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Sportomics: metabolomics applied to sports. The new revolution?

T. BONGIOVANNI[1], R. PINTUS[2], A. DESSÌ[2], A. NOTO[2], S. SARDO[3], G. FINCO[3], G. CORSELLO[4], V. FANOS[2]

[1]Nutrition, Hydration & Body Composition Department, Parma Calcio 1913, Parma, Italy
[2]Neonatal Intensive Care Unit, Neonatal Pathology and Neonatal Section, University of Cagliari, Policlinico Universitario, Cagliari, Italy
[3]Department of Medical Sciences and Public Health, University of Cagliari, Policlinico Universitario, Cagliari, Italy
[4]Operative Unit of Pediatrics and Neonatal Intensive Therapy, Mother and Child Department, University of Palermo, Palermo, Italy

Tindaro Bongiovanni and *Roberta Pintus* contributed equally to this review

Abstract. – Sportomics is the application of metabolomics in sports to investigate the metabolic effects of physical exercise on individuals, whether they are professional athletes or not. Metabolomics is one of the "omics" sciences that provide a picture of the metabolic state of a person in physiological or pathological conditions. This is achieved through the analysis of metabolites present in a biological fluid, such as saliva, blood, feces, and urine. The authors revised the recent literature concerning this topic and discussed the useful information that sportomics can provide and the limits of the current experimental settings. Furthermore, in the future, sportomics analyses could be used to prevent and manage injuries as it would be known in advance if an athlete is more prone to experience muscular damage or fatigue. Following more trials, it would also be possible to set the best diet and training programs to get the best performances out of the athletes. Moreover, based on their metabolic profiles, both adults and children could choose tailored physical training in order to preserve and improve their health.

Key Words:
Sportomics, Metabolomics, Exercise physiology.

Introduction

It is well known that participation in sports and exercise determines modifications in the human physiological state.

Exercise, as an external challenge to the human metabolome, creates an immediate response (the turn-over rate being only some seconds) across this biological matrix, which unlike studies investigating the effects of fasting, have been shown to exhibit large inter-subject variability[1].

Physical stress response during or after a training session or a sport challenge alters the equilibrium of the biochemical internal environment, which means that it affects the rate of production or synthesis and the kinetics of the metabolites that are related to exercise intensity and/or muscle damage[2].

Nowadays, the number of metabolomics studies in sport is limited, but they show that resistance exercise indicates that continue and intense training lead to modifications in hundreds of metabolites in biological fluids, in particular, those associated with extensive lipid mobilization and oxidation. Metabolomics is an all-encompassing approach even in this type of investigation and could provide new insights and novel biomarkers with applications to the individual athlete level. Furthermore, the effects on the athletes' metabolism during or after a particular nutritional regimen or a given training session can be extensively studied with this technique[3].

For instance, intense training leads to increased levels of metabolites associated with carnitine metabolism and long chain, dicarboxylate, and essential fatty acid metabolism[4], while short-term extensive exercise causes shifts of metabolites related to muscle energy consumption and production[5].

Metabolomics is a holistic, hypothesis-free approach to the investigation of metabolome modification in different pathophysiological situations

Corresponding Author: Roberta Pintus, MSc; e-mail: gomberta@icloud.com

through powerful data acquisition and advanced data processing techniques that detect a large number of metabolites at the same time. In this context, it can give further insights concerning biochemical modifications during physical exercise[6].

Through the analysis of biomolecules present in tissue samples, cellular extract and biological fluids, such as saliva, urine, blood, and sweat, several hypotheses can be made concerning the effects of physical exercise or nutrition on the metabolic pathways[7].

The analytical methodologies used in metabolomics studies are: liquid chromatography coupled with mass spectrometry (LC-MS) and gas chromatography coupled with mass spectrometry (GC-MS and nuclear magnetic resonance (^{1}H-NMR).

