-
ing on a treadmill to failure at a temperature of 22–23 °C and 60% humidity.

Blood samples were taken before exercise and two days after exercise on an empty
stomach from the cubital vein and collected into invacutainers containing 3.8% sodium
citrate anticoagulant (IMPROVACUTER, Guangzhou Improve Medical Instruments Co.,
Ltd., Guangzhou, China); the samples were centrifuged at 3000 rpm for 6 min at room
temperature. Each plasma sample (500 μL) was collected into two dry Eppendorf-
type polypropylene test tubes, frozen, and stored at −80 °C prior to analysis.

Mass spectrometric studies of the blood plasma samples of participants obtained be-
fore exercise and two days (48 h) after exercise were performed to determine changes in
the bodies of the athletes at the molecular level. The focus of our study was on metabolites
and hormones. In addition, immunological reactions or hyperreactivity to certain food
allergens was assessed using ELISA.

It was found that 48 h is not enough time for the complete recovery of the body for
the athletes in the study sample; therefore, repeated and constant monitoring of the bio-
chemical parameters of an athlete’s body and the intake of macronutrients and microele-
ments can help identify individual deficiencies and track changes, particularly as training
volume and nutritional needs increase.
2. Data Description

The pilot study consisted of conducting a mass spectrometric analysis of the composition of the blood plasma of athletes after a recovery period (48 h). Mass spectrometric analysis of the blood plasma samples revealed 25 amino acids differing in content (DOI: 10.17632/nkn4skdbgr, file Supplementary S1, Table S1) and 6 hormones (DOI: 10.17632/nkn4skdbgr, file Supplementary S1, Table S2) whose level after recovery differed by 1.5 times relative to the level “before the load”.

The metabolites, whose level after recovery differed by 1.5 times relative to the level “before the load” are presented in Table 1.

Table 1. Amino acids and hormones detected in the blood plasma of athletes before exercise and after recovery (FC > 1.5).

<table>
<thead>
<tr>
<th>No.</th>
<th>Metabolite</th>
<th>Mean Concentration, (μM/L) BL *</th>
<th>Mean Concentration, (μM/L) AR **</th>
<th>SD BL *</th>
<th>SD AR **</th>
<th>Fold Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>3-Methylhistidine</td>
<td>0.56</td>
<td>1.57</td>
<td>0.33</td>
<td>1.08</td>
<td>↑ 2.77</td>
</tr>
<tr>
<td>2.</td>
<td>Arginine</td>
<td>18.19</td>
<td>4.85</td>
<td>9.45</td>
<td>0.76</td>
<td>↓ 0.26</td>
</tr>
<tr>
<td>3.</td>
<td>Carnosine</td>
<td>0.47</td>
<td>1.04</td>
<td>0.15</td>
<td>0.89</td>
<td>↑ 2.21</td>
</tr>
<tr>
<td>4.</td>
<td>cis-Aconitic Acid</td>
<td>0.61</td>
<td>0.79</td>
<td>0.76</td>
<td>1.03</td>
<td>↑ 1.28</td>
</tr>
<tr>
<td>5.</td>
<td>Fumaric Acid</td>
<td>1.97</td>
<td>4.09</td>
<td>0.81</td>
<td>0.14</td>
<td>↑ 2.06</td>
</tr>
<tr>
<td>6.</td>
<td>Histidine</td>
<td>21.51</td>
<td>37.08</td>
<td>–</td>
<td>–</td>
<td>↑ 1.72</td>
</tr>
<tr>
<td>7.</td>
<td>Hydroxyproline</td>
<td>1.41</td>
<td>2.33</td>
<td>0.26</td>
<td>1.91</td>
<td>↑ 1.6</td>
</tr>
<tr>
<td>8.</td>
<td>Lactic Acid</td>
<td>12.48</td>
<td>33.91</td>
<td>1.50</td>
<td>4.53</td>
<td>↑ 2.71</td>
</tr>
<tr>
<td>9.</td>
<td>Methionine Sulfoxide</td>
<td>38.20</td>
<td>69.82</td>
<td>–</td>
<td>–</td>
<td>↑ 1.82</td>
</tr>
<tr>
<td>10.</td>
<td>Ornithine</td>
<td>2.58</td>
<td>6.26</td>
<td>–</td>
<td>–</td>
<td>↑ 2.42</td>
</tr>
<tr>
<td>11.</td>
<td>Oxalic Acid</td>
<td>7.31</td>
<td>3.68</td>
<td>–</td>
<td>–</td>
<td>↓ 0.50</td>
</tr>
<tr>
<td>12.</td>
<td>Phenilalanine</td>
<td>10.92</td>
<td>21.33</td>
<td>–</td>
<td>–</td>
<td>↑ 1.95</td>
</tr>
<tr>
<td>13.</td>
<td>Succinic Acid</td>
<td>0.08</td>
<td>0.34</td>
<td>0.09</td>
<td>0.34</td>
<td>↑ 4.28</td>
</tr>
<tr>
<td>14.</td>
<td>Tryptophane oxidized</td>
<td>468.14</td>
<td>271.65</td>
<td>112.73</td>
<td>25.68</td>
<td>↓ 0.58</td>
</tr>
</tbody>
</table>

