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Sportomics: Building a new concept in metabolic studies and exercise science

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ABSTRACT

For more than a decade, we have used alternative approaches to understand metabolic responses to physical stress. In addition to classic laboratory studies (cell and animal models), we have used elite athletes and sports to examine metabolic stress. Our central question involves the ability of the body to protect the central nervous system from high and toxic ammonemia during acute and chronic exercise. Information about this problem can aid in understanding important signaling pathways, which may yield better ways to protect people who suffer from diseases that lead to hyperammonemia, such as liver failure, or to hypermetabolic states, such as cancer or thermal injury. We proposed a Sportomics approach to mimic the real challenges and conditions that are faced during sports training and competition. Sportomics is non-hypothesis-driven research on an individual's metabolite changes during sports and exercise. It is similar to metabolomics and other "-omics" approaches, but Sportomics focuses on sports as a metabolic challenge. Our study is holistic and top-down; we treat the data systematically and have generated a large computer-searchable database. We also propose that in-field metabolic analyses are important for understanding, supporting and training elite athletes. In this review, we discuss Sportomics history, problems, benefits and results. We included different weather conditions, such as temperature, wind and humidity, and diverse metabolic responses due to uneven sleep and eating behaviors near the time of the experiment. We are currently generating databases as well as data-mining principles and procedures to improve metabolomics and proteomics studies as well as adding genomics and transcriptomics studies to the Sportomics approach. We believe that this approach can fill a methodological gap between systems biology and translational medicine similar as a bench to the field approach.

1. Introduction

The biological roles of molecules have been of scientific interest since researchers began to study biochemistry. Advances in biochemical and biophysical methods have enabled us to better understand cells and organisms. In addition, during the past few decades, several new tools have become available further our understanding of biological molecules.

The genomics era initiated an increased capability to discover and analyze genes and their sequences. Proteomics is the study of protein expression in a predetermined biological matrix for a given state. This concept has been credited to Marc Wilkins and was first proposed in 1994. Due to the enormous amount of data generated by large-scale analytical techniques, the "-omics" sciences require a proportional effort in informatics data analyses. Mass spectrometry techniques in biological research have provided a new investigative approach to understanding life. With the advantages of new computational capabilities to manage large quantities of data, we began to use non-targeted analyses to compare different experimental conditions.

For more than a decade, we have used alternative approaches in our laboratory to understand metabolic responses to physical stress. We use exercise and diet protocols to promote changes in metabolism and study these changes. In addition to classical laboratory studies (cell and animal models), we have used elite athletes and established sports to examine metabolic stress.

Abbreviations: GC–MS, gas chromatography/mass spectrometry; ketoanalogues, herein, a commercial combination of amino acids, ketoacids and essential amino acids; NMDA, N-methyl-D-aspartate; Non-Target Analysis (NTA), mass spectrometry analysis of chemical components in a given matrix; leupeptin, N-acetyl-L-leucyl-L-leucyl-L-argininal, naturally occurring protease inhibitor; VO_{2max}, maximum oxygen consumption.

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"-Omics" approaches are high-throughput, data-driven, holistic and top-down methodologies. The suffix "-ome" indicates that all constituents are considered collectively in a given state; for example, genomics is the study of all genes, and metabolomics is the study of all metabolic processes. Thus, Sportomics is the use of "-omics" sciences with classic clinical laboratory analyses. In general, our study is holistic and top-down; we treat the data systemically and generate large quantities of data through great computational effort. We proposed a Sportomics approach to mimic the real challenges and conditions faced during sports training and competition. We believe that the studies we perform are -omics studies because they are part of a broad investigation using different techniques. 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 [1].

Here we describe the events that led us to propose the concept of Sportomics and how we developed it. We describe how an investigation of proteolysis inside of a neuron led us to study hyperammonemia and to pursuit a good model for investigating the effects of ammonia on the central nervous system (CNS). We attempt to understand cell metabolism as an integrated system and the relationships among many measured molecular species and cells. This investigation led us to use exercise as a feasible model and compel us to face the differences between laboratory protocols and field studies. We intend to discern certain advantages, disadvantages and considerations required to analyze these data. We also propose that in-field metabolic analyses are important for understanding, supporting and training elite athletes. In this review, we will discuss the history, problems, benefits and results of Sportomics.

2. In the beginning, the cell

The concentration of the neurotransmitter glutamate (Glu) is five orders of magnitude higher in the neuronal cytoplasm than