There can be two types of metabolomics investigations: targeted and untargeted. Targeted analyses are used to detect metabolites related to specific biological processes/pathways that affect a biological function of interest. These methodologies have been developed for the identification and quantification of amino acids, glycerophospholipids, and acylcarnitines, that are related to cell energy metabolism. While, non-targeted metabolomics (also known as unbiased, global, or discovery metabolomics) employs a wide analytical collection and measurement techniques whereby all detectable metabolites are semi-quantified, collected, and prospected for biomarkers that are indicative of predefined conditions (for instance, metabolites that discriminate between diagnosis of diseased and healthy individuals). These detected biomarkers could indicate causative and/or associative relationships with a physiological state of interest, allowing the investigation of the effects on the metabolism of a given stimulus, providing an early diagnosis or prognosis and even to detect the efficacy of a specific therapeutic treatment. One of the biggest challenges of non-targeted metabolomics is the precise detection, identification, and quantification of a metabolite of interest. The application of modern high-resolution mass analyzers, such as time-of-flight and the Orbitrap, has improved analyte identification with the ability to measure mass-to-charge ratios (m/z) with an error of fewer than 10 parts per million (<0.001%). Highly accurate measured masses can then be compared to open-source databases and consequently be given a tentative identification for a molecule's name/structure[8].

The first GC-MS based metabolomics studies were performed in the 1970s, but in 2010 it was still considered an emerging field. When some of the earliest exercised-based metabolomics studies with human athletes were published, sportomics was born[3].

Sportomics: a Sneak Peek on Physical Exercise

Sportomics is the use of "-omics" sciences in sports and can be combined with clinical laboratory analyses. In general, a sportomics approach mimics the real challenges and conditions faced during sports training and competition. This holistic approach (*ex post facto* design) is a non-target analysis using a top-down study model that requires the analysis of large datasets.

An important issue in sportomics is statistics: useful and fundamental statistical option is to use non-target analysis (NTA), principal component analysis (PCA), and probabilistic latent semantic analysis (PLSA); these techniques can be combined to facilitate a better understanding of metabolites and biomarkers during sportomics research[9].

In 2011, Resende et al[10] proposed the concept of sportomics for the first time in a publication, which examined metabolism and cellular responses to different interventions in a windsurfer. The study showed that the branched chain amino acids (BCAA) isoleucine, leucine, and valine were consumed, and the concentration of these BCAAs in the blood decreased by 50% compared to the resting level during the first round of windsurfing. Even after rest, these concentrations did not increase, reaching approximately 30% of their resting concentration after the second round of exercise. Aromatic amino acids were consumed at lower levels and decreased less than BCAAs.

A brief summary of the most important studies concerning sportomics with the main results are listed in Table I.

Since untargeted sportomics is non-hypothesis-driven research on an individual's metabolite changes during sports and exercise, can aid athletes, coaches, technical, and medical staff[9]. For example, de Almeida et al[26] and Prado et al[27] proposed that acute use of KAAA (ketoanalogues associated with amino acids) can lower the ammonemia increase caused by endurance exercise in humans and 28. Gonçalves et al[28] showed that an increase in ammonemia and a corresponding rise in lymphocytes are both decreased through arginine supplementation.

Table I. Most important sportomics studies with main findings.