* BL—before loading; ** AR—after recovery.

Table 1 shows that after the load, in the recovery period (48 h), in the blood plasma of athletes, an increase and decrease in the content of 12 and 3 amino acids and carboxylic acids, respectively (FC > 1.5), was detected. The largest increase in content (FC > 2) was observed for 3-methylhistidine, carnosine, fumaric and lactic acid, ornithine, and succinic acid.

Table 1 also shows that after exercise and during the recovery period in the blood plasma of athletes, an increase in the content of 11-Deoxycortisol and estriol (approximately two times) and a decrease in the content of allopregnenolone and DHEA (approximately two times) were observed.

During exercise, amino acid oxidation and protein breakdown are increased, and protein synthesis is suppressed, despite the minor role of protein as an energy source. In response to exercise-induced stimulation, free amino acids in skeletal muscle and blood plasma undergo various changes to meet the physiological requirements [13].

In this study, we noted an increase in the content of molecular participants of the tricarboxylic acid (TCA) cycle, namely, 3-Methylhistidine, carnosine, and arginine, as well as hormones, namely, 11-Deoxycortisol and estriol (FC > 1.5), during the recovery period compared to that in the state before exercise.
The TCA is an important pathway for providing energy to athletes during prolonged exercise. TCA is intensified during exercise, which is reflected by an increase in the content of pyruvic acid, malic acid, and aconitate [14].

In our study, an increase in the levels of cis-aconitic, succinic, and fumaric acids was observed 48 h after exercise (Figure 1). This phenomenon can be attributed to compensatory adaptive changes owing to the influence of physical activity. The use of succinic acid has a pronounced protective effect and optimizes the energy metabolism of muscle tissue during physical exertion [15].

Figure 1. Mitochondrial metabolism: an increase in the content of metabolites of the tricarboxylic acid cycle and glycolysis was observed. The green arrows indicate the TCA components, the content of which was increased in the study participants after exercise and recovery, and the blue arrow indicates an increase in the level of lactate, the end product of the anaerobic breakdown of glucose and glycogen (glycolysis) in glycolysis.

Pyruvic acid is the binding site for glycolysis, lactic acid, acetyl-CoA, oxaloacetic acid, malic acid, and various amino acids [16] and plays a key role in energy metabolism. Almost all physical activity uses skeletal muscle glycogen to produce ATP, the concentration of which rapidly decreases in skeletal muscles with intensified physical activity [17]. At the same time, lactic acid is formed in skeletal muscles as the end product of anaerobic glycolysis, and its concentration in the blood is one of the indicators in clinical stress testing and in assessing the fatigue of athletes [18]. Our study showed that elevated plasma lactate levels in athletes can persist for up to two days after endurance exercise.

