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Review

Footballomics: urinary metabolomics in adolescents and athletes playing football (soccer) – Review of the literature and practical approach

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Abstract

Sportomics constitutes a true "athlete's biological passport" for the systematic study of sport-induced responses and adaptations at any biological level. Specifically, metabolomics is a powerful investigative technology that allows the study of changes in an individual's metabolome in real time, whether under physiological or pathological conditions and/or as a result of individual exposure to epigenetic factors such as environment, diet, and drug use. The specific subject of this contribution is metabolomics applied to football (soccer), which we call "footballomics". In particular, metabolomics has the potential to provide new information and insights into the complex biological mechanisms that contribute to sports injuries and identify new targets for intervention.

The purpose of this review is to focus on the 12 studies of urinary metabolomics in football players in both adolescence (n = 3) and adulthood (n = 9, of which 1 included adolescents) in order to identify a panel of urinary metabolites useful in practice to prevent injury, monitor functional recovery after fatigue, and optimize athletic training and sports performance.

A practical table suggests important metabolites increased or decreased in urine (and other biofluids) specific to football players (acylcarnitine, alanine, citric acid, 2- and 3-hydroxybutyric acid, 5-hydroxytryptophan, guanidoacetic acid, hypoxanthine, hippurate, lactate, methylhistidine, trimethylamine-N-oxide), that can help in monitoring fatigue and recovery, prevent injuries, optimize performance.

Important elements to consider are: baseline value of metabolites, changes over time (e.g., weekly), changes before and after game, changes during the season, abrupt changes in concentration, correlations with Global Positioning System (GPS) and subjective Rating of Perceived Exertion (RPE), relationship with diet, relationship with any supplement, overall metabolite picture, anticipation of injury and/or illness.

It is not difficult to foresee a bright future for metabolomics in the world of football.

Keywords

Metabolomics, sportomics, footballomics, football, soccer, injury prevention, fatigue, recovery, performance, review, practical approach.

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Introduction

The "omics" sciences encompass a broad spectrum of disciplines that study the relationship between genotype and phenotype. Sportomics combines "omics" sciences (genomics, transcriptomics, proteomics, metabolomics, etc.) with classical clinical laboratory analyses to assess the actual biological conditions faced during sports training and competition [1, 2].

Sportomics constitutes a true "athlete's biological passport" for the systematic study of sport-induced responses and adaptations at any biological level [3, 4].

Specifically, metabolomics is a powerful investigative technology that allows the study of changes in an individual's metabolome in real time, whether under physiological or pathological conditions and/or as a result of individual exposure to epigenetic factors such as environment, diet, and drug use. The metabolome, i.e., the set of metabolite changes over time, can be analyzed by noninvasive methods, as we make use of the collection of biological fluids such as urine, blood, saliva, and feces. Sample analysis techniques can be qualitative or quantitative, such as magnetic resonance spectroscopy and gas or liquid chromatography coupled with mass spectrometry (GC-MS or LC-MS, respectively) [5, 6].

The potential applications of this technique in the medical field are many, and in recent years there has been an exponential increase in studies regarding the application of metabolomics in sports [7] and medicine.

Hints on workload management

Workload or "load" is defined as the cumulative amount of physiological, psychological and/or mechanical stress applied to the human biological system as a whole during multiple training sessions or competitions over a defined period of time [8]. Allostatic load is price for the organism to continuously adapt to stimuli from the external and internal environment to maintain homeostasis. Allostatic load is the accumulation of the body's response to stress applied (sport-related and no sport-related) that leads to wear and tear of tissues and organs that could lead to disease [8].

Workloads can be measured as external or internal loads or a combination of both.

External load quantifies the amount of work done by the athlete such as training frequency and time, distance traveled, accelerometer loads, jumps performed, balls thrown or tossed, number of games played, movement analysis over time, neuromuscular function or watts produced. It is commonly measured with the Global Positioning System (GPS). It has been shown that there is a direct correlation between external load and muscle damage in male football players [9].

Internal load measures physiological and psychological stresses for the athlete. These include objective parameters (such as heart rate) and subjective parameters (such as the Rating of the Perceived Exertion [RPE] scale). Metabolomics (understood as changes in the levels of specific metabolites in response to exercise) is the most appropriate tool for assessing internal load [10]. Indeed, metabolomics provides a unique "fingerprint" of cellular activity, offering a broader spectrum than traditional assays, on individual metabolites. The potential of metabolomics in bridging the gap between genotype and phenotype is remarkable [11-13].