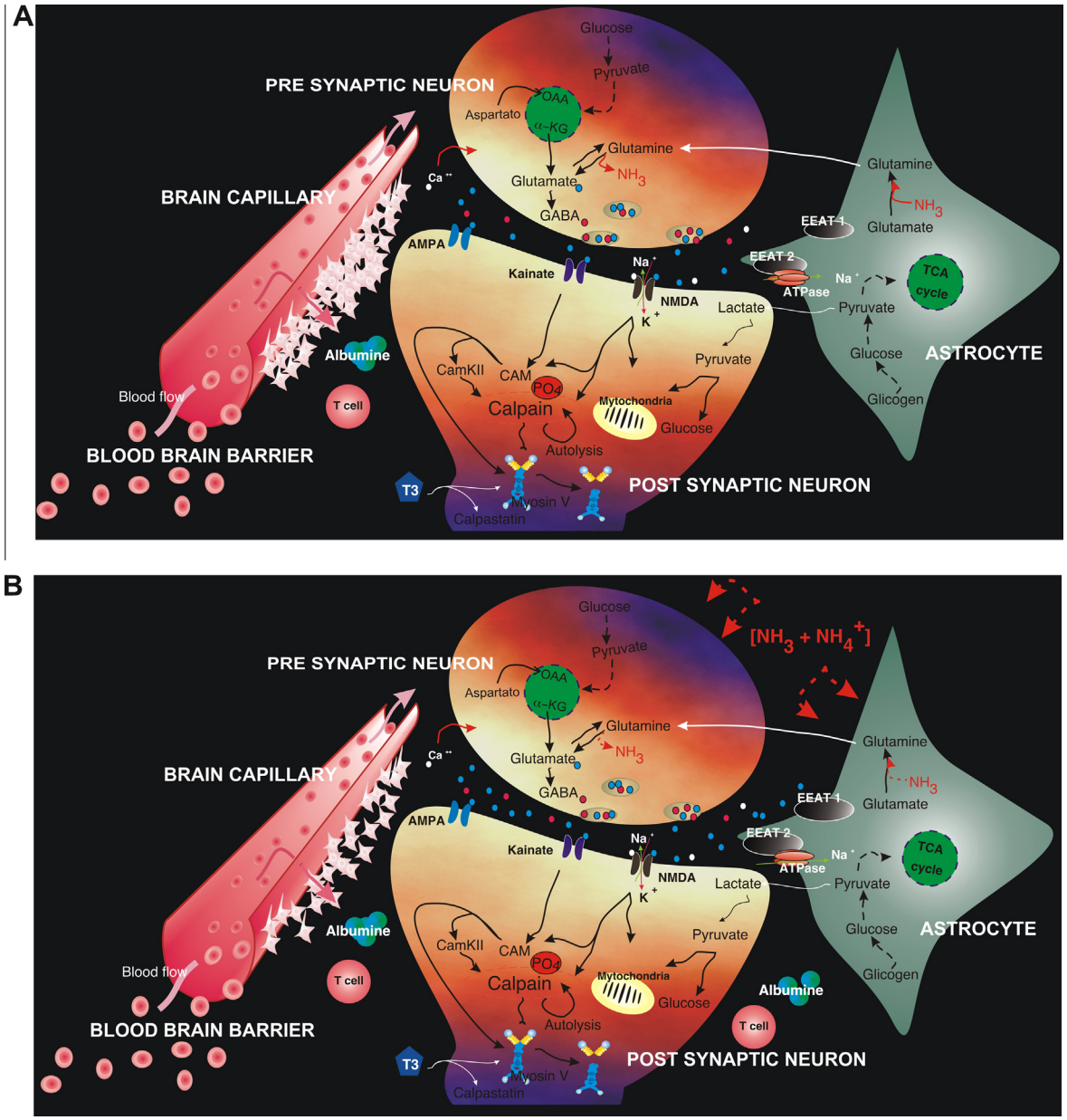

Fig. 1. Moving from the cell to Sportomics. Our working model on excitotoxicity and ammonia. (A) Rest; (B) Hyperammonemia. The model shows the activation of neuronal glutamate receptors and the effects of hyperammonemia that promote lower glutamate reuptake from the synaptic cleft and metabolic changes in the astrocyte-neuron metabolic relationship.

at the synaptic cleft (10 mmol L^{-1} compared with 20 nmol L^{-1}). In synaptic terminal vesicles, Glu is stored at levels as high as 100 mmol L^{-1} [2]. During hypoxia and hypoglycemia, unbalanced Glu release from vesicles subsequently activates non-NMDA and NMDA receptors and opens both Na^+ and Ca^{++} channels. This effect leads to neuronal death due to swelling and the activation of lipases and proteases, enhanced free radicals and cytoskeletal damage through secondary messengers [3]. Cell death increases the Glu levels at the synaptic cleft, thereby enhancing the effect. These combined effects are the main cause of neuronal cell death after a stroke.

We have studied the cytoskeleton and myosin calpain-induced proteolysis though glutamate excitotoxicity. We determined that myosin 5a is a target for proteolysis in both NMDA and kainate glutamate receptors. Proteolysis is inhibited by leupeptin, which is a broad protease inhibitor, and the specific calpain inhibitor I. These inhibitors also significantly improve the neuron morphology appearance, which suggests that the motor is a target for calpain I during excitotoxic injury [4]. These findings are consistent with results for depolarized nerve endings [5] and indicate that calpain-mediated myosin 5a proteolysis can be increased through calmodulin (CaM) release [6] and subsequent CaM-kinase II activation, which is a calpain regulatory protein [7]. In addition, we used different techniques to show that myosin 5a is regulated by T3, which is a stress-released hormone that is synthesized in response to nerve injury [8]. Our hypothesis was that myosin 5a is an important target for proteolysis during neuronal injury, and without this protein's function, axonal transport and synaptic function are affected (Fig. 1).

3. From the cell to exercise

Ammonia is a base (NH_3) that forms a conjugated pair with the ammonium ion (NH_4^+). The pKa of the reversible reaction $NH_3 + NH^+ \Longleftrightarrow NH_4^+$ is near 9.3. Thus, in the human body, most ammonia is protonated (ammonium). Henceforth, we will refer to ammonia as the sum of both its forms ($NH_3 + NH_4^+$) for clarity. In humans, ammonia reaches the bloodstream after absorption from enteric microbiota and as a metabolic result of either amino acid or purine deamination (AMP deaminase; EC 3.5.4.6). Ammonia requires an efficient detoxification system. Humans convert ammonia to urea mainly in the hepatocytes, and different cells can decrease ammonemia by synthesizing amino acids (mainly glutamine) as a safety mechanism via posterior excretion as urea. As a result, ammonemia ranges from 50 to 100 μM L^{-1} in the resting state (for a comprehensive review, see Adeva [9]).

Different metabolic events can increase Glu at the synaptic cleft, including hypoglycemia and hyperammonemia. Ammonia is a toxic metabolite to the CNS due to its role in inhibiting synaptic glutamate re-uptake, among other effects. In diseases, the blood ammonia concentration can rise to levels greater than 300 μM L^{-1}, depending on the degree of liver failure. In certain diseases, the ammonia concentration in the brain can reach 1000 μM L^{-1} [10]. Ammonia accumulation in the brain has long been implicated in brain pathogenesis during liver failure [11].