The ammonia concentration increases in proportion to the increase in load power; therefore, it can be concluded that the degree of increase in the ammonia concentration in the blood is determined by the energy needed to perform the full work and the individual’s response to aerobic exercise [19]. Since the concentration of ammonia in the blood significantly correlates with lactate content [20], this suggests that the production of ammonia and lactate in the muscles may be associated with the general process of short-term energy supply during muscle fatigue, which is consistent with the results of our study.
which showed a stable increase in ornithine even during the recovery period (Table 1, Figure 2).

![Figure 2. Ornithine cycle. An increase in the content of ornithine after exercise was observed.](image)

With prolonged muscle activity, in the liver, the ornithine cycle intensifies with the formation of urea, which is excreted in urine and sweat. The blood urea concentration was normal for each adult individual. It can increase due to a significant intake of proteins from food, damage to the excretory function of the kidneys, and after long-term physical work, due to increased protein catabolism [21]. In sports medicine, this indicator is widely used to assess an athlete’s tolerance for training and competitive physical activity, course of training sessions, and body recovery processes [22]. If the performed physical activity is adequate for the functional capabilities of the body and there has been a relatively rapid recovery of metabolism, then the urea content returns to normal, which is associated with balancing the rate of synthesis and breakdown of proteins in the tissues. However, an increased urea content indicates incomplete recovery of the body or the development of fatigue.

Delayed muscle soreness is primarily associated with damage and inflammation in muscle connective tissues, the main element of which is collagen and not the muscle fibers themselves [23,24]. The collagen triple helix is stabilized by the non-proteinogenic amino acid hydroxyproline (Hyp) [25]. Some studies have shown an increase in the concentration of Hyp in urine [26] and blood [27] after maximal unconventional knee extensor exercises, which can be used as an indicator of muscle collagen breakdown [28,29].

Phenylalanine (Phe), a tyrosine precursor, is an essential amino acid and substrate for tyrosine hydroxylase, an enzyme that catalyzes the rate-limiting step in catecholamine synthesis [30]. Due to its insulinotropic effect, Phe stimulates increases in insulin, corticosteroid, and glucagon concentrations by regulating the synthesis and secretion of gluca
gon-like peptide-1 (GLP-1), which positively regulates glucose metabolism [31]. Subse
dually, the gradual depletion of the circulating glucose source is replenished by the breakdown of glycogen or gluconeogenesis from glycogenic amino acids (alanine, threo
nine, and serine) induced by glucagon and rapidly delivered to the muscle tissue because of the positive inotropic and chronotropic effects of glucagon. In turn, extreme exercise leads to an increase in the production of steroid hormones in the adrenal cortex through the activation of the hypothalamic-pituitary-adrenal axis, which is affected by GLP-1. This
is reflected in the increased level of 11-deoxycortisol in our study and is consistent with previously obtained data [32]. Steroid hormones can be attributed to anabolic elements that stimulate energy metabolism, muscle protein synthesis, and neuronal excitability, which may explain the increased endurance of athletes.

Tryptophan can be metabolized in various ways, mainly through the kynurenine pathway, which negatively regulates the immune response and positively regulates alanine synthesis. In contrast, the decrease in tryptophan in our study may be associated with the muscle fatigue that occurs after intense exercise, due to the activation of indolamine-2,3-dioxygenase-1 in enhanced tryptophan catabolism and the kynurenine pathway, which is known as an immunoregulatory event [33] associated with the development of regulatory T cells and inflammation [34]. In addition, the delicate balance of tryptophan may be shifted along the kynurenine pathway towards the production of alanine as a substrate for gluconeogenesis and niacin for the generation of NAD/NADP required in oxidative glycolysis and the pentose phosphate shunt.

Carnosine is a dipeptide found in mammalian skeletal muscles. High concentrations of carnosine were found in individuals with a high proportion of fast-twitch fibers. Athletes who train short distances show significantly high levels of muscle carnosine; however, the acute effects of prolonged training on muscle carnosine are limited [35].