Authors	Athletes	Samples	Methods	Metabolites results
Yan (2009)[11]	16 male professional rowers of different ages, 12 controls	Serum	GC/TOF-MS	Metabolites responsible for classification based on age and training level: alanine, lactate, beta-dimethyl glucopyranoside, pyroglutamic acid, cysteine, glutamic acid, citric acid, free fatty acids, valine, glutamine
Pechilvanis (2010)[6]	12 men, short run	Urine	^{1}H-NMR	Modification in lactate, pyruvate, alanine, and BCAA
Lehmann (2010)[12]	29 healthy non-professional runners	Plasma	LC-MS	Octanoyl-, decanoyl-, and dodecanoyl-carnitine
Lewis (2010)[13]	25 Boston marathon runners *vs.* 302 longitudinal cohort study subjects	Plasma	LC-MS	↑Glycerol, niacinamide, glucose-6-phosphate, pantothenate, succinate
Enea (2010)[5]	22 women 10 untrained 12 trained short term intense physical exercise	Urine	^{1}H-NMR	↓Acetate in trained athletes
Ciborowski (2010)[14]	20 divers	Plasma	LC-MS	↑Lysophosphatidylcholines, lysophosphatydiletanholamine, lysoplasmalogens, and hemolysis related compounds
Zauber (2012)[15]	1060 km no stop cycling events	Saliva	LC-MS	↑Creatinine, glucose and antioxidative mechanisms related metabolites
Yde (2013)[16]	12 milk protein *vs.* 8 control in high resistance exercise	Plasma	^{1}H-NMR	No difference
Morris (2013)[17]	214 healthy adults divided according to their fitness levels	Urine	^{1}H-NMR	↑Amino acids in the low fitness group
Mukherjee (2014)[18]	9 competitive cyclists *vs.* 8 minimally active males 50-60 years old	Urine	^{1}H-NMR	Different concentration of: 2-hydroxyisovalerate, acetate, malonate, hypoxanthine, formate, glycine, fumarate, tryptophan
Nieman (2014)[19]	19 trained male cyclists	Plasma	GC-MS LC-MS	13- and 9-hydroxyoctadecanoic acid
Peake (2014)[20]	20 cyclist and triathletes, different training protocols	Plasma	GC-MS	↑ TCA intermediates, monounsaturated fatty acids after high-intensity training ↓Amino acids in both moderate and high-intensity training
Lou (2014)[21]	6 healthy sedentary man from 0 to 3000 m altitude	Urine	LC-TOF/MS	1-methyladenosine, 5-methylthoiadenosine 3-indoleacetic and L-glutamic acid
Wang (2015)[22]	12 professional half pipe snowboarders	Serum	GC/ TOF-MS	TMAO, phenylalanine lactate, alanine, trimethylamine, malonate, taurine, glycine
Daskalaki (2015)[1]	3 physically active males	Urine	LC-MS	Modification in purine, tryptophan, carnitine, cortisol and androgen pathways, amino acid oxidation and gut microbiota related metabolites
Karl (2017)[23]	25 male soldiers, 4 days ski-march	Plasma	UPLC-MS	↑Free fatty acids, TCA metabolites and BCAA ↓Monoacylglycerols, lipid metabolism

Table continued

Table I. *Continued.* Most important sportomics studies with main findings.

Authors	Athletes	Samples	Methods	Metabolites results
Messier (2017)[24]	20 endurance exercise at sea level and 2150 m above	Plasma	^{1}H-NMR	↓Glucose, glutamine, alanine, and BCAA at 2150 m
Berton (2017)[25]	10 young male athletes, resistance exercise	Serum	^{1}H-NMR	↑ 2-hydroxybutyrate, 2-oxoisocaproate, 3-hydroxyisobutirate, alanine, hypoxanthine, pyruvate, succinate ↓Isoleucine, leucine, lysine, ornithine, valine
Nieman (2017)[3]	24 male runners to exhaustion	Plasma	UHPLC-MS/MS	↑Carnitine, 13 methylmuristic acid, sebacate

Camerino et al[29] suggested that KAAA supplementation decreases ammonia concentration during high-intensity exercise but does not affect physical or cognitive-motor performances under a low heat stress environment.

Smith and Hill[30], observed changes for compounds related to ATP-PCr and glycolytic systems and recently Dudzinska et al[31] showed that a standardized physical exercise with increasing intensity leads to an increase in IMP (inosine nucleotides) concentration in red blood cells immediately after exercise which, with a significant increase in the ATP/ADP and ADP/AMP ratios, indicates an increase in the phosphorylation of AMP and ADP to ATP.