Hyperammonemia impairs long-term potentiation (LTP), which is the basis for certain forms of learning and memory. NMDA receptor activation is involved in both deaths due to acute ammonia toxicity and cognitive impairment due to chronic ammonia poisoning. Treating hyperammonemia involves lower ammonemia levels and greater ammonia clearance (to better understand hyperammonemia and CNS metabolism, refer to the elegant work of Vicente Felipo's group).

Physical exercise is considered to be a good model for studying metabolism and the effects of ammonia (for an elegant review, see Wilkinson et al. [12]). During exercise, ammonemia can rise three- to fivefold compared with the resting state. We have employed different models to understand ammonia production, metabolism and clearance. We have demonstrated that ammonia blood concentrations can increase up to 600 μM L^{-1} [13]; this increase depends on physical exercise and is related to intensity [14,15]. Furthermore, we have demonstrated that using certain amino acids and ketoanalogues can affect changes in ammonia concentration [16–18].

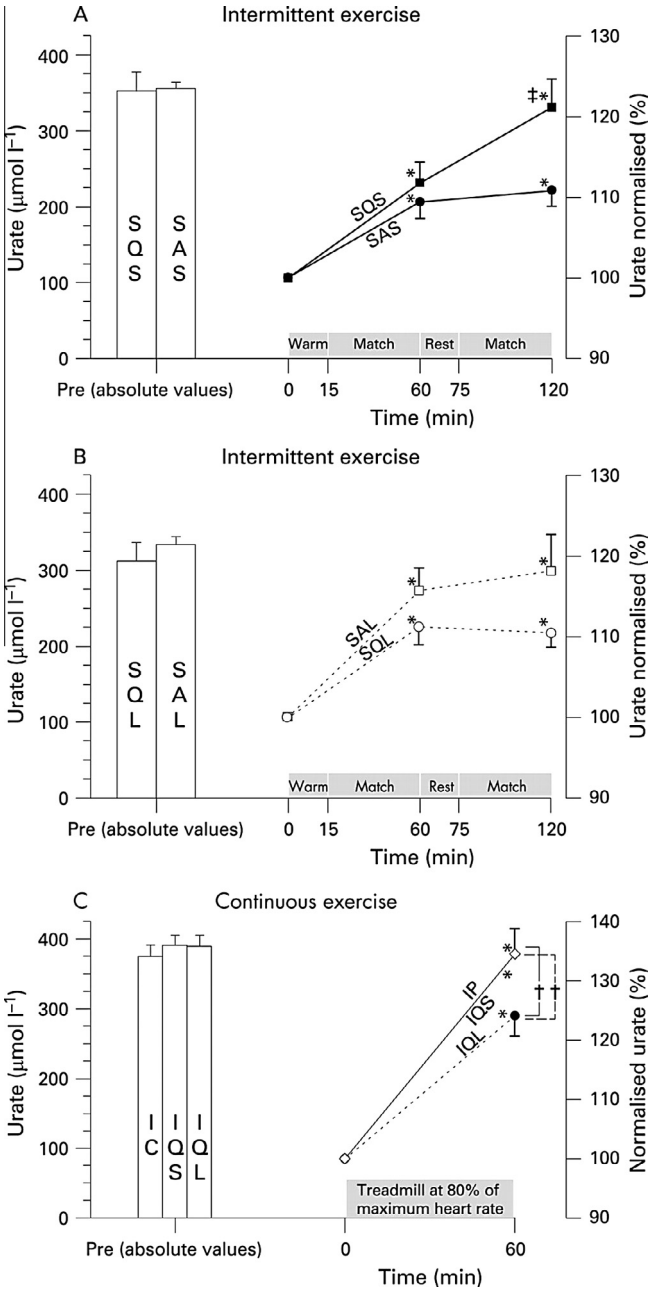

Fig. 2. From the laboratory to Sportomics. Ammonia production in the same athletes in response to a real match and to a laboratory test. Ammonemia during intermittent (A and B) and continuous exercise protocols (C). (A) Short-term amino acid supplementation. (B) Long-term amino acid supplementation. Absolute pre-exercise values are shown using bars. The line scatter shows the data normalized to pre-exercise values. IP, indoor placebo; IQL, indoor glutamine long-term supplementation; IQS, indoor glutamine short-term supplementation; SAL, soccer (football) alanine long-term supplementation; SAS, soccer (football) alanine short-term supplementation; SQL, soccer (football) glutamine long-term supplementation; SQS, soccer (football) glutamine short-term supplementation; $^*p < 0.05$ compared with pre-exercise; and $^\dagger p < 0.05$. Extracted from [19].

Our central question lies in the ability of the body protect the CNS from high and toxic ammonemia during acute and chronic exercise. Information on this problem can aid us in understanding the important signaling pathways to generate better methods that protect individuals suffering from diseases leading to hyperammonemia, such as liver failure, or hypermetabolic states, such as cancer and thermal injury.

To explore the maximum possible effect of exercise on CNS protection, we used elite athletes to better understand metabolic shifts during metabolism-stressing conditions. It is important to emphasize that the metabolic response during high intensity exercise is similar to the metabolic reaction to extensive trauma and sepsis. Through this comparison, we predicted that we can use exercise studies to understand the real metabolic challenges faced by patients during hypermetabolic states.

4. Exercise

We used exercise to induce metabolic stress and understand the CNS protection in response to a rise in ammonemia. We realize that comparing in-field exercise and laboratory protocols is challenging.

Our understanding of exercise metabolism is slowly growing. We recognize that to answer our original question, we must understand exercise under the same conditions that athletes are exposed to. We measured metabolism and tissue integrity using two different protocols; the first protocol involved soccer players and measured the effects of caffeine as a catecholamine modulator, and the second involved studying the effect of high-intensity ultra-endurance on metabolism and white blood cell signaling [14,15].

We showed that athletes' metabolic response during a soccer match differed from the response to treadmill exercise. We learned that continuous indoor treadmill exercise produced an approximately fourfold greater ammonia increase compared with intermittent field exercise. The following metabolites did not differ in either protocol: urate, which is the final metabolite for IMP (indicating that AMP is deaminated and ammonia is produced with direct stoichiometry) and urea, which is the final metabolite for ammonia (indicating the total ammonia produced) [19] (Fig. 2). In many different experiments, we compared metabolism during judo, taekwondo and the modern pentathlon and found differences in ammonia, urea, urate and glucose metabolism during indoor and competition protocols [20,21].

