



**UNIVERSIDADE FEDERAL DE UBERLÂNDIA
INSTITUTO DE GENÉTICA E BIOQUÍMICA
PÓS-GRADUAÇÃO EM GENÉTICA E BIOQUÍMICA**

**ALTERAÇÕES METABÓLICAS E DE BIOMARCADORES DURANTE O
TREINO E COMPETIÇÃO EM ATLETAS DE ALTO RENDIMENTO:
UMA ANÁLISE ESPORTÔMICA.**

Aluno: Alexandre Magno Vieira França

Orientador: L.C. Cameron

**UBERLÂNDIA - MG
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L.C. Cameron

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Dados apresentados no Congresso do American College of Sports 104
Medicine, 2013: **“A sportomic study of ammonia and aminoacids
metabolism in competing world-class judo athletes”**.

Dados apresentados no Congresso do European College of Sports 112
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elite athletes using a 100m dash race as a model”**.

SEÇÃO B

Dados apresentados no Congresso do American College of Sports 118
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analysis”**.

SEÇÃO C

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rendimento”**; e **“Esportômica e hidratação”**.

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analysis of world-class canoeists during a two-day training
protocol”**.

RESUMO

O exercício físico tem sido usado como modelo na investigação das interações metabólicas fornecendo informações essenciais sobre o controle e sinalização do metabolismo em níveis sistêmico e celular. Esse mesmo modelo pode auxiliar na formação de estratégias e metodologias para a melhora de performance de atletas de alto rendimento. Os estudos apresentados nessa tese foram desenvolvidos sob a ótica da esportômica, conceito criado num cenário em que as amostras, geralmente sangue e urina, são coletadas *in loco*, diretamente nos campos de competição e treinamento, simulando os desafios reais e as condições enfrentadas por atletas de elite durante as performances esportivas. A esportômica é o uso das ciências “-ômicas” em conjunto com as análises clínicas laboratoriais clássicas para entender as modificações induzidas pelo esporte. Neste trabalho serão apresentados estudos acerca das alterações metabólicas e de biomarcadores utilizando a metodologia esportômica em investigações conduzidas em protocolos de treino e competição em atletas de alto rendimento, a fim de contribuir para a melhora de seu rendimento e compreender o metabolismo sistêmico e celular.

Palavras-chave: esportômica; medicina personalizada; treinamento.

ABSTRACT

Physical exercise has been used as a model to investigate metabolic interactions providing essential information about controlling and signaling of systemic and cellular metabolism. This model can also help us to develop strategies and methods to improve the performances of elite athletes. The studies here presented have been developed from a sportomics viewpoint, a concept that proposes taking blood and urine samples direct in the field of play, mimicking the real challenges faced by elite athletes in training and competitions. Sportomics is similar to other “-omics” approaches combined with classic clinical laboratory analyses, allowing us to better understand the metabolic changes that are induced by exercise and sports. In this paper we will present studies about metabolic and biomarkers changes during training and competitions protocols of elite athletes, aiming on improving their performances and understanding systemic and cellular metabolism.

Key words: Sportomics; personalized medicine; training.

APRESENTAÇÃO

O exercício físico tem sido usado como modelo na investigação das interações metabólicas em momentos de saúde, doença e estresse metabólico, uma vez que as situações vividas sob essa condição fornecem informações essenciais sobre o controle e sinalização do metabolismo em níveis sistêmico e celular.

A nível secundário, esse mesmo modelo pode auxiliar no desenvolvimento de estratégias e metodologias para a melhora de performance de atletas de alto rendimento. Nos últimos oito anos o grupo de estudos do Laboratório de Bioquímica de Proteínas (LBP) da Universidade Federal do Estado do Rio de Janeiro (UNIRIO) iniciou o suporte a atletas de elite, numa parceria com o Laboratório de Ciência do Esporte (LCE) do Comitê Olímpico Brasileiro (COB). O trabalho inovador desenvolvido pelo LBP a atletas brasileiros laureados nas duas últimas edições de Jogos Olímpicos e Pan-americanos tem sido largamente citado pela comunidade olímpica como um dos fatores contribuintes para a evolução do país no quadro de medalhas.

Um dos diferenciais da pesquisa desenvolvida pelo LBP nessa área é a proposta do conceito de esportômica, desenvolvido num cenário em que as amostras, geralmente sangue e urina, são coletadas *in loco*, diretamente nos campos de competição e treinamento, simulando os desafios reais e as condições enfrentadas durante as performances esportivas. O sufixo “-ômica” significa que todos os constituintes são considerados coletivamente, assim como a *genômica* é o estudo de todos os genes e a *metabolômica* é o estudo de todos os processos metabólicos. Nesse sentido, esportômica é o uso das ciências “-ômicas” em conjunto com as análises clínicas laboratoriais clássicas (p. ex., determinação enzimática, ELISA, *Western Blotting* e outros procedimentos analíticos) para entender as modificações induzidas pelo esporte. O estudo é holístico e *top-down*, sendo os dados tratados de forma sistêmica e gerando uma grande quantidade de dados, com um enorme esforço analítico computacional.

Nesta tese serão apresentados estudos acerca das alterações metabólicas e de biomarcadores utilizando a metodologia esportômica. Para tanto, as investigações foram realizadas por meio de protocolos de treino e competição em atletas de alto rendimento, a fim de contribuir para a melhora de seu rendimento e compreender o metabolismo sistêmico e celular.

OBJETIVOS

1. Objetivo Geral

Discutir, utilizando a abordagem esportômica, as diferenças entre as respostas sistêmicas e celulares de atletas de elite de alto rendimento em diferentes etapas de treinamento e competição, enfatizando a necessidade da individualização na compreensão dos fenômenos envolvidos.

2. Objetivos específicos

- 2.1 Comparar a resposta metabólica de indivíduos diferentes a protocolos similares de exercício;
- 2.2 Comparar a resposta metabólica de indivíduos e modalidades diferentes a testes padrão de avaliação de performance em exercício;
- 2.3 Comparar a resposta metabólica de atletas em diferentes etapas de treinamento e competição; e
- 2.4 Estudar respostas anômalas a protocolos esportômicos.

Para responder ao Objetivo 2.1

- Foram avaliados quatro canoístas do sexo masculino de nível mundial durante uma sessão combinada de treinos. Após seu aquecimento, os atletas foram submetidos a um protocolo de treinamento que consistiu de diversos sprints de remada com três minutos de intervalo entre cada tiro, cobrindo variadas distâncias e intensidades. A distância total percorrida foi de 16km e o protocolo durou 210 min. Após 20 min de intervalo, os atletas participaram de uma sessão

de musculação que durou 50 min, sendo empregados grandes grupos musculares dos membros superiores e inferiores. Cinco amostras de sangue foram coletadas nesse período: antes (T1) e depois (T2) da sessão de sprints; antes (T3) e depois (T4) da sessão de musculação; e após 45 min de recuperação (T5). (Capítulo II).