Footballomics

The specific subject of this contribution is metabolomics applied to football (soccer), which we call "footballomics". As early as today, metabolomic analysis can enable important goals to be achieved in the world of football, shown in **Tab. 1**.

It relies on analytical methods with high processing power to analyze large volumes of data and is a promising field of investigation in translational sports medicine [1-4].

Table 1. Important goals in the world of football (soccer) achievable with metabolomics.

• Injury prevention
• Anticipation of performance potential
• Assessment of the athlete's fitness and stamina (aimed, for example, at considering the possibility of playing the full 90 minutes of a football match effectively)
• Improvement of sports performance through personalized nutrition
• Improvement of sports performance through personalized use of supplements, particularly probiotics
• Optimization of physiotherapy strategies
• Choice of the right sport for the child based on individual metabolic characteristics
• Identification among junior players of the potential "champions" of tomorrow (identifying "the makings of a champion" based on an outlier metabolome)

The use of omics data provides a comprehensive understanding of the biological processes and systems underlying athletic performance and injury risk in order to finally identify new targets for intervention. Futbol Club Barcelona (FC Barcelona) used sportomics to understand exercise-induced metabolic changes and the relationship with acute and chronic fatigue in its players. This helped to study the association between internal and external load indicators during training or playing sessions and to estimate the risk of injury [4]. Consecutive intense exercise leads to symptoms of fatigue in athletes that need to be closely monitored.

In particular, omics data have the potential to provide new information and insights into the complex biological mechanisms that contribute to sports injuries and identify new targets for intervention [14].

However, injuries have multiple causes and risk factors, and the debate about which omics variables to study (and under which conditions) is ongoing. The most important question of predicting injury risk is still unresolved.

In addition, the analyses must be gender-specific, as there are substantial biological and injury rate differences between men and women [10]. Unfortunately, the most relevant omics variables for women have not yet been established, as largely existing models are biased toward men or a mix of both sexes.

When analyzing data from female athletes, it is necessary to incorporate specific factors such as menstrual cycle dynamics and hormonal fluctuations. The menstrual cycle profoundly influences various physiological processes, including metabolism, immune function, and musculoskeletal health, which are linked to injury susceptibility. Therefore, integrating menstrual cycle data into predictive models can provide valuable information on the dynamic interaction between hormonal fluctuations and injury risk profiles. In addition, considering the impact of hormones, such as estrogen and progesterone, on ligament laxity, muscle strength, and neuromuscular control is critical to a comprehensive understanding of injury susceptibility in female athletes [4].

Literature review

A detailed systematic review on metabolomics and physical activity in football has been published very recently. It consists of 21 experimental studies involving 637 individuals (491 male, 123 female, while one study did not report gender). The authors studied different biological matrices: urine (n = 12), serum (n = 2), plasma (n = 2), saliva (n = 5), sweat (n = 1), and erythrocyte membrane (n = 1). It should be noted that, in one study, plasma, urine and saliva were investigated simultaneously. As can be observed, urine was the most studied biofluid [15].

This systematic review is not without limitations (including variability in biological matrices, analytical methods, and types of interventions) that limit the ability to conduct a meta-analysis. In addition, the literature produced to date has predominantly focused on male athletes [15]. Finally, there are very few studies involving adolescents who would potentially need more studies and controls regarding fatigue endurance compared to adults.

The purpose of the present review is to focus on the 12 studies of urinary metabolomics in football players in both adolescence (n = 3) and adulthood (n = 9, of which 1 included adolescents) in order to identify a panel of urinary metabolites useful in practice to prevent injury, monitor functional recovery after fatigue, and optimize athletic training and sports performance. It is not surprising the amount of study by far prevalent in urine, given the ease of collection even in the locker room, the easier way of storing and transporting samples, and the reliability in assessing the body's overall metabolism [16]. In the near future, sportomics could be applied in a personalized way to choose the best diet and training program for the individual to achieve the best possible performance and

prevent injuries in athletes in all sports disciplines, including football [1, 2].