5. From laboratory and in-field exercise protocols to Sportomics

As biochemists, we were accustomed to test tube experimentation. We assumed that both controlling and reproducing training and competition conditions would be almost impossible. Different experiments taught us that we must follow athletes in their setting. Our first problem was how to conduct these analyses without losing information. Typically, plasma ammonia must be quickly analyzed, and this information was important to the study. Therefore, we brought the laboratory to the field. Since the experiments began, we have prepared and preserved certain metabolites to avoid mistakes. For example, we learned how to treat samples to

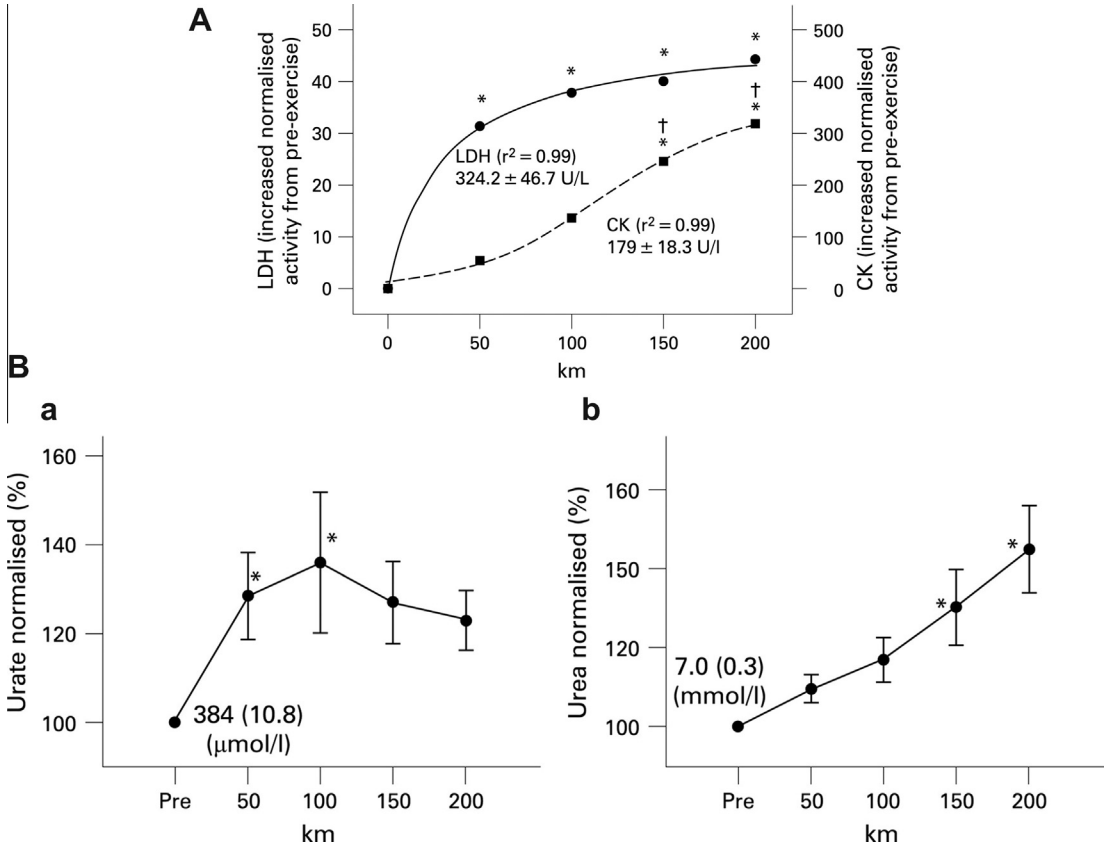

Fig. 3. Muscle injury biomarkers; urate and urea increase in a Sportomics experiment. Main graphic: Creatine kinase (CK) and lactate dehydrogenase (LDH) blood kinetics. The data plotted are the blood creatine kinase (square, CK) and lactate dehydrogenase (circle, LDH) against distance cycled. The lines show non-linear fitting curves. The data are the average (SE) increase in normalized activity from pre-exercise (0%). Absolute pre-exercise values are shown within the graphs. $^{*}p < 0.05$ compared with pre-exercise; $^{\dagger}p < 0.05$ compared with 50 km. (A) Urate concentrations increased during the first part of the protocol, but the urea (B) maintained a constant slope increase. The data were normalized (average ± SE) to pre-exercise values (100%), and the absolute pre-exercise values are shown in the graphs (U/l). $^{*}p < 0.05$ compared with pre-exercise. Extracted from [15].