The decrease in arginine levels after exercise is evident because the phosphate groups of ATP represent the chemical energy required for biochemical reactions involved in almost all synthetic metabolic pathways and muscle contractions [36].

3-Methylhistidine (3-MeHis) is a post-translationally modified histidine analog found in skeletal muscle contractile proteins (actin and myosin). During the intracellular breakdown of these proteins, 3-MeHis is released and excreted in the urine, which often serves as an indicator of the rate of myofibrillar protein breakdown in muscles and, to some extent, reflects the intensity of nitrogen metabolism [37]. Thus, it can be assumed that the level of 3-MeHis may increase during the recovery period, that is, after more than 24 h, which corresponds to the data of our study where an increase in the concentrations of MeHis and histidine was observed on the second day after exercise [38].

Any abnormal reactions in the body after eating food are defined as adverse food reactions. These include food hypersensitivity, food intolerance, food allergies, and food aversion [39]. IgG-mediated food intolerance is believed to be caused by increased intestinal permeability, which leads to the release of food components into the bloodstream and the production of specific IgGs [40]. The increased production of IgGs combined with the low production of anti-inflammatory cytokines (IL-10 and TGFβ1) is associated with irritable bowel syndrome [41].

Physical activity is beneficial for human health; however, heavy training and excessive physical activity in professional athletes increase the permeability of the gastrointestinal tract, which is reflected in a high sensitivity to food intolerance and negatively affects the immune system [42].

In our study, we assessed the hyperactive response of athletes to food allergens mediated by immune processes. It was found that the immune system of athletes is in a state of hyperreactivity both before and after exercise. The results obtained demonstrate that even in the “before exercise” state, athletes have a hyperactive reaction to certain foods (DOI: 10.17632/nkn4skdbgr, file “Supplementary S2”, Figure S1); most often, an increased immune response to products such as beef protein, brewer’s yeast, and barley groats are recorded, whereas after the load and during the recovery period, athletes also react to hazelnuts and almonds (DOI: 10.17632/nkn4skdbgr, file “Supplementary S2”, Figure S2).

In addition, it was found that the immunological reaction (manifestation of a hyperactive reaction to food components) is personalized in nature. Therefore, it is important for coaches and sports doctors to analyze and control the eating behavior of athletes to timely identify food intolerances or food allergies and then develop an individual elimination diet.
3. Methods

Data acquisition was performed using a high-resolution quadrupole time-of-flight (Q-TOF) Xevo G2-XS mass spectrometer (Waters, Inc., Wexford, Ireland) equipped with a Z-spray ionization source coupled with a UPLC Acquity H Class (Waters, Inc., Ireland) system. The instrument was operated with a positive ionization source in ESI mode. Data were acquired in continuous MSe over a 50–700 m/z mass range within a 1.875 s scan time using the SONAR mode setting with a quadrupole peak width of 20 m/z. Precursor ions were decomposed at a low collision energy level of 5 eV and a high collision energy ramping between 12 and 40 eV using argon as a collision gas. The cone voltage was fixed at 16 V, with a source voltage offset of 21 V and a capillary voltage of 2.8 kV. The desolvation gas and cone gas (nitrogen) were adjusted to 700 and 50 L/h, respectively, and the source temperature was adjusted to 320 °C. The lock mass (warfarin, m/z = 309/1127) was acquired over the data collection for a 70 ms interval of 10 s and averaged over three scans with a mass window of 0.1 Da. Chromatographic separation was performed on an Acquity Premier HSS T3 column (2.1 × 150 mm, 8 μm particle size) heated at 50 °C with a 0.4 mL/min flow rate in a gradient of mobile phase A (water with 0.1% formic acid and 0.005% heptfluorobutyric acid) and mobile phase B (acetonitrile with 0.1% formic acid) for 8.6 min, starting from 2% of B for 0.4 min, then increased to 18% of B at 0.65 min, then to 47% of B at 5.5 min, and finally to 95% of B at 5.7 min and held for 7.0 min. Thereafter, the gradient was returned to the initial condition at 7.2 min and the column was equilibrated for the next 1.4 min.