In one of our studies [17], we performed an untargeted nuclear magnetic resonance spectroscopy (1H-NMR) analysis of the urine of 21 professional football players (age 25 ± 4 years), all male, collected at 3 different times during the pre-season preparation period (second, sixth, and sixteenth day of training) before the start of the Serie A (first division) championship in Italy. To our knowledge, this is the first study performed on urines collected from elite soccer players during the preparation phase. The urine profile of the players changed during the observation period. In particular, significant changes were observed for trimethylamine-N-oxide (TMAO), dimethylamine (DMA), methylhistidine, hippuric acid, hypoxanthine, guanidinoacetic acid, 3-hydroxybutyric acid, citric acid, and creatine. These metabolites have been associated with diet, training, and microbiota.

TMAO results from gut microbiota metabolism of carnitine, choline, and choline-containing compounds in the diet. In addition, its urinary concentration has been associated with urinary nitrogen excretion and thus protein intake. TMAO has also been proposed as a biomarker of cardiac function. Therefore, changes, particularly if abrupt, in its concentration could also be an indication of changes in cardiac function, although not necessarily of pathological origin. This phenomenon could lead to a consequent oxygen deficit in muscle tissue that may make athletes more prone to muscle accidents or even sudden death on the field.

DMA is a primarily food-derived metabolite. Its urinary concentration is associated with fish ingestion. Human consumption of fish can lead to at least a 4-fold increase in urinary excretion of DMA in humans. DMA is derived from TMAO but is also present in fibroblasts. This metabolite has been associated with rhabdomyolysis, which is caused by extreme muscle exertion and impaired renal function. Therefore, it may also be a potential indicator of muscle suffering, and monitoring this metabolite in the urine of athletes could be useful in evaluating their muscle status and achieving an optimal exercise program.

3-hydroxybutyric acid is a ketone body derived from fatty acid metabolism. It is synthesized in the liver from acetyl-CoA. Ketone bodies are the main source of energy for the brain, skeletal muscles, and heart in hypoglycemia and during endurance exercise. Its production increases under conditions of anoxia. In a study of professional swimmers, increased urinary levels of ketone bodies were found after training sessions. It can be considered as an indicator of proper nutritional and physical preparation.

Hypoxanthine is derived from purine metabolism and is of plant origin (coffee and cocoa consumption). It is found in skeletal muscle and is the only metabolite that can be converted back into the adenine pool by the muscle enzyme. The net efflux of purine bases may reflect the loss of adenine nucleotide precursors in muscle. Several authors have argued that purine metabolism may reflect exercise-induced muscle adaptation. Indeed, the duration and intensity of exercise are the key parameters that determine post-exercise hypoxanthine concentrations. Hypoxanthine has been proposed as a predictor of sports performance in elite athletes, regardless of discipline, especially in sports characterized by sprinting.

Hippuric acid is a normal component of urine, and its excretion increases after consumption of phenolic compounds such as tea, wine, and fruit juices. Therefore, it is mainly related to diet, being a marker of host-gut microbial co-metabolism. It has also been associated with cardiovascular disease and obesity. A significant decrease in urinary hippuric acid was observed in soldiers after extensive military training, suggesting symptoms of developmental stress and gastrointestinal permeability.

Citric acid is a metabolite that belongs to the tricarboxylic acid cycle (TCA cycle). Its levels in urine may also be due to the consumption of sports drinks and fruits. This metabolite, along with DMA, has been linked to rhabdomyolysis.

Guanidinoacetic acid is involved in the urea cycle and is a precursor of creatine, an essential substrate for muscle energy metabolism. There are limited studies in the literature. It has been suggested to be a serum biomarker of intense exercise and fatigue. In addition, guanidinoacetic acid administration to physically active men increased creatine in the right vastus medialis by about 15%, suggesting possible use as a performance enhancer.

Quintas et al. [14] studied the association between external load, defined by variables obtained using Electronic Performance Tracking Systems (EPTS), and the urinary metabolome as a surrogate marker of metabolic adaptation to training.

Untargeted metabolic data from the urine and EPTS of 80 male professional football players (ages 16-21 years) included in a 5-point observational longitudinal study (preseason and at 3, 5, 8, 10 months of competition) were analyzed with ultra-high-performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry

(UHPLC-QTOF-MS). Steroid hormone metabolites, hypoxanthine metabolites, acetylated amino acids, intermediates in phenylalanine metabolism, tyrosine, tryptophan metabolites, and riboflavin were identified among the most relevant variables associated with external load. The ratio of players showing deviation from the partial least squares (PLS) model of exercise adaptation was higher among those who had sustained a muscle injury than among those who had not. These data may allow the development of metabolic models to identify professional football players at risk of developing muscle injury.