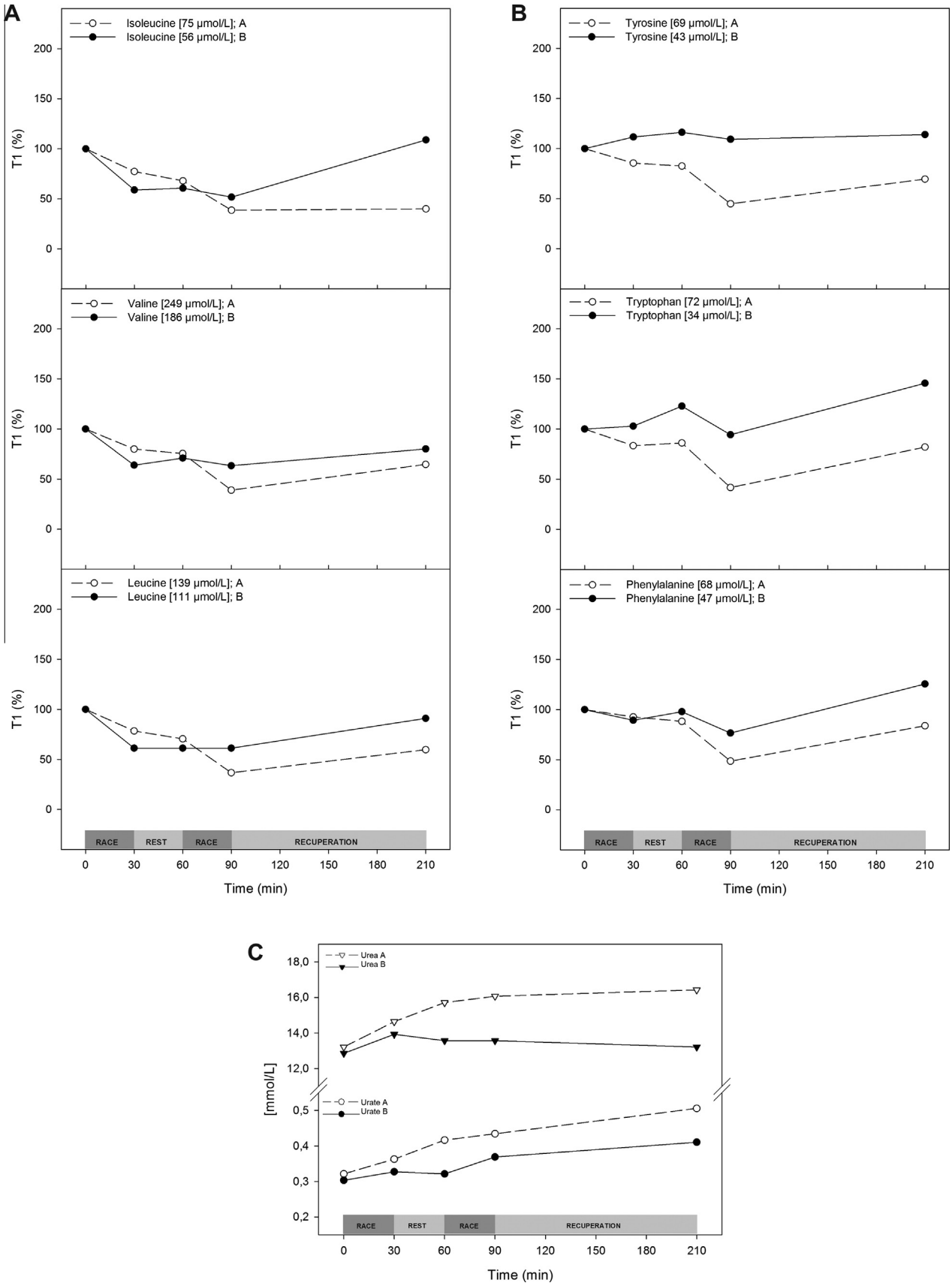

Fig. 4. Branched-chain and aromatic amino acid concentration changes in the blood during two Sportomics analyses. (A) Blood isoleucine, leucine and valine responses to exercise and intervention; (B) Phenylalanine, tryptophan and tyrosine exhibited a greater recovery than the BCAA; (C) Both the urate and urea concentrations decreased due to the intervention after the Sportomics evaluation. The empty symbols are the data measured before the Sportomics-driven intervention. Extracted from [24].

preserve ammonia and how to discuss data by considering different non-controllable variables.

The Sportomics protocols started in 2008 [15] and have been accepted by the scientific community [12,22,23]. To find a model that follows the metabolic changes and tissue responses to exercise, we monitored and analyzed a high-intensity, ultra-endurance (HIU) exercise as an extreme challenge to human metabolism. HIU is defined as repeated bouts of high-intensity exercise (75% VO_{2max}) with a limited recovery. We studied an entire cycling team that biked 800 km in ~23 h. Using this protocol, we followed the creatine kinase (CK) and lactate dehydrogenase (LDH) blood kinetics. We fitted a sigmoidal curve for CK and a hyperbolic curve for LDH, which suggests cooperativity in the CK blood levels. To the best of our knowledge, this is the first time that increased blood CK activity was measured at twice the resting level in a timeframe as small as 6 h [15] (Fig. 3A). Additional interesting features from this study are the urate and urea kinetic measurements. It is important to emphasize here that the athletes performed exercise followed by rest and feeding. During the first hours of exercise, the blood urate levels increased followed by urea levels (Fig. 3B). These findings provide certain clues to the metabolic basis of both AMP and amino acid deamination in metabolic stress.

Due to our predator characteristics, fatigue is much more understandable in humans than exhaustion. As predators, we learn to calculate the exercise levels that are necessary (energetic cost) to conquer prey (energy return) during evolution. Following this reasoning, exercise is a voluntary activity for human beings. In our opinion, understanding Sportomics lies in the idea that we do not believe in voluntary exercise to exhaustion. It has been shown that metabolic challenges are much greater during Sportomics protocols than exhaustion-based protocols [20,21]. In general, athletes do not train in controlled environments, such as inside a laboratory. They endure changes in temperature, humidity and wind, among other changes. These training differences are reflected in different metabolic adaptations. This reasoning indicates that Sportomics is most similar to real conditions, which will aid understanding the real metabolic changes encountered during exercise.

Using Sportomics protocols, we can better understand the metabolic changes that are induced by exercise and sports. In 2011, in an analogy to other "-omics" sciences, we proposed the concept of Sportomics for the first time in a publication [24]. We examined metabolism and certain cellular responses to different interventions in a windsurfing athlete. Windsurfing is a high-intensity exercise, and Olympic-level windsurfers reach 70–80% of VO_{2max} during sail pumping [25]. We studied metabolism in an Olympic athlete under conditions that mimicked an Olympic windsurfing competition. Our study showed that the branched chain amino acids (BCAA) isoleucine, leucine and valine were consumed, and the concentration of these BCAAs in blood decreased by up to 50% compared with 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. As expected, the aromatic amino acids phenylalanine, tryptophan and tyrosine were consumed at lower levels and decreased less than the BCAAs (Fig. 4A and B). A combined intervention in nutrition and training decreased anaplerosis, cataplerosis, gluconeogenesis and the use of amino acids as carbon donors. This effect was easily observed through a decrease in both urate and urea synthesis in response to exercise [24] (Fig. 4C).