- Foram testados quatro canoístas de calibre olímpico, incluindo campeões mundiais, no experimento 1 e cinco atletas de alto rendimento com níveis de performance semelhantes no experimento 2. O sangue foi coletado em jejum (T1); antes e após uma sessão de canoagem (T2 e T3); depois de uma sessão de musculação (T4) e após recuperação (T5) de 70 min em E1 e 45 min em E2. O tempo gasto para cada experimento foi de aproximadamente 350 min. O volume geral remado em cada sessão foi de 16km, percorridos em cerca de 50 min. Os protocolos de treinamento utilizados foram testados por um período de quatro anos e acompanhados por diferentes treinadores durante a fase de pré-temporada de treinamento. (Material Complementar, Seção A)

- Foram avaliados pela metodologia esportômica dois atletas de alto rendimento do sexo masculino da modalidade judô. Foram realizados dois experimentos, sendo o primeiro em período de pré-competição (E1) e o segundo durante a competição (E2). Na primeira avaliação (E1) foi utilizado um modelo de competição internacional, em cinco combates com diferentes intervalos de descanso entre eles. Participaram desse experimento os dois atletas, pertencentes às categorias peso pesado (J1) e até 73 quilos (J2). No segundo experimento (E2) somente o atleta J2 foi avaliado durante um dia de treino de alta intensidade. (Material Complementar, Seção A)

- Duas velocistas de alto rendimento da modalidade atletismo foram avaliadas durante um treinamento de 100m. Para esse experimento foram coletadas seis amostras de sangue em diferentes tempos: jejum (T0); pré-corrida (T1); imediatamente pós-corrida (T2); e após recuperação de 2, 5 e 10 minutos (T3, T4 e T5, respectivamente). Foram analisados aproximadamente 40 analitos

presentes no sangue para entender as diferentes respostas individuais ao exercício (Material Complementar, Seção A).

Para responder ao Objetivo 2.2

- Um ultramaratonista de elite foi testado em um protocolo de oito horas de corrida contínua, tendo o atleta percorrido nesse período um total de 83 quilômetros. Foram coletadas dez amostras de sangue, da seguinte maneira: a cada hora de corrida, totalizando oito coletas; uma hora após o término do experimento; e depois de 12 horas de recuperação (Capítulo III).
- O perfil metabólico de duas atletas de alto rendimento foi avaliado durante competições e sessões de treinamento. As modalidades avaliadas no desenho experimental foram Pentatlo Moderno (MP) e Taekwondo (F1), sendo utilizado o teste de VO₂Max como variável de interesse para comparar as duas modalidades. No primeiro experimento (E1) a atleta da modalidade de Taekwondo (F1) foi avaliada durante um treinamento de combate; no segundo experimento (E2), a atleta do Pentatlo Moderno (MP1) foi avaliada durante o Campeonato Brasileiro da modalidade. Por fim, no terceiro experimento (E3), ambas atletas foram avaliadas no mesmo protocolo de VO₂Max. (Material Complementar, Seção B)

Para responder ao Objetivo 2.3

- Uma atleta foi monitorada durante 34 meses (2010 a 2013), em dez diferentes coletas semanais (3-6 dias cada). Os períodos de coleta foram: jejum, pré e pós treinamento, e recuperação de 90 minutos. (Material Complementar, Seção C).

Para responder ao Objetivo 2.4

- Foi conduzida uma análise esportômica em quatro ciclistas de alto rendimento de nível nacional, combinada com o uso de banco de dados para diagnosticar, intervir e acompanhar a evolução de hepatotoxicidade causada pelo uso de grandes doses de acetaminofeno para tratar dor lombar. (Capítulo IV)

CAPÍTULO I
FUNDAMENTAÇÃO TEÓRICA

O exercício físico é considerado um modelo valioso para entendimento do estresse metabólico (1-3). Estudos durante o exercício permitem a compreensão da resposta celular e sistêmica diante de alta demanda energética (2). Nas últimas décadas, as reações induzidas pelo estresse provocado pelo exercício tornaram-se importantes ferramentas de apoio ao estudo e pesquisa de diversas áreas da ciência como a bioquímica e a biologia celular (4-6).

Durante o exercício físico adaptações no equilíbrio bioquímico são intensas e contínuas. Assim, à semelhança das reações provocadas por diversas doenças, diferentes respostas metabólicas são iniciadas para manter a homeostase e proteger os indivíduos contra os efeitos deletérios do exercício, (7-9).

Ao longo dos últimos 20 anos, nosso grupo vem buscando abordagens alternativas para entender as respostas metabólicas ao estresse metabólico e à compreensão de estados hipermetabólicos (2, 10-13). Essa nova visão busca conectar estudos clássicos realizados em laboratório às ciências “-ômicas”, tais como genômica, metabolômica e proteômica analisando a totalidade (ou a grande maioria) de constituintes de uma amostra em determinados estados e suas modificações. O modelo esportômico apresenta a fusão da ótica das ciências -ômicas com as análises laboratoriais clássicas aplicada aos estudos dos efeitos do exercício em campo, dentro de um contexto esportivo (2, 14, 15). Esta abordagem permite o estudo *ex-post-facto* dos fenômenos biológicos que ocorrem durante o exercício em condições reais de treino e competição. Essa abordagem busca compreender situações reais a que o organismo humano é submetido durante a atividade física.

Diferentemente dos estudos realizados em laboratório em condições extremamente controladas, durante os estudos esportômicos as amostras são coletadas em situação de campo e em ambiente não-controlado, reproduzindo os desafios apresentados em sessões reais de treinamento e competição (13, 15, 16). Vale salientar que esportômica se baseia na análise *non-target* de fluidos biológicos coletados de indivíduos em diferentes tempos durante o exercício.

Destaca-se que essa abordagem não é orientada por hipótese, visto que o seu principal objetivo é caracterizar os estados e alterações metabólicas do indivíduo durante a prática de esportes e exercícios, bem como buscar suas interações com a saúde e doença. Todos os dados coletados são tratados sistematicamente, com a geração de uma imensa base de dados num estudo holístico e com enfoque *top-down*. Assim, busca-se reduzir a lacuna existente entre a biologia sistêmica e a medicina translacional da mesma forma que o fazemos na abordagem da bancada do laboratório para o campo (2, 3, 11, 17, 18).