The ratio of metabolite concentrations after recovery and exercise was determined for each study participant. If the value belonged to the range [0.67, 1.5], the data were excluded from further analysis, that is, the calculation of the mean and standard deviation. Statistical analyses were performed using R Project for Statistical Computing [10]. A semi-quantitative assessment of hormone content was then performed, taking into account the chromatographic peak area.

4. User Notes

Physical activity under conditions of extreme tolerance (pronounced fatigue) is accompanied by a sharp drop in the tissue level of macroergic phosphates (creatine phosphate and ATP), accumulation of ammonia and lactic acid, and the appearance of shifts in the acid-base balance towards acidosis, as well as several other interrelated biochemical and pathophysiological changes that significantly limit the performance of the human body.

Amino acids are the building blocks of hormones and all body tissues. Evaluation of the intake of “essential” amino acids with food, their sufficiency, the correct balance between them, and the activity of enzymes that convert them into hormones is fundamental to clarifying the underlying cause of many pathological processes, including metabolic and nutritional disorders, nitrogen balance, and chronic fatigue. We found that, after exercise, metabolites with significant fluctuations were mainly associated with amino acid metabolism.

Factors such as exercise regimen, intensity or duration, and nutrition must be considered to improve specific metabolic or biochemical dysfunctions in athletes. Nutrition is considered to be one of the fundamental factors of athletic performance, and the implementation of recommendations related to nutrition after training is key to the effectiveness of recovery and adaptive processes. Therefore, completing the results of the pilot study, in the future, will allow one to choose an effective recovery strategy between workouts or during competitions that can maximize the adaptive response to various fatigue mechanisms, improve muscle structure and function, and thereby increase exercise tolerance.
5. Limitations
The main limitation of this study was the lack of experiments performed immediately after exercise testing. This is due to the presence of errors at the pre-analytical stage: the resulting biomaterial had a 3–4 degree of hemolysis. The use of hemolyzed specimens may lead to false results. Performing a semi-quantitative assessment of hormone levels is necessary due to the lack of commercial standards for quantitative analysis.

In addition, we did not have data on the diets of athletes to substantiate in more detail the relationship between hyperactive reactions to foods and physical activity.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/data8010003/s1. The repository contains files with the results of the mass spectrometric analysis (Supplementary S1) and the results of the ELISA (Supplementary S2). Supplementary S1 includes two tables: Table S1. Amino acids detected in the blood plasma of athletes before exercise and after recovery, and Table S2. Hormones detected in the blood plasma of athletes before exercise and after recovery. Supplementary S2 contains information about the ELISA technique and results of the determination of hyperreactive reaction to food allergens. Table S3. Food allergens in the Reagent Kit for semi-quantitative enzyme immunoassay for allergen-specific IgG antibodies. Table S4. Interpretation of ELISA results. Figure S1. Results of quantitative determination of the content of IgG class antibodies in the blood plasma of athletes (the number of technical repetitions is 3). Allergens are presented for which the IgG concentration was greater than 0.88 μg/mL (hyperreactivity to food allergens). An asterisk indicates allergens for which the calculated IgG concentration was 0 μg/mL. The yellow and blue zones correspond to a mild and moderate negative allergic reaction, respectively. The green and pink zones are high and very high allergic reactions, respectively. Figure S2. The occurrence of an allergic reaction to food components before exercise (a) and after a recovery period of 24 h (b).


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Institutional Review Board Statement: This study was approved by the Board for Ethical Questions at the A. I. Burnazyan State Research Center of the FMBA of Russia (Protocol №40 from 18.11.2020).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: Data were deposited at the Mendeley Data, V1, Malsagova, Kristina (2022), “Assessment of the state of the athlete’s molecular profile after short-term exercise”, doi: 10.17632/nkn4skdbrgr.1.

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Conflicts of Interest: The authors declare no conflict of interest.

References


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