Kim et al.'s work [18] done in the Republic of Korea (2022) focused on the fact that during the off-season, Korean football players participate in the Winter Training Season (WTS) to develop endurance in running for the following season. For young football players (in this study, 14 males aged 10-13 years), adequate recovery time is necessary to prevent injury or muscle damage.

In this study, urinary metabolites in young players after 1, 5, and 10 days of WTS were analyzed using 1H-NMR in an untargeted manner, combined with multivariate analysis to suggest adequate recovery times for improving their football skills. A total of 79 metabolites were identified using PLS discriminant analysis (PLS-DA). Of these, 15 metabolites, including 1-methylnicotinamide, 3-indoxyl sulfate, galactarate, glutamate, glycerol, histamine, methylmalonate, maltose, N-phenylacetylglycine, trimethylamine, urea, 2-hydroxybutyrate, adenine, alanine, and lactate were significantly different from those from before WTS and were mainly involved in urea, purine nucleotide, and glucose-alanine cycles.

In this study, most of the selected metabolites increased 1 day after WTS and then returned to normal. However, 4 metabolites (adenine, 2-hydroxybutyrate, alanine, and lactate) had increased during the 5-day recovery period following WTS. Based on the excessive levels of ammonia, adenine, and lactate in urine, at least 5 days of recovery time may be considered adequate.

The objectives of the Rodas et al.'s study [19] of 51 professional athletes from FC Barcelona (28 females, age 25 ± 5, range 17-36; 23 males, age 25 ± 5, range 18-33) were 2-fold: a) to study the association between external loading (EPTS) and urinary metabolites as a surrogate for training adaptation and b) to evaluate the effect of sex on player load adaptation in professional football players. Progressive changes in the urinary metabolome associated with external loading were found in the analysis of amino acids and metabolites of tryptophan and phenylalanine.

Significant differences were demonstrated between the metabolic profiles in male and female teams regarding the effect of training.

The article by Gonzáles et al. [10] focused on elite women's football, aimed to develop an injury prediction model based on clinical, GPS and multiomics (genomics and metabolomics) data to better understand the factors associated with injuries in elite female football players. The prospective cohort study included 2 seasons (2019-20 and 2021-22) of noncontact injuries in 24 elite female players (ages 17-31) in the Spanish Premiership competition. The authors used GPS data to determine external workload, genomic data to capture genetic susceptibility, and metabolomic data to measure internal workload. As for the results, 40 noncontact injuries were recorded, the most frequent of which were muscle (63%) and ligamentous (20%) injuries.

Six genetic polymorphisms located in the *DCN*, *ADAMTS5*, *ESRRB*, *VEGFA*, and *MMP1* genes were found to be associated with the injuries. Regarding metabolomics, three metabolites were injury-related: beta-alanine, serotonin and 5-hydroxy-tryptophan (5-HTP). The model including baseline variables, genetic score and player load showed the best predictive ability (C-index: 0.74).

Dietary supplementation with beta-alanine may help reduce the risk of injury by improving muscle endurance and reducing muscle fatigue.

In addition, beta-alanine supplementation may increase fatigue carnosine concentration in muscles, which may buffer the accumulation of hydrogen ions during high-intensity exercise. This may delay the onset of muscle fatigue and improve muscle endurance, also helping to reduce the risk of injury.

In addition, high levels of 5-HTP, the serotonin precursor, have been associated with an increased risk of injury because it may increase serotonin production in the brain. Finally, high levels of 5-HTP may cause drowsiness and fatigue, reduce attention and reaction time.

The study underscores the fact that injury prevention is a crucial aspect of sports, particularly in high-performance settings such as elite football.

Gouveia et al.'s study [20] describes the untargeted urinary metabolomic profile of 14 professional female football athletes from an elite team in Brazil (ages 19-32 years). Identification of discriminatory metabolites occurred at 3 different pre- and immediate post-match times during one season of the league. Urine samples were ana-

lyzed using 1H-NMR. Forty-three metabolites were identified in the samples. Orthogonal PLS-DA (OPLS-DA) analyses demonstrated progressive separation between pre- and post-game conditions.