Há dez anos, adicionalmente ao uso do exercício como modelo de estudo, utilizamos os dados obtidos em nossos estudos para auxiliar o desenvolvimento de estratégias e metodologias para a melhora de performance de atletas de alto rendimento. Este fato nos levou à associação com o Comitê Olímpico do Brasil para o suporte a atletas de elite. Nos últimos cinco anos foram analisados pelo nosso grupo mais de 400 atletas de nível internacional em cerca de 25 modalidades distintas. Com base nisso, criou-se um repositório de dados compreendendo o acompanhamento de atletas por intermédio da coleta de diferentes matrizes biológicas, realizadas em diferentes situações de treinamento ou competição, de maneira a fornecer a cientistas e treinadores uma perspectiva real da evolução do treino e do desempenho. A esportômica tem permitido a introdução de alterações na dieta, de estratégias de recuperação e programas de treinamento, corroborando com uma melhora na performance dos sujeitos estudados.

A maioria dos artigos sobre ciência do esporte é focada em atletas comuns e amadores, ou ainda simplesmente em pessoas ativas fisicamente. Há pouquíssimos dados e literatura disponível apresentando situações com atletas de elite porque esse material normalmente é protegido por cláusulas de confidencialidade, a fim de resguardar quaisquer associações com suas performances. Dessa forma, é de extrema importância prover à comunidade científica acesso às raras informações metabólicas de atletas de elite de alto-rendimento, para melhor entender o metabolismo.

Um grande número de estudos demonstrou que as respostas individuais ao exercício físico não podem ser previstas a partir dos valores médios da população. Portanto, estudar o metabolismo de atletas de elite em diferentes estágios de formação e desempenho, em tempo real, é extremamente valioso para a compreensão de seu metabolismo único.

Devido ao pequeno número de atletas de calibre olímpico com quem trabalhamos, a maior parte dos experimentos que realizamos contém um número de sujeitos reduzido, muitas vezes composto por apenas um sujeito. A esportômica busca evitar a utilização do método tentativa-erro, à semelhança do que se realiza na medicina personalizada, considerando a importância do papel da variabilidade da resposta metabólica entre indivíduos. Normalmente, os padrões investigativos utilizados para examinar as performances de atletas são baseados em informações empíricas coletadas por treinadores, conectadas por sua vez a testes de laboratório e dados clínicos obtidos por cientistas. Em muitos casos temos sujeitos submetidos exatamente às mesmas rotinas de treinamento, dieta e recuperação, mas com respostas metabólicas completamente diferentes ao analisar sua resposta sistêmica (11).

A era das ciências “-ômicas” tem nos permitido utilizar a ampla massa de dados gerada pelas análises proteômica e metabolômica (2, 10, 12, 19-21) para compreender as respostas individuais a diferentes formas de estresse. Ademais, um dos desafios atuais enfrentados pela medicina é desenvolver terapias adaptados ao exato estado biológico do indivíduo. Os resultados de intervenções terapêuticas ligados a padrões globais de apresentação de doenças têm sido questionados por considerarem apenas a visão sistêmica e não a variabilidade de parâmetros biológicos dos indivíduos (22, 23). A abordagem apresentada pela medicina personalizada utiliza os enormes bancos de dados providos pela genômica e metabolômica para cotejar informações que definam elementos para a compreensão e a avaliação de doenças, diagnósticos e tratamentos de um determinado perfil de indivíduo.

As evidências sobre esse tema, apresentadas em diversas revisões nos últimos anos, tem levado pesquisadores mais e mais a defender a tese dos ensaios clínicos de “n=1” (24-27). Os achados desses estudos, assim como os apresentados pelo nosso grupo podem não ser aplicados a outros sujeitos e requerem cautela para serem extrapolados (2).

Não obstante, a relevância científica das amostras coletadas em situações reais de estresse metabólico poderá levar-nos a entender melhor, por exemplo, a ação protetora do exercício no sistema nervoso central em situações de hiperamonemia (18, 28-31). Os efeitos deletérios da amônia sobre o sistema nervoso central já foram identificados como uma das potenciais causas da fadiga central. As concentrações de amônia encontradas durante o exercício podem chegar a quase 20 vezes os valores normais de um indivíduo em repouso. Embora tal concentração seja correlacionável com valores encontrados em encefalopatias e estado de coma, o mecanismo de proteção ativado durante o exercício ainda não é conhecido. (32)

Dado o exposto, verifica-se que atletas de alto rendimento apresentam performances próximas de situações-limite em treinamentos e competições, o que claramente não pode ser aplicado continuamente a sujeitos voluntários ou atletas amadores. Nessa mesma linha, a ciência pode utilizar o exercício para encontrar vias sinalizadoras que contribuam para a compreensão de doenças como a falência hepática ou renal, ou ainda estados hipermetabólicos, como o câncer e queimaduras extensas (2, 9, 15, 33-36).

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CAPÍTULO II

COMPARAÇÃO DA RESPOSTA METABÓLICA DE INDIVÍDUOS DIFERENTES A PROTOCOLOS SIMILARES DE EXERCÍCIO

**INVESTIGATING THE CELLULAR AND METABOLIC RESPONSES OF
WORLD-CLASS CANOEISTS TRAINING. A SPORTOMICS APPROACH.**

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Investigating the Cellular and Metabolic Responses of World-Class Canoeists Training: A Sportomics Approach

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Abstract: (1) Background: We have been using the Sportomics approach to evaluate biochemical and hematological changes in response to exercise. The aim of this study was to evaluate the metabolic and hematologic responses of world-class canoeists during a training session; (2) Methods: Blood samples were taken at different points and analyzed for their hematological properties, activities of selected enzymes, hormones, and metabolites; (3) Results: Muscle stress biomarkers were elevated in response to exercise which correlated with modifications in the profile of white blood cells, where a leukocyte rise was observed after the canoe session. These results were accompanied by an increase in other exercise intensity parameters such as lactatemia and ammonemia. Adrenocorticotrophic hormone and cortisol increased during the exercise sessions. The acute rise in both erythrocytes and white blood profile were probably due to muscle cell damage, rather than hepatocyte integrity impairment; (4) Conclusion: The cellular and metabolic responses found here, together with effective nutrition support, are crucial to understanding the effects of exercise in order to assist in the creation of new training and recovery planning. Also we show that Sportomics is a primal tool for training management and performance improvement, as well as to the understanding of metabolic response to exercise.

Keywords: metabolism; biochemistry of exercise; ammonia; urate; exercise intensity biomarkers; physical stress response

1. Introduction

Physical stress response due to a sport challenge is implicated in many metabolic modifications which affect the equilibrium of the biochemical internal environment [1,2]. This includes changes in the amount and kinetics of diverse biomarkers that are correlated with exercise intensity and muscle damage [3,4]. Some of these changes in metabolism can be assessed using blood as a biological matrix. For more than one decade, our group has dedicated research efforts towards understanding changes in metabolism using exercise as an induced-stress metabolic model [5–17]. The Sportomics approach targets metabolic and signaling molecule evaluations during either mimicked or real conditions faced in sports situations; it combines “-omics” technique with classic clinical laboratory analyses in order to understand sport-induced modifications [17]. These approaches represent a powerful tool to understand changes in physical

and metabolic stress [18–20] and allow researchers to propose interventions in order to optimize athletes' performance [21–23]. The approach is also a useful investigation tool for studying the effects of nutrition supplementation on physical training in different physiological or clinical conditions, such as type 2 diabetes mellitus [24]. Therefore, the analysis of world-class athletes in a field perspective allows us the possibility of understanding metabolic and signaling responses during high metabolic stress. Similar to a personalized-medicine approach, the Sportomics method allows us to better understand individual changes and to propose individualized interventions.