Important issues were raised in this report that we would like to highlight. The first was the number of subjects in the investigation. We only used one subject in this study, and we attempted to publish it as a research article and not as a case report. This led us to another concern about using Sportomics protocols.

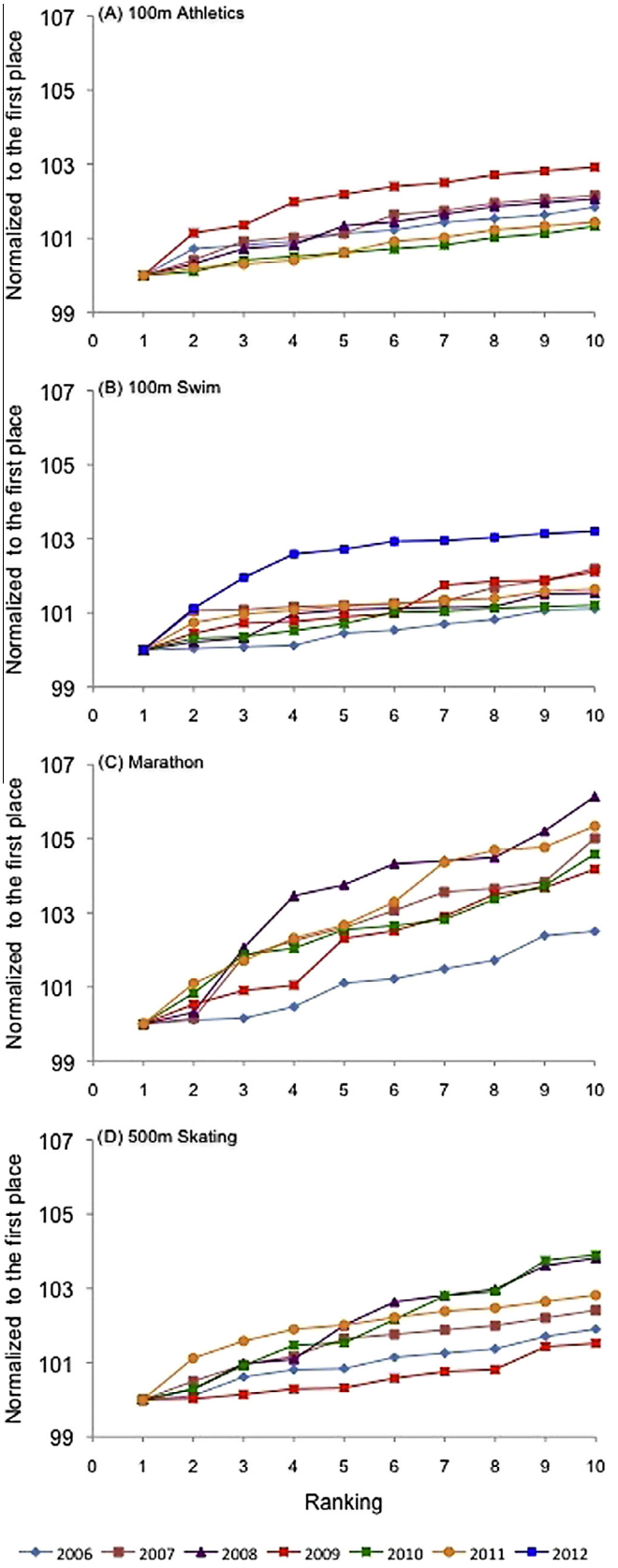

Fig. 5. Time difference measured from the first to tenth athlete in different sports over seven years. (A) 100-m athletics; (B) 100-m swimming; (C) NYC marathon and (D) 500-m speed skating. This graph was constructed based on a talk presented by Prof. Ron Maughan at the American College of Sports Medicine. His brilliant conference helped us advance one of the biggest statistical problems in Sportomics.

The conditions are nearly impossible to reproduce. Sportomics is an *ex post facto* type of science. We address different weather conditions, such as temperature, wind and humidity, and diverse metabolic responses due to uneven sleep and feeding behaviors near the time of the experiment. Herein, we discuss certain important problems. In our approaches, the presented data were normalized to the basal value for each state of the athlete. This normalization provided an ability to follow each individual's response to both the exercise and different conditions (at basal state and during the exercise). Because different states were analyzed, we were required to normalize the responses to the initial states. Each initial state for the athlete was different, and in our view, the only way to understand these phenomena was to normalize the results. When necessary, we also measured the area under the curves and included these data as additional information.

6. Sportomics limitations

Most papers in exercise science have focused on non-elite athletes, serious recreational athletes or active persons. Few data are available on world-class competing athletes because the physical data must be secret, as competitors could use it. Thus, we believe it is important that the scientific community has access to the metabolic information on top-level athletes. It is important to note that the values of several measured variables differed at the beginning of different experiments, which suggests that the physiological state of the athlete is not comparable in these experimental times. We want to emphasize the perception that each athlete was under different conditions (*e.g.*, feed, rest and hydration) in each studied protocol. We prefer to perform our studies on elite level world-class athletes, this can be viewed as a limitation of our protocols due to a lower number of subjects; however, it is also a merit. We are using a limited but significant sample. Considering that we work with a sample from a very stratified population in a given protocol, certain analyses indicate that we can work with 10–20% of the population. Thus, we believe it is important that the scientific community can access metabolic information on top-level athletes and use that information to understanding metabolism through cutting-edge methodologies.