Eight metabolites were found to discriminate consistently at the 3 time points: formate, glycine, dimethylglycine, citrate, urea, trimethylamine, 3-hydroxyvalerate, and glycolic acid.

It should be noted that all of these metabolites, which are related to energy and protein metabolism and likely correlate with fatigue accumulation, were found to be higher in urine before the football game than after the game.

Formate, a metabolite involved in the processes of metabolic acidosis and energy production, is considered a marker of fatigue after exercise. In this study, formate was higher in urine before the game and decreased after the game in all 3 games played.

Glycine was also elevated before the game compared to after the game. This behavior, also confirmed by other authors, suggests a possible indication of impaired renal function related to post-exercise lactic acidosis.

Dimethylglycine also had the same trend, likely due to protein catabolism resulting from physical and muscular exertion.

Like creatinine, urea, trimethylamine, and 3-hydroxyvalerate may be related to renal function. Instead, they increase in renal dysfunction due to strenuous exercise.

Glycolic acid is an intermediate in glyoxylate metabolism, related to the Krebs cycle.

Lactate values (such as pyruvate and succinate) were found to be increased as expected. Citrate, on the other hand, which is considered a marker of post-exercise fatigue and is normally increased after exercise, unlike other studies, was found to be decreased after matches. The authors explain this finding as being due to the high participation of citrate in reduced aerobic metabolism (Krebs cycle), as witnessed by metabolic acidosis (elevated lactate) that reduces aerobic metabolism.

Marinho et al.'s study [21] aimed to assess the metabolic impact of Brazilian elite male players under 20 years of age (U-20) using the RPE assessment for the first time in the literature to discriminate urinary metabolomic sensitivity after 2 football matches separated by a short recovery interval.

Urine was collected immediately and then 20 hours after 2 football matches of elite Brazilian U-20 players. RPE was collected after the matches.

Results showed that metabolic pathways related to energy production, cellular damage and organic stresses were changed immediately after the match; 20 hours after matches, antioxidant and anti-inflammatory pathways related to cellular recovery (e.g., gallic acid, ascorbate, and betaine) were identified. The matrix of positive correlations between metabolites was more predominant and stronger after match 2 than after match 1. Athletes with higher RPE values showed a high metabolic profile related to muscle damage (e.g., creatine, creatinine, and glycine) and energy production (e.g., creatine, formate, pyruvate, 1,3-dihydroxyacetone) 20 hours after the football match. The key message is that a different metabolic profile was found between athletes with higher and lower RPE values.

In conclusion, the metabolomic analysis allowed us to observe the metabolic impacts of energy production and muscle damage. The correlation matrix indicated a greater predominance of positive and strong correlations between metabolites in the second batch than in the first, as expected.

Some clarifications are useful. Although formate is related to energy and anaerobic metabolism, its presence immediately after the match may be associated with alpha oxidation of branched-chain fatty acids given the presence of ketone bodies in urine. The appearance of 2-hydroxybutyrate, 3-hydroxybutyrate, and 3-hydroxyvalerate supports a possible change in the ketone body pathway, taking into account that these metabolites may represent an alternative source of energy during exercise, especially if intense and prolonged as that which occurs in a football match. Regarding changes in DMA, evident suggest that this metabolite is related to muscle stress during the football season [17]. Therefore, its change immediately after a match and 20 hours later may represent an increase in the muscle stress process due to the proximity with the match. This argument is consolidated by the presence of creatine and creatinine in urine. Gallic acid is a phenolic compound found in blackberry and grape, related to antioxidant action. Its activity may be associated with cell regeneration and maintenance processes essential for maintaining the integrity of muscle cell damaged during a match. Finally, regarding glycine, dimethylglycine, and taurine, these metabolites may be related to protein catabolism due to constant eccentric movements, e.g., accelerations and decelerations, a factor highly correlated with muscle tissue damage in sports.