Several recent investigations have focused on the ammonemia changes resulting from a physical effort, which may be modified due to different causes [25–27]. Amino acids play a central metabolic role as an energetic source during exercise, which requires their deamination in order to be transduced into chemical energy. Increased muscle contraction rate also can contribute to changes in ammonemia, through adenosine monophosphate (AMP) deamination [28–31]. During intense or prolonged exercise, the reduced ability to resynthesize ATP promotes accumulation of ammonia and inosine monophosphate (IMP) which is metabolized to urate [32]. An intensity relationship has been proposed between ammonemia and exercise, as ammonia rapidly increases at intensities greater than 50%–60% of $\text{VO}_{2\text{max}}$ [33,34]. However, ammonia production and release is not solely restricted to intense exercise. During prolonged (>1 h) submaximal exercise (60%–75% $\text{VO}_{2\text{max}}$), ammonia could be produced through the breakdown of branched chain amino acid (BCAA) for additional energy provision [35–37].

Ammonia may cross the blood brain barrier causing neurotoxic effects including neuropsychiatric disorders, convulsion, and death [38], and may be implicated in central fatigue [27]. Therefore, ammonia accumulation may be avoided through a detoxification system. Humans convert ammonia to urea mainly in hepatocytes, and different cells can decrease ammonemia by synthesizing amino acids as a mechanism for further excretion of urea [10,12,39]. Therefore, an increase in urea levels reflects both AMP and amino acid deamination. On the other hand, urate is the final metabolite of the purine metabolism; hence, its measure can be stoichiometrically related to IMP deamination. Since urea and urate are, respectively, the final products of ammonia and purine metabolism, the study of the kinetics of those blood analytes leads to a better understanding of the metabolic pathways of ammonia origin and the response to exercise [39]. For this reason, our group has proposed nutritional and training interventions to promote metabolic adaptations in elite athletes to enhance their performance in training and competitions [3,40].

Canoeing has been featured as an Olympic sport since the Summer Olympic Games of 1936 in Berlin. Currently, men's and women's competitions cover distances of 200 m, 500 m, and 1000 m either solo, in pairs, or in crews of four. Canoeing contests are sprint events requiring sustained bursts of speed and power, leading to intense mechanical and metabolic stress. Little is known about these athletes' metabolic responses during training sessions or competitions, therefore, the aim of this study was to evaluate four world-class canoeists during a training session through a Sportomics approach. As far as we know, this is the first metabolic investigation in the field, coming from our unique opportunity to investigate world-class athletes. This investigation will help enlighten us about the metabolism behavior in elite athletes.

2. Materials and Methods

This study assessed the metabolic response of four male world-class canoeists during a combined training session. All athletes were currently engaged in international elite competitions (including world championships, Pan-American, and Olympic games). During the trials, the athletes were instructed to maintain their typical hydration and food ingestion habits. Additionally, clinical evaluation, anthropometric measurements, and laboratory tests of collected blood samples were

performed to assess health status. A Sportomics evaluation and analysis was performed to understand the metabolic effects of a training session. Subjects were fully instructed about the testing procedures and each signed a written informed consent. This study was conducted according to all procedures involving human subjects approved by the Ethics Committee for Human Research at the Federal University of the State of Rio de Janeiro (117/2007, renewed in 2011, 2013 and 2016) and met the requirements regulating research on human subjects (Health National Council, Brazil, 1996) the proper written informed consent was read and signed by the athletes.

2.1. Experimental Designs

After a regular warm up, the athletes were subjected to a training protocol that consisted of several canoe sprint bouts, with three minute intervals between each bout, covering different distances and intensities. The total distance totaled 16 kilometers. This first part of the protocol had a duration of 210 min followed by a rest period of 20 min during which they ingested a 500 mL beverage consisting of about 20% carbohydrate (short and medium absorption); 2% lipids; 5% proteins (casein and whey proteins). Next, they performed a weight lifting training session for 50 min focusing on exercises that recruit large muscle groups for both upper and lower body, followed by a 70 min of recovery. See the experimental trial depicted (Figure 1).

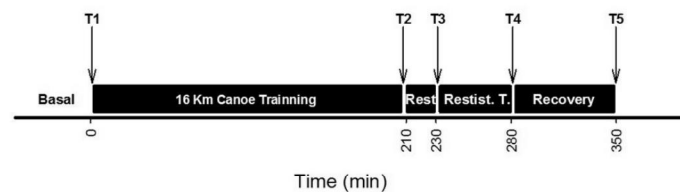


Figure 1. Experimental trial. Blood samples of the athletes were collected at the time points indicated in the Figure and as described in materials and methods.

2.2. Blood Collection

Blood samples were collected following an antecubital vein puncture before (T1) and after (T2) the 16 km canoe training session; before (T3) and after (T4) the resistance training; and after the recovery period (T5) (Figure 1). Samples for hematological analysis assays were collected into tubes with K₂-EDTA (Vacuette, Greiner Bio-One, Frickenhausen, Germany). White blood cell (total and differential), erythrocyte, and thrombocyte counts were measured in whole blood within a two-hour time frame after collection. Blood was immediately centrifuged to obtain either plasma or serum that was aliquoted, centrifuged (3000× g; 10 min; 4 °C), and stored in liquid nitrogen for later analysis (never more than eight hours). Samples were analyzed in duplicate or triplicate, when necessary, and measured against a standard curve with no less than five points.

2.3. Blood Analysis

A range of hematological and biochemical analyses was carried out totalizing around 100 analytes. The large amount of data generated was used in a non-target analysis linked to an ex-post facto study design. We chose near 20 analytes that could be relevant for our study of the athlete's performance. Among others, our data set included a broad spectrum of metabolites and biomarkers related to different cellular and systemic signaling processes like inflammation and both muscle and hepatic injury.

Alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), γ -glutamyltransferase (γ GT), creatine phosphokinase

muscle-brain fraction (CKMB), creatine phosphokinase (CK), ammonia, urea, blood urea nitrogen (BUN), creatinine, urate, glucose, lactate, and 2-hydroxybutyrate were measured by the enzymatic kinetic method [41] in an automatic analyzer (ADVIA 1200—SIEMENS, Erlangen, Germany/Autolab 18 Boehringer Mannheim, Ingelheim am Rhein, Germany). Myoglobin was evaluated by the Hybridization Signal Amplification Method [42]. Albumin and total protein were assessed by electrophoretic analysis [43]. High-density lipoprotein (HDL), low-density lipoprotein (LDL), very-low-density lipoprotein (VLDL), total lipids, triacylglycerols (TG), and total cholesterol were assessed by the Chabrol & Charonnat method [44]. Amino acids were measured by high performance liquid chromatography (HPLC) [45]. CKMB-mass, insulin, adrenocorticotrophic hormone (ACTH), and cortisol levels were assessed by chemiluminescence (Immulite 2000 Siemens, Erlangen, Germany) [46].

2.4. Statistical Analysis

Statistical analyses were performed using the software SigmaPlot 11.0 integrated with SigmaStat 3.5 packages (Systat, CA, USA). Due to the nature of the experiment, including the similarity of subjects and the controlled experimental conditions (diet, sleep, training and major physical condition variables), the data were expressed as mean \pm standard error (SEM). Data were normalized to pre-training results (T1) for clarity and analyzed by Analysis of Variance (ANOVA) using the condition and time as the repeated measured variables, which were confirmed using Tukey's post hoc test. $p < 0.05$ was defined as the limit for statistically different mean values.

3. Results

Anthropometric characteristics of the individuals are presented on Table 1. Approximate averages of the values measured were as follows: 1.77 m of height, 82.9 kg of weight, 9.8 kg of fat weight, 73 kg of fat-free mass, 11.5% of body fat percentage, indicating that all tested individuals presented typical body composition, fat distribution, and weight profiles. We assessed the lipid profiles and serum protein levels of the individuals to characterize their nutritional status. As observed in Table 2, the assessed lipid profiles were in accordance with the healthy status of the general population. Table 3 presents the results regarding serum protein levels. Despite the fact that these data are considered normal values for the general population, it is worth noting that the assessed albuminemia was low considering a world-class team of athletes. Due to the lack of knowledge of world-class biomarker levels we chose to show all the data as a reference for future studies [3,22,23,40].

Table 1. Anthropometric parameters of the athletes were measured and are shown here as mean \pm standard error.

Anthropometry	
Height (m)	1.77 \pm 0.02
Weight (Kg)	82.9 \pm 5.0
Fat weight (Kg)	9.8 \pm 2.4
Fat-free mass (Kg)	73.0 \pm 2.6
Fat percentage (%)	11.5 \pm 2.0
BMI (Kg/m ²)	26.2 \pm 1.2

Table 2. Lipid panel values – high-density lipoprotein (HDL); low-density lipoprotein (LDL); very low-density lipoprotein (VLDL) - were assessed as described in materials and methods and are shown here as mean \pm standard error.

Lipid Panel (mg/dL)	
Serum cholesterol	173.6 ± 24.2
Serum triacylglycerol	83.3 ± 20.0
HDL	55.3 ± 5.0
LDL	95.0 ± 26.5
VLDL	16.6 ± 3.8
Cholesterol/HDL ratio	3.2 ± 0.7
LDL/HDL ratio	1.9 ± 0.6
Non cholesterol lipids	118.3 ± 28.9

Table 3. Protein fractions were assessed as described in materials and methods and are shown here as mean ± standard error.

Protein Fractions (g/dL)	
Total proteins	6.7 ± 0.12
Albumin	2.7 ± 1.23
α-1 globulin	0.4 ± 0.14
α-2 globulin	0.5 ± 0.05
β-1 globulin	0.4 ± 0.02
β-2 globulin	0.2 ± 0.003
γ globulin	1.2 ± 0.12

3.1. Muscle Stress Biomarkers

Well-established metabolic stress biomarkers were assessed in order to characterize the training intensity of the proposed trial. Compared to basal levels, AST showed a statistically significant increase after the resistance training (T4) by 30%, and continued to increase by up to 40% after the recovery period. Other biomarkers, such as ALT, ALP, and γGT, did not change throughout the trial (Figure 2, panels A and B). CK activity in blood samples, a classic muscle injury marker, was significantly higher by approximately 60% at T4. Compared to the pre-exercise state, it kept increasing to nearly two-fold at T5 (Figure 3, panel A). Despite the fact that no significant change was observed in CKMB and LDH blood activity, the CKMB mass activity, a very specific muscle injury parameter, reached an increment of 170% at T4 and was up regulated by three-fold at T5 (Figure 3, panel A). Blood levels of myoglobin were significantly increased after the first session of exercise, with an increment of 170%, and kept increasing throughout the trial to reach six-fold values at T5 when compared to basal (Figure 3, panel B).

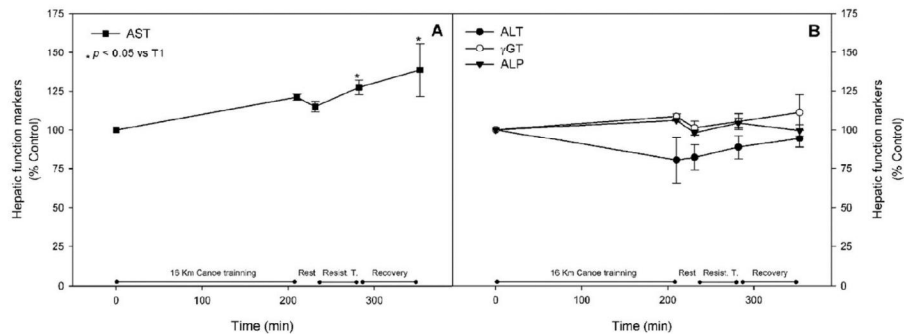


Figure 2. Hepatic injury biomarker. Aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ -glutamyltransferase (γ GT), and alkaline phosphatase (ALP) were measured as described in materials and methods and are represented as mean \pm standard error of percentage values against control. * Indicates statistical difference against control values ($p < 0.05$). **Panel A** shows AST results and ALT, γ GT, and ALP results are presented in **panel B**.

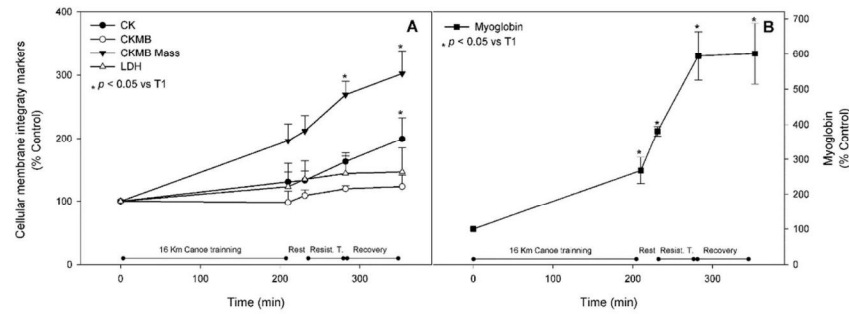


Figure 3. Cellular membrane integrity markers. Creatine phosphokinase (CK), creatine phosphokinase muscle-brain fraction (CKMB) activity and mass, lactate dehydrogenase (LDH) (A), and myoglobin (B) were measured as described in materials and methods and are represented as mean \pm standard error of percentage values against control. * Indicates statistical difference against control values ($p < 0.05$).