Yan et al[11] demonstrated that long-term endurance and strength training exert different effects on the metabolism of athletes of different exercise seniority, and training stage-related trajectory of the two groups of athletes was clearly shown along with training time. However, most of these variations were not observed by common biochemical parameters. The identified metabolites contributing to the classification included alanine, lactate, beta-d-methylglucopyranoside, pyroglutamic acid, cysteine, glutamic acid, citric acid, free fatty acids, valine, glutamine, phenylalanine, tyrosine, and so on, which were involved in glucose metabolism, oxidative stress, energy metabolism, lipid metabolism, and amino acid metabolism.

Santone et al[32] investigated the variation in metabolic content of saliva in the presence of a physical stress (the Yo-Yo IR test) in fourteen professional soccer players from the Italian Lega Pro team (C1). For every athlete two samples of saliva were collected: one before and one after the test. The NMR spectra of saliva offer a metabolites profiling which was analyzed by Principal Component Analysis as a blind test. Among the meaningful metabolite variations, authors identified glucose, citrate, acetate, leucine, ornithine, myo-inositol, lactate, and glutamate.

Enea et al[5] explored the profile of metabolite changes due to acute physical exercise[5]. They described a global picture of the effects of two types of exhaustive exercise (short term and prolonged) on the metabolic urinary profile of untrained (ten recreational women) and (12 elite athletes) trained women. They found that creatinine excretion is not affected by physical exercise in the urine. Creatinine concentration is associated with diet and muscle mass, but they observed the difference in creatinine excretion between trained and untrained women. TMAO (trimethylamine-N-oxide, a biomarker of ischemic medullar renal injury) was at a very low level both at rest and after exercise. They observed an increase in urine excretion of alanine, hypoxanthine, lactate, acetate, and lactate/pyruvate ratio (suggesting tissue hypoxia) after the short-term intensive exercise test. Prolonged exercise does not change urinary metabolic profiles.

A pilot study was conducted on three physically active, non-smoking males, engaged in a two-day (37 h) well-designed trial, where aerobic exercise was performed on the first day and athletes rested on the second day[1]. The urine samples were collected at regular intervals across five time points every day and showed that significant metabolites involved in different pathways were affected by exercise: purine pathway, tryptophan metabolism, carnitine (increase of fatty acid carnitine conjugates), glucose metabolism with an increase of pyruvate after exercise, increase of some microflora's synthesis metabolites, as well as an increase of keto acids. The final major group varying between first pre-exercise and post was a series of urinary steroid metabolites as urocortisol glucuronide[1].

A non-targeted, 1H NMR-based metabonomic analysis of urine samples obtained from twelve young, moderately trained, healthy males before and after two exercise sessions (repeated sprints), differing in the duration of the rest interval (10 seconds or 1 minute) between repeated efforts, showed[6] that physical exercise caused dramatic changes in the urine metabolome; hence, post exercise urine provided very different profiles compared to pre-exercise urine. Even after the exclusion of the lactate signal, PCA alone could differentiate from pre-exercise to post-exercise urine. Separation of the 10 seconds from the 1-minute rest interval was mainly due to lactate, pyruvate, alanine, compounds of the Krebs cycle, 2-oxoacids of BCAA, and 2-hydroxybutyrate. All of these metabolites increased more with the short compared to the long interval, thus supporting the hypothesis that the former elicited greater metabolic disturbances than the latter as a result of the very limited time available for recovery[6].

Coelho et al[2] used the sportomics approach to evaluate biochemical and hematological changes in response to a combined training session in four male world-class rowers.

More recently, a metabolomic study by Messier et al[24] found that, at similar exercise intensity, substrate use during endurance exercise differed between SL (sea level) and moderate altitude of 2150 meters. It appears that BCAAs were used to maintain glycaemia during endurance exercise at moderate altitude.

Recent research has generated fascinating metabolomic data; Lou et al[21] found changes in energy pathways (lipid metabolism), and an increase in purine metabolic products at altitudes of 3,000-4,000 meters in six healthy, sedentary men, when compared with a condition of normoxia.