França et al.'s study [22] used untargeted urinalysis of urine samples to study changes in metabolism

during a football game in 30 male junior professional football players. Samples were collected before and after the game and analyzed by LC-MS. The results showed significant changes in tyrosine metabolism. Exercise caused under-regulation of the metabolites 4-maleylacetoacetate and succinylacetone to 20% and 16%, respectively. 4-hydroxyphenylpyruvate was found to be upregulated by 26%. The concentration of hawkinsin and its metabolite 4-hydroxycyclohexyl acetate increased 6-fold. Several metabolic pathways of dihydroxyphenylalanine (DOPA) were also affected by exercise: DOPA and dopaquinone (increased 4- to 6-fold); 3-methoxytyrosine, indole-5,6-quinone, and melanin (downregulated by 1% to 25%); dopamine and tyramine (decreasing by up to 5% or 80%). Blood total carbon dioxide (TCO_2) decreased as did urinary glutathione and glutamate (40% and 10%, respectively) associated with a 2-fold increase in pyroglutamate. The study found unexpected similarities between exercise-induced changes in metabolism and the hereditary disorder hawkinsinuria, suggesting a possible transient condition called exercise-induced hawkinsinuria.

In Cao et al.'s paper [23], 12 adolescent football players, aged 14 to 16 years, underwent 3 combined training groups using a cycle ergometer, with subjective RPE as the fatigue criterion. Then the following indicators were measured in each training group: maximal oxygen consumption (VO_2max), maximal anaerobic power, and average anaerobic power. Urine samples were collected before and after training. GC-MS was performed for metabolomic analysis of the samples. There was no significant difference in VO_2max among the 3 groups. Compared with group 1, the maximal and average anaerobic power in group 3 decreased significantly ($p < 0.05$) at the end of training. Twenty-five important metabolites (3 upregulated, 22 downregulated) were finally selected. These different metabolites belonged to 5 metabolic pathways: glycine-serine-threonine metabolism, citrate cycle, tyrosine metabolism, nitrogen metabolism, and glycerophospholipid metabolism.

In the combined exercise of aerobic and anaerobic metabolism, adolescent football players showed a significant decrease in anaerobic metabolism capacity after fatigue. The metabolic mechanism of exercise fatigue was related to disturbances in amino acid and energy metabolism.

The study by Zhao et al. [24] evaluated high-intensity interval training (HIIT), which can elicit a greater training stimulus to improve maximal aerobic capacity and is often applied in professional sports training. Longitudinal multi-omics profiling, including metabolome and proteome, was performed on urine samples of 23 healthy young football players before and after HIIT exercise. Metabolomics revealed metabolomic changes during HIIT, including steroid hormone metabolites, amino acid biosynthesis, and relevant metabolites. A significant association was found between HIIT and urinary omics profiles, with alteration of metabolic pathways associated with long-term adaptation to training.

The work of Alzharani et al. [25] involved professional football players from South Arabia. Urine, plasma and saliva samples were collected 2 days before and 2 days after training. It was demonstrated for the first time, using a metabolomics approach in different biofluids, the response of metabolic profile after short-term training in young professional football players.

The most important finding of the work is that acylcarnitine may increase after exercise in football players, suggesting that they may burn lipids rather than glucose. The level of carnitine metabolites in plasma after exercise may therefore be an important indicator of fitness. Purine metabolites are considered an important indicator of the effects of exercise, especially hypoxanthine: activation of this metabolic pathway requires high physical activity. Hypoxanthine is increased more than twice in plasma and saliva and more than 3 times in urine as well as xanthine, the oxidation product of hypoxanthine, the concentration of which is doubled in plasma, confirming previous studies, and occurs in high intensity training and is higher in urine than in plasma. Adenine has also been found to be elevated in plasma and saliva. The majority of metabolites were found to be increased; however, this increase in metabolites was less than in other studies. In Rodas et al.'s 2022 work [19], the lower increase in urinary hypoxanthine concentrations as a function of total training load in the men's team compared to the women's team could indicate greater degradation of purine nucleotides or a less efficient hypoxanthine recovery process. Decreased carnitine activity has been associated with increased frailty in the elderly person. In conclusion, although metabolic changes can be observed after exercise in saliva and urine, plasma gives a more adequate picture in this paper [25].

Prado et al. [26] studied 30 male semi-professional soccer players (18-20 years old) over 2 consecutive days. Blood and urine samples were collected before and immediately after the matches. In capillary blood, glucose increased significantly by 35%, while urate increased by 16% and uremia by 17% without any change in creatinine.