3.2. White Blood Cells

During the trial, blood leukocytes rose by $40.0\% \pm 16.1\%$ and $62.1\% \pm 26.8\%$ after the canoe (T2) and weight lifting (T4) sessions, respectively, showing a discrete decrease after recovery and reaching levels of $43.2\% \pm 21.5\%$ higher than basal. These results were mainly due to the increment in the neutrophil count, which showed a significant increase by $54.3\% \pm 22.3\%$ and $166.2\% \pm 71.4\%$ after T2 and T4, respectively, and was still 136.0 ± 58.2 higher than basal levels after the recovery. Despite the slight increase after the canoe training, the levels of lymphocytes showed a significant decrease of approximately 40% after the 20 min rest between the training sessions and remained significantly lower until the end of the protocol (Figure 4). Eosinophils measurements tended to accompany the lymphocytes pattern, presenting an increment of approximately 30% at T2 followed by an acute reduction that remained until the trial was terminated. Monocytes acutely responded to exercise stress and the recovery periods, increasing by about 30% after both exercise sessions with a rapid restoration of the original values. Thrombocyte levels responded positively and significantly to the canoe training sessions; they increased 30% compared to the control at T2, acutely returned to basal levels after the 20 min rest prior following resistance training, and remained similar to the original value for the rest of the trial (Figure 5).

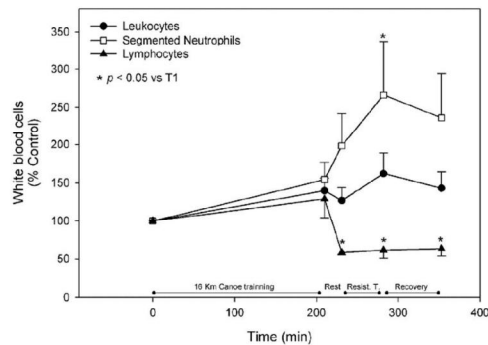


Figure 4. White blood cells. Leukocytes, segmented neutrophils, and lymphocytes were measured as described in materials and methods and are represented as mean \pm standard error of percentage values against control. * Indicates statistical difference against control values ($p < 0.05$).

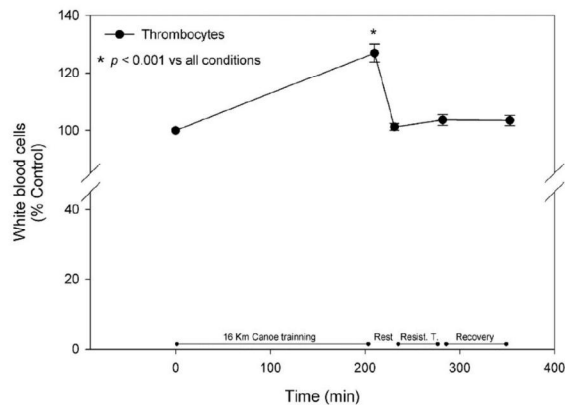


Figure 5. Thrombocytes. Thrombocyte levels were measured as described in materials and methods and are represented as mean \pm standard error of percentage values against control. * Indicates statistical difference against any other condition ($p < 0.05$).

3.3. Branched Chain Amino Acids

Plasma branched chain amino acids (BCAA), which are important substrates either as metabolic fuel or as protein synthesis precursors, decreased right after both physical stimuli. Leucine showed the most prominent, significant decrease after the canoe training, reaching almost 50% of the basal value, and continued to decrease by approximately 22% after the resistance training. These decreases did not return to the original values, even after the recovery period. Both isoleucine and valine plasma concentrations seemed to be down regulated after canoe training, however, this decrease was not significantly different. After the 20 min rest between training sessions their levels returned to original values (Figure 6, panel A).

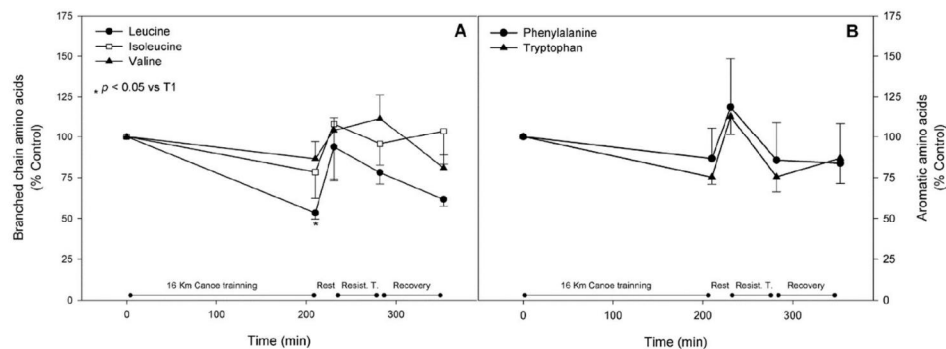


Figure 6. Branched chain (A) and aromatic (B) amino acids. Amino acid parameters were assessed as described in materials and methods and are represented as mean \pm standard error of percentage values against control. * Indicates statistical difference against control values ($p < 0.05$).

3.4. Aromatic Amino Acids

The three amino acids comprising the aromatic amino acids (AAA), phenylalanine, tryptophan and tyrosine, are ketoglucogenic amino acids that may be deviated to the gluconeogenic pathway in hepatocytes. Our results showed that these amino acids decreased in the range of 15%–25% after both the canoe and the weight lifting exercises (Figure 6, panel B).

3.5. Gluconeogenic Amino Acids

Many amino acids may serve as both substrates in anaplerotic reactions replenishing intermediates of the tricarboxylic cycle and as gluconeogenic substrates. Hence, many amino acids serve as energy sources in metabolic pathways. Interestingly, the plasma concentration of some of these amino acids increased right after the first exercise bout. Alanine showed a two-fold increment after the canoe training and also showed a slighter increment of approximately 20% at T4, after resistance training. Glutamate was up regulated by 63% at T2, but regained the original levels at T4. Ornithine plasma levels were enhanced by approximately 20% at T2. Methionine was elevated by approximately 86% after the canoe training session and decreased for the remainder of the trial. Taurine followed the methionine response, which is one of its precursors, rising 56% at T2. Glycine showed a later increase, with levels elevated by about 35% at T3 and with measurements similar to control at all other time points. On the other hand, arginine blood levels were down regulated to 60% of control values at T2 and 69% at T4. Glutamine presented a similar but slighter response, reaching 85% of basal values at T2 and returning to control levels thereafter. Lysine showed an approximately 30% decremented level at T2 when compared to control. Other assessed amino acids, such as asparagine, aspartate, serine, and threonine, did not fluctuate throughout the trial.