As scientists, we believe that our studies cannot be extrapolated without significant studies wherein the number of observations is greater. To the best of our knowledge, our study is interesting because we use a small portion of the population (people that have achieved a world-class level in sports) and follows the metabolic changes in response to modifications to training, diet or both. We attempted to use a combination of different *ex post facto* analyses, which was similar to a petroleomics study [26]. It is impossible to determine the relationship between the original organic matter and the oil in petroleum analyses; similarly, we cannot change

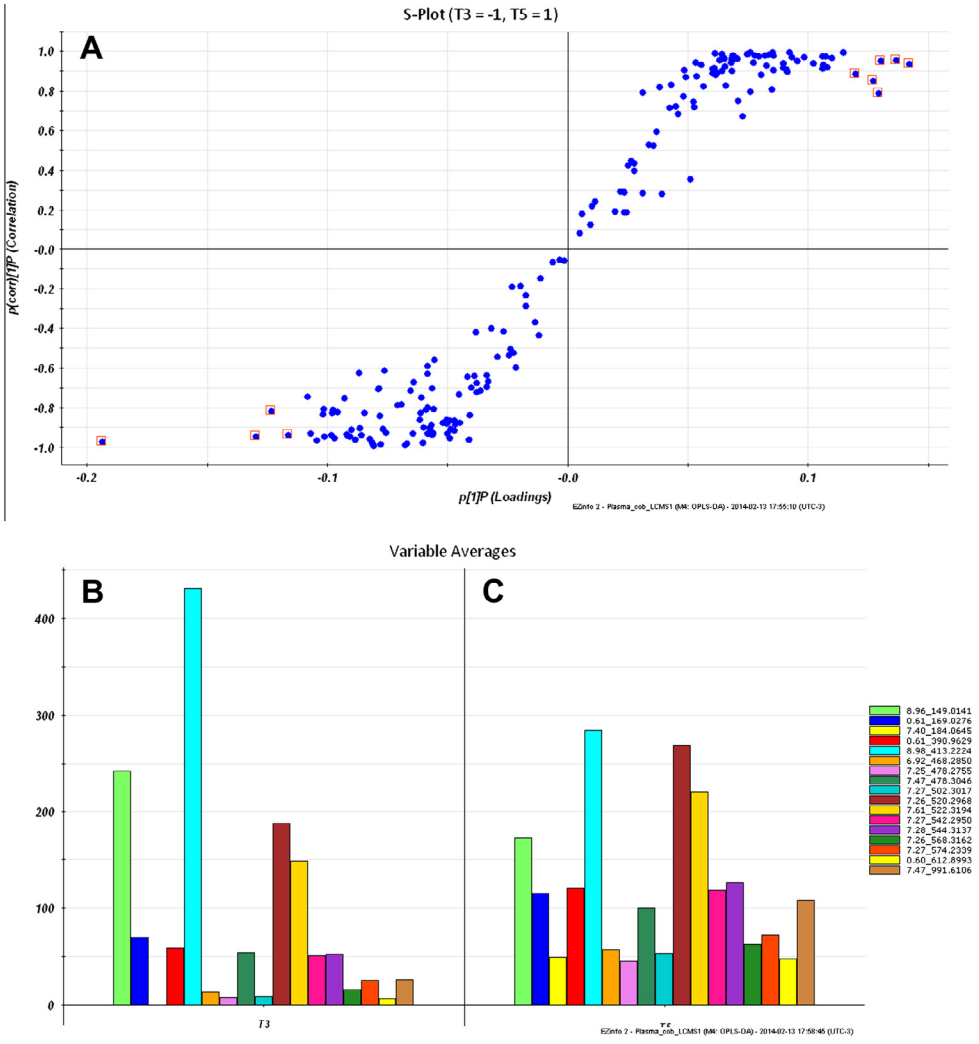

Fig. 6. Non-Target Analysis (NTA) with principal component analysis (PCA) used to analyze the metabolite differences measured during post-exercise and resting states using GC–MS. (A) PCA analysis of replicates; urate and lipid differences (B) post-exercise and (C) at rest. Previously unpublished data.

the initial differences in our studies. Several variables are beyond our control in a Sportomics study. Therefore, it is difficult for us to control (through we can observe) the feed, hydration and rest states. Furthermore, it is impossible to control with the environment; we can only observe and try to reproduce the experiments under similar ambient conditions.

7. Sportomics and statistics

Statistics is an important issue that should be discussed here. As scientists, we were trained to believe in statistics. I would like to slightly disagree with the established statistics in certain cases using sports examples. The average difference in times between the first place athlete to the tenth place athlete during 100-m running athletics over seven years was less than 2%. The same evaluation showed a 4.6% difference for the New York City marathon. It is important to emphasize that we are discussing the difference between first and tenth place and that medal disputes do occur. When considering the first (medal) to the fourth (no medal) places, the difference is less than 2% in all sports examined (Fig. 5). An important and useful 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 (Fig. 6).

We must always address the decision to work with and responsibility of working with a small number of subjects, even if they compose a representative sample from a small population. We must refrain from proposing general rules or mechanisms. The Sportomics approach rests on many variables, and we must be careful.

8. How Sportomics can aid athletes

Glycogen depletion increases ammonia production and subsequent ammonemia during exercise [27]. For the first time, using a standard animal protocol, we demonstrated that acute supplementation using ketoanalogues associated with amino acids (KAAA) can reduce the increase in ammonia levels due to resistance exercise [17]. Similar data from Sportomics protocols using a ketogenic diet to deplete glycogen show that KAAA equally decreased the ammonemia increase in response to exercise. Our data suggest that the effect of KAAA during exercise was not due to ammonia removal via urea synthesis but to use of carbon bodies [18]. Considering both studies, we propose that acute use of KAAA can lower the ammonemia increase caused by endurance exercise in humans.

We have used Sportomics protocols to aid in understanding metabolism. We recently showed that a corresponding rise in lymphocytes was associated with an increase in ammonemia and that both are decreased through arginine supplementation [13]. These findings revealed interesting possibilities for therapeutic arginine use in either sports or disease (Fig. 7).