Hypoxanthine and related metabolites were up-regulated in urine after a soccer match, suggesting increased deamination of adenosine monophosphate (AMP), producing ammonia and urate. Ammonia is known to be highly toxic and is related to fatigue during exercise. The authors conclude that during the football match there was an increase in the use of adenosine triphosphate (ATP) provided by the synthesis of adenosine diphosphate (ADP) via myokinase.

Overall, the Rodas et al.'s 2024 work [4] results demonstrate that the development of metabolic patterns of adaptation in professional football players can benefit from the separate analysis of women's and men's teams, demonstrating different adaptation to external load.

There is also the question of whether and how much the transition from adolescence to adulthood with regard to sports activity can lead to significant changes in metabolism and particularly in the metabolome of athletes.

Practical approach

A summary of the important urinary (and other biofluids) metabolomic markers specific to football players currently identified is shown in **Tab. 2**: it is a practical table that suggests metabolites increased or decreased in urine (and other biofluids) specific to football players (acylcarnitine, alanine, citric acid, 2- and 3-hydroxybutyric acid, 5-HTP, guanidoacetic acid, hypoxanthine, hippurate, lactate, methylhistidine, TMAO), that can help in monitoring fatigue and recovery, prevent injuries, optimize performance.

These markers can complement currently available biomarkers [27], significantly enhancing diagnostic and predictive capabilities for performance, disease, and injury.

Important elements to consider are: baseline value, changes over time (e.g., weekly), changes before and after game, changes during the season, abrupt changes in concentration, correlations with GPS and subjective RPE, relationship with diet, relationship with any supplements, overall metabolite picture, anticipation of injury and/or illness.

Relationships between metabolomics and microbiomics in sports exercise

The discussion of the relationships between metabolomics and microbiomics in sports exercise is beyond the scope of this contribution, but we feel it is necessary to make this mention. Over the past few years it has been shown that the gut microbiota and exercise are interconnected [28-31]. Moderate exercise has positive effects on athletes' health, such as a reduction in inflammation and gut permeability, along with improved body composition. It also induces positive changes in gut microbiota composition and microbial metabolites produced in the gastrointestinal tract. Conversely, intense exercise can increase intestinal permeability and associate with decreased intestinal mucus thickness, potentially allowing pathogens to enter the bloodstream and contributing to increased levels of inflammation. However, elite athletes appear to have higher gut microbial diversity, shifted toward bacterial species involved in amino acid biosynthesis and carbohydrate/fiber metabolism, consequently producing key metabolites such as short-chain fatty acids. In addition, rodent studies have shown a bidirectional relationship, with exercise affecting the composition of the gut microbiota while the microbiota can influence sports performance [31, 32]. As a result, there is extraordinary interest in the personalized use of probiotics in sports. Although scientific research has not yet assessed the complexity of the ergogenic effect of probiotics, they can certainly improve the health of the individual and modify athletic performance through: improved recovery from fatigue (it is very important to recognize fatigue before it turns into exhaustion), improved immune response, maintenance of gastrointestinal and lower respiratory tract health, and reduced muscle inflammation.

We suggest reading Pérez-Castillo et al.'s very recent and beautiful review from 2024 [33] in collaboration with Real Madrid Club de Fútbol (Real Madrid CF) staff, which analyzes the available data on the athlete's gut microbiota, discusses mechanisms involved in the bidirectional association between exercise and the gut environment, and assesses the role of the athletes' diet in this interaction and proposes a practical approach to doing so. The integration of microbiomics, metabolomics, and big data is of paramount importance, as anticipated in the past [28, 29].

Conclusions

Metabolomics is definitely a very promising technique in the world of football, and the new biomarkers could be used in clinical practice as a predictive diagnostic tool or to assess the efficacy and toxicity of a drug, the positive contribution of a

Table 2. Important metabolomic markers increased in urine (and other biofluids) specific to football (soccer) players.