3.6. Metabolic Pathway Substrates, Intermediates, and Products

The metabolism of amino acids results in the production of nitrogen compounds, including ammonia; the increase in the levels of these compounds in the blood is tightly related to exercise intensity and duration. Ammonemia was significantly up regulated after the canoe training session by 78% and remained significantly enhanced (by about 71%) even after the recovery period. Urea blood level was slightly higher by about 21% at T4 and tended to reach normal values after the recovery period. Urea concentration may reflect total ammonia excretion. IMP production is

correlated to urate appearance in the blood, which is the final product of purine catabolism. Urate blood concentration rose significantly in blood by 24% and 20% at T4 and T5, respectively, when compared to the control. Blood levels of creatinine, a muscle damage indicator that may also suggest hemoconcentration alteration, responded acutely to both exercise sessions, augmenting significantly by 24% at T2. This was followed by a restoration of control values at T3, then a significant enhancement right after resistance training by 20% when compared to the control, and then a return to normal values at the end of the recovery period (Figure 7). BUN concentration remained unchanged throughout the trial.

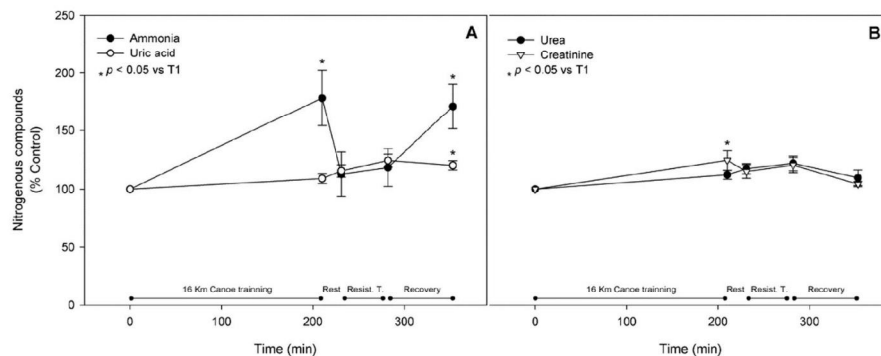


Figure 7. Nitrogenous compounds. Ammonia and uric acid are shown in **panel A**. Urea and creatinine fluctuations are presented in **panel B**. Nitrogenous compounds were evaluated as reported in materials and methods and are shown as mean \pm standard error of percentage values against control. * Indicates statistical difference against control values ($p < 0.05$).

Serum glucose, insulin, ACTH, and cortisol fluctuations during exercise are also related to the destinations of amino acid metabolites and, therefore, were measured. Due to the stress caused by the canoe training session, the hypothalamus activated the production of Corticotropin Releasing Hormone (CRH), which in turn stimulated the anterior pituitary gland to produce ACTH, and then the adrenal gland to produce cortisol. While ACTH serum levels increased by 82% at T2, cortisol rose only 12% in comparison to initial levels. After exercise, the HPA axis was suppressed, and blood levels of both hormones diminished significantly when ACTH reached values of 36% at T4, and measured cortisol was 39% at T5 when compared to T2 (Figure 8).

Insulin presented a slight decrease in response to the canoe exercise by about 10% and a significant increase of 137% after the 20 min of rest due to the food and hydro-electrolyte reposition; it returned to the approximate basal levels after the resistance training session and presented a drop by 50% of the original concentration. Glycemia was significantly enhanced by 78% after the canoe bout and tended to decrease progressively throughout the trial while maintaining its blood level slightly higher than basal. The ketone body 2-hydroxybutyrate blood levels were also significantly augmented after the canoe bout by 29%, followed by a slighter increment of 11% after the second exercise training session (Figure 9, panels A and B). It is well known that the lactate blood levels increase according to the exercise intensity. Our results showed a significant increment of 360% and 255% after the canoe and resistance training sessions, respectively (Figure 9, panel A).

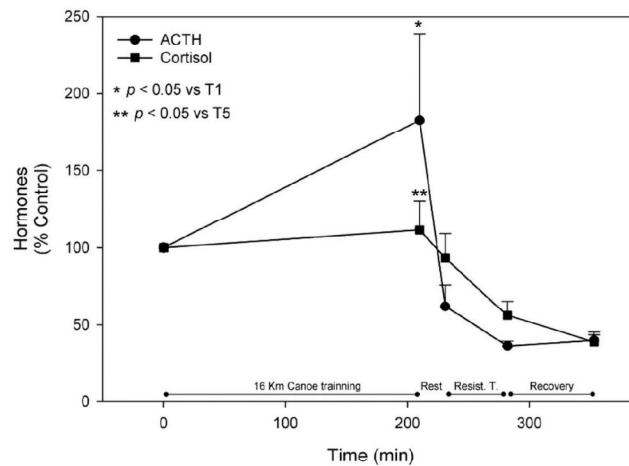


Figure 8. Hypothalamic-pituitary adrenal axis hormones. Adrenocorticotrophic hormone (ACTH) and cortisol were measured as described in materials and methods and are represented as mean \pm standard error of percentage values against control. * Indicates statistical difference against control values ($p < 0.05$). ** Indicates statistical significance when compared to T5 values ($p < 0.05$).

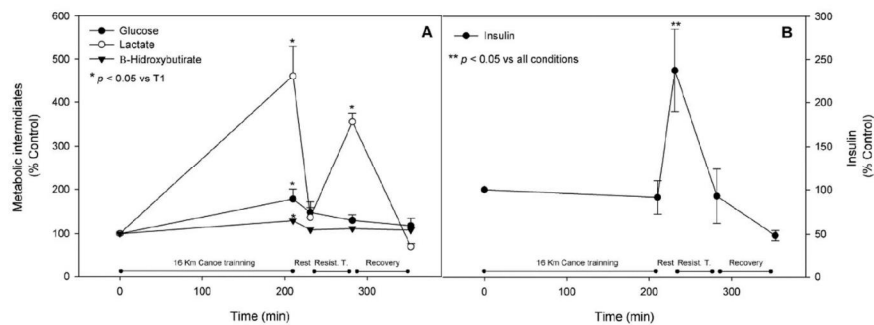


Figure 9. Metabolic intermediates and Insulin. Glucose, lactate, 2-hydroxybutyrate (A), and insulin (B) were assessed as described in materials and methods and are represented as mean \pm standard error of percentage values against control. * Indicates statistical difference against control values ($p < 0.05$). ** Indicates statistical significance when compared to all conditions ($p < 0.05$).