9. How Sportomics can aid metabolism studies

In another study, we used caffeine to interfere with amino acid and ammonia metabolism. Using the gold-standard GC–MS technique to measure caffeinemia after caffeine supplementation, we showed that the serum caffeine level increased with two different patterns. Higher concentrations of serum caffeine prevented exercise-induced increases in serum glutamine levels but did not affect exercise-induced increases in alanine and glutamate. In addition, caffeine decreased the availability of urea cycle intermediates. We suggest that caffeine might decrease systemic urea by decreasing the serums glutamine concentration, which in turn decreases the ammonia levels transported to the liver, which subsequently decreases urea synthesis [28] (Fig. 8).

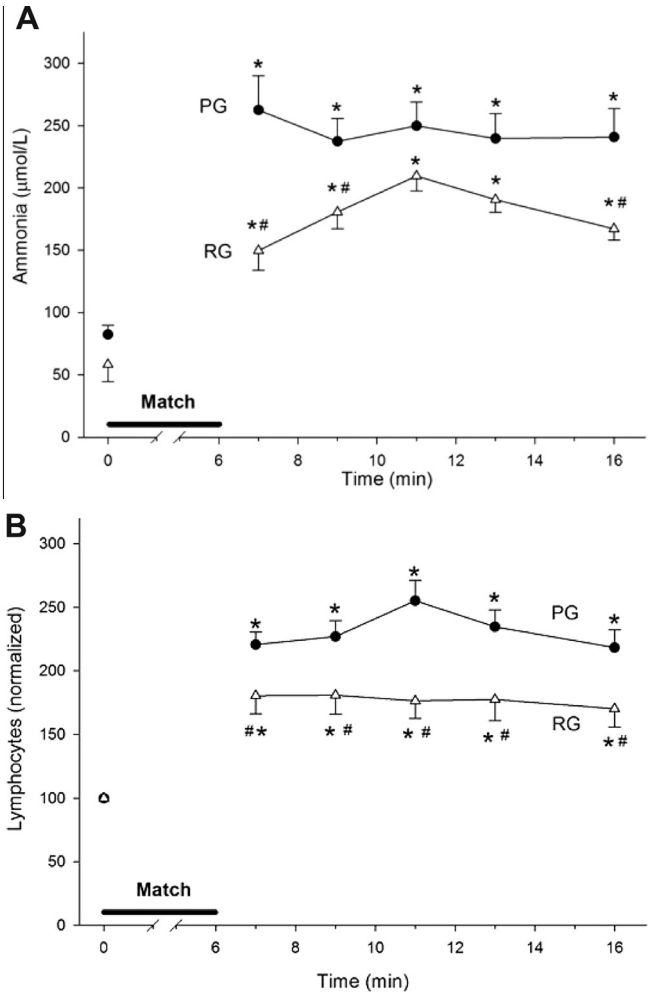

Fig. 7. The net ammonemia and lymphocyte levels decreased due to arginine during a Sportomics analysis. (A) The ammonemia response to exercise is indicated. The blood ammonia concentration increases after high-intensity exercise in an arginine supplement-dependent manner. A six-minute Jiu-Jitsu match was performed after a three-day LCD by athletes who had received either arginine (triangle, RG) or a placebo (circle, PG). Blood was collected before and after exercise. Control, $n = 23$; arginine, $n = 16$. (*) denotes that the average ± SE differs from the pre-exercise values; (#) denotes a difference between the two experimental groups. The calculated area under the curve was 3397 µmol/L^{min-1} for the placebo group and 2366 µmol/L^{min-1} for the arginine group. (B) Lymphocyte count. Exercise induces a lymphocyte increase in an arginine supplement-dependent manner. (*) denotes that the average ± SE differs from the pre-exercise values. The absolute pre-exercise values are shown within the graphs. The absolute pre-exercise values for lymphocytes are $2.2 \pm 0.1 \times 10^9$ cells/L^{-1} for the PG and $2.9 \pm 0.3 \times 10^9$ cells/L^{-1} for the RG (no statistically significant difference, $p = 0.07$).

Sportomics is a useful tool for managing athlete training and performance. In a recent study, we accidentally identified muscle injuries followed by acetaminophen hepatotoxicity [29]. We showed that collecting and analyzing physiological data during training could provide important information on an athlete's clinical condition and performance.

10. Future perspectives

We are currently establishing databases to facilitate more metabolomics and proteomics studies. We intend to add genomic and transcriptomic findings to further our Sportomics studies. In

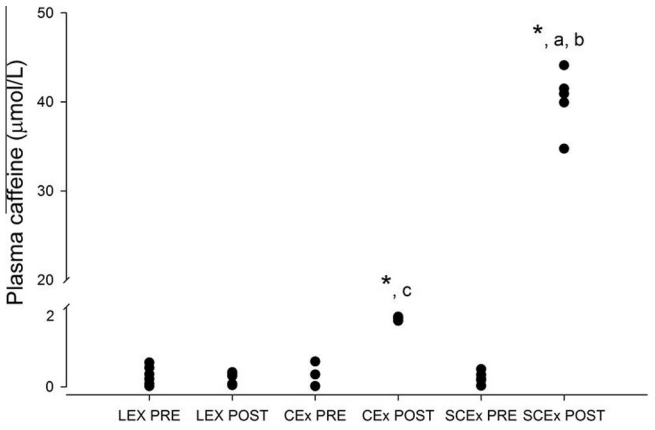

Fig. 8. Caffeinemia increases differently in response to supplementation and exercise. All groups were supplemented. One group received caffeine at 5 mg kg^{-1}, whereas the control group received lactose. One hour after supplementation, blood was collected, and caffeine was measured using GC–MS. The same procedure was repeated after the Sportomics protocol exercise. The subjects were distributed based on the level of caffeinemia. The figure shows individual measurements for each athlete (certain data are indistinguishable). *Significant difference between before and after exercise ($p < 0.05$). Significant differences relative to SCEx for [a]CEx and [b]LEx. [c]Significant differences relative to CEx versus LEx.

addition, we encourage combining data from cellular screening and mass spectrometry imaging in our studies [30].

We strongly believe that the Sportomics approach will facilitate different methods for scientific observations and experiments.

Acknowledgments

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