In 2017, Berton et al[25] postulated that, to improve the understanding of the metabolic response after resistance exercise, the employment of metabolomics can be essential, allowing for a broader and better understanding of the metabolic response in a biological system. In addition, also due to their untargeted approach, metabolomics may allow for the identification of changes in concentrations of metabolites that have not yet been described, as well as generating new hypotheses (e.g., mechanism of muscle hypertrophy and energy supply) and the discovery of novel biomarkers.

Several researches used a metabolomic approach to gain a comprehensive view of the metabolome and its modulation by different types, intensity, duration of exercise. Published investigations[12,13,16,25,33] have focused on the metabolic changes inducted from endurance exercises and resistance exercises. Metabolomics approach was also used for supporting medicinal value[26]. Metabolomics approach, using gas chromatography mass spectrometry (GC-MS) and orthogonal partial least square discriminant analysis (OPLS-DA) was used to discriminate between chronic localized pain and chronic widespread pain[34].

NMR-based metabolomics investigations were applied to depict the metabolic profiles of urine samples, which were collected from young athletes at three-time points including the time before exercises, the time before a series of exhaustive physical exercises followed by short-term rest, and after the treatment of acupuncture, or taking an extended rest[35].

Nieman et al[19] used a metabolomics approach to study the changes in plasma of two bioactive oxidized linoleic acid metabolites: 13-hydroxy-octadecadienoic acid and 9-hydroxy-octadecadienoic acid following a 75-km cycling bout in 19 male cyclists. In the same year, Mukherjee et al[18] used an NMR spectroscopy-based metabolomics to characterize the metabolic adaptive pathways altered by exercise in veteran athletes, 50-60-year-old males, and age-matched untrained individuals.

An untargeted metabolomics approach was used to develop a compliance measure in urine to distinguish between two dietary patterns[36].

To gain insights into adaptive physiological processes and to study the variations in metabolites of energy mobilization, Zauber et al[15] report the adaptive processes through the collection of saliva, from male and female athletes, at four time points in the daytime, throughout a 1060 km nonstop cycling event and taking the saliva over 3 days before the event and throughout the following two days. Using a mass spectrometry-based approach, they found that during the workout there was an increase of proteins with redox-regulatory function and of lysozyme and amylase; while during the recovery phase, there was an increase of immunoglobulins.

The metabolomic approach was previously used[14] to evaluate the effect of increased pressure equivalent to diving at 30 and 60 meters for 30 minutes in two groups of divers using an untargeted approach with LC-MS fingerprinting of plasma.

As reported in a recent review, the metabolomics analysis revealed exercise-induced changes in 209 metabolites, with a predominance of post-ex-

ercise increase in plasma long-and medium-chain fatty acids, fatty acid oxidation products (dicarboxylate and monohydroxy fatty acids, acylcarnitines), and ketone bodies. Perturbations in many of these metabolites were still apparent in endurance athletes after 14 h of recovery. The relationship between increases in plasma IL-6 and these lipid metabolites were also tested, but no viable statistical model could be established[3].

In a recent study, Nieman et al[4] use metabolomics profiling and bioinformatics technologies to evaluate the relationship of exercise-induced increases in plasma IL-6 and lipid-related metabolites in 24 athletes who ran on treadmills to exhaustion at high intensity.

Global measurement of metabolites could be applied to help understand the athlete's current state, such as fatigue, physical capacity, etc., or for use in aiding prediction of future events, such as talent identification, onset of illness, susceptibility to injury or impaired physical performance[8].

Karl et al[23] showed that high physical activity and a short-term but severe energy deficit, in 25 male soldiers, elicited pronounced changes in plasma metabolite profiles that were consistent with an increase in energy metabolism, lipolysis, fatty acid oxidation, and ketogenesis. Of 737 identified metabolites (performing nontargeted global metabolite profiling in plasma), 478 changed during the training (13 km/day for 4 days, cross-country ski march carrying a backpack that weighted around 45 kg). Increases in 88% of the free fatty acids and 91% of the acylcarnitines and decrease in 88% of the mono-and diacylglycerols detected within lipid metabolism pathways were observed. Smaller increases in 75% of the tricarboxylic acid cycle intermediates, and 50% of the branched amino acid metabolites detected were also observed[23].