Metabolite	Biological fluids	Biomarker of...	Clinical significance of variations
Methylhistidine	• Blood • Urine • Saliva	• Muscle protein turnover, optimization of nutrition and training programs	• Increased in case of muscle loss, intense training, fatigue • Decreased in case of injury recovery
2- and 3-hydroxybutyric acid	• Serum • Urine	• Physical preparation and nutrition	• Increased in case of physical activity, fasting, caloric restriction, ketogenic diet
Alanine	• Urine	• Functional recovery after fatigue • Injuries in females	• It increases with cumulative external load in both male and female players • After fatigue it increases for 3 days; after that, it decreases • Low concentrations: related to injuries • If supplemented: increase of muscle resistance and resistance to fatigue
Hypoxanthine	• Plasma • Urine • Saliva (It increases 3-folds in urine and 2-folds in plasma)	• Sport performance	• It reflects muscle adaptation to fatigue • Minor increase in males compared to females as a function of total external load
Acylcarnitine	• Plasma • Urine • Saliva (Plasma is more adequate compared to other biofluids)	• Fitness	• Increased in case of physical activity or fasting
5-hydroxytryptophan (5-HTP) (precursor of serotonin)	• Urine	• Injuires (no contact), especially in females	• An increased concentration indicates increased risk of injuries in females, since it can cause drowsiness and fatigue, reducing alertness and reaction • Possible differences between males and females
Lactate	• Serum • Urine	• The degree of tissue hypoxia • Injuries in females	• Increased concentration post-exercise • Especially increased in women, even in short games • After fatigue, it increases for 3 days; after that, it decreases
Trimethylamine-N-oxide (TMAO)	• Urine	• Diet and the intestinal microbiota • Cardiac function	• Significative variation: muscle injuries, sudden death (?)
Citric acid	• Urine	• Physical activity, post-exercise fatigue, rhabdomyolysis	• Elevated post exercise • It may be linked to consumption of sports drinks and fruit • Contrasting data: decreased post game
Guanidoacetic acid	• Urine	• Prolonged exercise and fatigue	• If supplemented: increase of muscle resistance and resistance to fatigue
Hippurate	• Urine	• Host/intestinal microbiota co-metabolism, associated to cardiovascular diseases	• It decreases after intense training, stress and intestinal permeability
Others: isoleucine, leucine, lysine, ornithine and valine	• Urine	• General metabolism	• Decreased after resistance exercise

supplement, so that a personalized treatment can be provided to the sportsman at his or her measure. In summary, we can conclude that footballomics is a great step toward personalized athletic preparation, to which football teams should pay great attention.

We have focused on urine because of the simplicity of collection in the locker room, the possibility of storing and transporting it at -20°, and the wealth of information about the body's overall metabolism.

Today there are already a dozen dosable metabolites available in urine that can be used in individualized athletic preparation for elite football players, optimization of sports performance, and injury prevention. Further studies are needed to translate this knowledge into daily practice. Their monitoring could be performed in elite football players in the preseason phase and then weekly once or twice, depending on commitments, ideally before or after the game.

Important elements to consider are: baseline value, changes over time (e.g., weekly), changes before and after game, changes during the season, abrupt changes in concentration, correlations with GPS and subjective RPE, relationship with diet, relationship with any supplements, overall metabolite picture, anticipation of injury and/or illness.

It is not difficult to foresee a bright future for metabolomics in the world of competitive sports, particularly in the world of football.

The concrete and applied interest of some top-level football teams, such as FC Barcelona and Real Madrid CF, is just one of the very important indicators of this prediction.

Abbreviations

1H-NMR: nuclear magnetic resonance spectroscopy
5-HTP: 5-hydroxy-tryptophan
ADP: adenosine diphosphate
AMP: adenosine monophosphate
ATP: adenosine triphosphate
DMA: dimethylamine
DOPA: dihydroxyphenylalanine
EPTS: Electronic Performance Tracking Systems
FC Barcelona: Futbol Club Barcelona
GC-MS: gas chromatography coupled with mass spectrometry
GPS: Global Positioning System
HIIT: high-intensity interval training
LC-MS: liquid chromatography coupled with mass spectrometry
OPLS-DA: orthogonal partial least squares discriminant analysis
PLS: partial least squares
PLS-DA: partial least squares discriminant analysis
Real Madrid CF: Real Madrid Club de Fútbol
RPE : Rating of Perceived Exertion
TCA cycle: tricarboxylic acid cycle
TCO_2: total carbon dioxide
TMAO: trimethylamine-N-oxide
UHPLC-QTOF-MS: ultra-high-performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry
VO_2max: maximal oxygen consumption
WTS: Winter Training Season

Declaration of interest

The Author declares that there is no conflict of interest.

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