Furthermore, Andersson Hall et al[37] used plasma NMR-metabolomics to investigate fat oxidation in different conditions (fasting, control and after an exercise and 2 h of fasting recovery). Authors showed for the first time that training after 2 hours of fasting recovery from a previous bout will increase fat oxidation showing that prior exercise is a potent stimulator of fat oxidation in a following training session.

Wang et al[22] explored how physical exercise (1-week of adaptative training followed by three weeks of training) impacts and changes metabolite profiles in twelve professional half-pipe snowboarders, using nontargeted 1H NMR-based metabolomics analyses of urine samples. Decreased levels of succinate were observed, as well as an increased TMAO, that may be due to increased food intake in daily diet. Moreover, heightened levels of hippurate and phenylalanine indicate a metabolic adaption in the athlete as a response to the elevated training load. Interesting, the PCA score plot showed the tendency to separate athletes with different training amounts and intensities.

Peake et al[20] compared the metabolic responses after high-intensity interval and continuous, moderately intense cycling matched for total workload. Targeted gas chromatography-mass spectrometry (GS-MS)-based metabolomic analysis was used to assess changes, in blood samples, in tricarboxylic acid (TCA) intermediates, fatty acids, amino acids, ACTH, cortisol, catecholamine, growth hormone, glucagon, IL-6, and insulin in ten well-trained male cyclists and triathletes. The results showed that of 49 metabolites identified and quantified in plasma, 11 changed after both two types of exercise: high-intensity interval training (HIIT) and continuous, moderate-intensity cycling training, 13 changed only after HIIT, and 5 changed only after moderate-intensity cycling exercises. Notable changes included substantial increases in tricarboxylic acid intermediates and monounsaturated fatty acids after HIIT and marked decreases in amino acids during recovery from both trials. Plasma ACTH, cortisol, and GH were all higher immediately after HIIT. Morris et al[17] examined the relationship between fitness level, substrate oxidation rates, and the metabolic profile in 214 healthy adults. The metabolomic analysis showed that adults with increased fitness levels and raised fat oxidation rate during exercise excreted less amino acids than adults with lower fitness levels and higher insulin resistance.

Conclusions and Future Perspectives

Over the past 7 years sportomics was used to investigate a wide range of sports and physical exercise, highlighting the metabolic shifts in athletes of all ages and with different level of fitness. Several biomarkers of oxidative stress, muscular damage, and energy deficit could be proposed in the future in order to prevent injuries and fatigue syndrome.

In addition, recent advantages in metabolomics will add metabolites with autocrine, paracrine or endocrine function to the contraction-induced secretome of the skeletal muscle. The identification

of these metabolites will lead to a more comprehensive view described by a new myo(metabo) kinome consisting of peptides, proteins, and metabolites[38].

The sportomics approach may be complementary to the current methods of studying and monitoring and athlete's state of fatigue and physical performance. It could also be used to predict future events, such as onset of illness, talent identification, and injury susceptibility[39]. Hence, it would be possible for everyone to choose the best physical exercise or sport (particularly important during infancy and childhood to preserve and improve the health status) and personalized nutrition tailored on someone's metabolomic picture[40].

Further research must be carefully performed to correctly collect and analyze samples, allowing a reliable statistical interpretation of the data. The major problem for these types of analyses in exercise and sport science is the high cost of the purchase and maintenance of NMR spectroscopy and MS systems, with trained personnel required for the every-day functioning of the machinery. A further issue is the space and provision required to operate these forms of machinery. Efforts are necessary to overcome these issues, and portable, compact mass spectrometers can be a solution to reduce costs and to translate from laboratory to field investigations in exercise and sport.

Conflict of Interests

The Authors declare that they have no conflict of interests.

Authors' contributions

TB reviewed the recent literature. RP analyzed and organized data in table. All other authors read and approved the final manuscript.

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