4. Discussion

Canoeing competitions are sprint events requiring sustained bursts of speed and power, leading to intense biochemical and metabolic stress. Many metabolites produced due to physical effort may be implicated in fatigue and impairment of physical performance, hence, the understanding of these responses is crucial to upgrade training sessions and optimize athlete performance. Here, we applied a Sportomics approach to study and evaluate different metabolic and cellular responses during a training session of world-class canoe athletes. Sportomics can help us in the understanding of the metabolism and signaling events that occur in response to exercise and allow us to perform interventions increasing both metabolic and sportive performances [20,40,47].

We measured serum levels of known exercise intensity biomarkers to characterize the applied training protocol magnitude and assure the possibility of correlating exercise intensity to the metabolic responses. Exercise intensity can be inferred by increases in plasma levels of CK, CKMB mass, CKMB, and LDH [3,48–50]. Several studies have investigated the increase in CK in response to exercise [3,22,23,40]. In this study, CK and CKMB mass blood levels increased continuously throughout the exercise trial, rising significantly at T4 and T5 when compared to basal levels. This suggests that the exercise stress and duration represented enough stimuli to cause such changes; a similar result was described by Siegel et al. [51]. Despite these observations, CKMB levels did not elevate significantly at any time point throughout the exercise session. However, it is worth noting that the blood basal levels of this enzyme were higher than expected due to exercise accumulation along the regular training season of the athletes, and this fact may have limited the furthest increment along the executed protocol. Additionally, immune assays show more analytical sensitivity when compared to enzymatic activity measurements. Therefore, it is necessary to separate immune assays from enzymatic assays. It is important to emphasize that most protocols measure the enzymatic activity of CK (also LDH and others) as a way to understand its increase or decline in response to exercise. These enzymes are also being subjected to blood environment changes that can lead to an increase in the specific activity (i.e., the ability to an enzyme to catalyze a reaction in a given unit of time), so it is our understanding that the preferable way to measure muscle cellular injury is to use a direct measurement of the enzyme content using immunological quantitative methods (such as ELISA or Western blot or mass spectrometry). For us, this is an important statement because we believe that researchers should carefully analyze the results of any increase of enzymes during exercise by the way of enzymatic activity.

Blood levels of LDH also remained constant throughout the exercise trial, and this result is in accordance with other studies which have shown a classic delayed response of LDH blood levels to strenuous exercise [52–54]. Myoglobin release from muscle to blood is a well-known biochemical marker of muscle injury [55]. In our study, myoglobin significantly increased due to the exercise stress, confirming skeletal muscle damage induced by the exercise protocol. This confirms the CK findings and corroborates our interpretation concerning the difference in the CK and CKMB increase.

Classical biochemical hepatic damage markers such as AST, ALT, ALP, and γ GT can be increased as a result of liver and/or muscle injury after strenuous exercise [56,57]. In the present study, the only enzyme that presented a significant increase throughout the protocol was AST, while the serum level of the other enzymes remained unchanged throughout the exercise. Since these enzymes are also present in muscles, the origin of this increase could not be differentiated between muscle or liver cell disruption. However, we have recently proposed that muscle damage can be distinguished from liver damage by using the more specific liver biomarkers such as ALT and γ GT [22], which remained constant in the present study. These data together suggest that the increases of these proteins are more likely from muscle rather than liver cellular injury.

White blood cell counts increase after many types of exercise and the release of neutrophils is directly correlated with exercise intensity and duration [3,18,19]. In this study, we reported an increment in total leukocytes in response to both exercise sessions, which can be attributed to the release of neutrophils. Interestingly, after a discrete increment in lymphocyte levels at T2, it returned to basal values and continued decreasing reaching a significant reduction between the training sessions. Previous studies indicated that the response of white blood cells to exercise is dependent on both cytokine and myokines modulation [58–60]. Taken together these results may suggest that white blood cell mobilization is due not to a non-specific exercise-induced spleen release, but rather to a specific signal. Additionally, we reported an increase in platelet count without any change in erythrocyte count, indicating that this effect occurs in a spleen-independent manner. As previously suggested, these results may indicate that both

thrombocytosis and leucocytosis observed during the exercise bout are induced by either muscle cell damage or differential cell signaling [3,19].

Muscle cell damage is known to stimulate immune cell mobilization to the bloodstream and migration to muscle tissue [22]. Exercise has been proposed to be a physiological way to modulate immunity; while acute severe exercise usually impedes immunity, chronic moderate exercise improves it [61,62]. Although the evidence to support these concepts is inconclusive, it supports the idea that exercise-induced immune suppression increases susceptibility to symptoms of infection, particularly around time of competition [63]. Moreover, metabolic stress is correlated with exercise induced white blood cell response, as carbohydrate supplementation and availability have been proposed to affect neutrophil count after intense exercise [64–66]. We previously described that a combination of training, rest and nutritional intervention could have an important impact in amino acid availability, muscle cellular injury, and immune response in another world-class athlete [40]. Therefore, the immune responses reported here may be directly correlated with the alterations in the nutritional status and metabolic availability as observed during the present experimental trial.

In this sense, it is important to maintain plasma level amino acids during training sessions, since many amino acids serve both as anabolic and energetic precursors. In addition, it has been proposed that blood fluctuations in the concentration of BCAAs may affect its ratio in the brain [67]. In our study, the levels of many amino acids presented a blood concentration decrease during the sport trial. Leucine showed the most important decrease after the canoe training session. Isoleucine and valine concentration also decreased in a smaller range. Aromatic amino acids, which are generally metabolized in the liver, were slightly consumed during the canoe exercise session; similar results were described before [40]. Glutamine levels presented a similar response; they decreased after the canoe trial and were restored after the recovery period. This could be the result of two processes: glutamine exportation from muscle to decrease its ammonia levels; and the use of glutamine as both a gluconeogenic substrate and a urea cycle feeder in the liver. On the other hand, alanine was up regulated after the first exercise bout, showing a two-fold increment at T2. This response may be attributed to a metabolic attempt to offer gluconeogenic substrates for further oxidation. The depletion of glycogen storage is related to exercise intensity, duration, and nutritional status, which in turn may increase the use of amino acids as energy substrates, thereby increasing ammonia and the production of other nitrogen compounds [68]. Both glutamine and alanine are anaplerotic and gluconeogenic substrates and contribute to ATP and glucose synthesis. The ergogenic properties of glutamine have been extensively studied [6,66,69], and we have recently reported the metabolic effects of alanine in comparison to long-term glutamine supplementation during an intermittent exercise protocol. Long term administration of glutamine is capable of reducing ammonia production during intermittent exercise, hence, it is postulated to be a protector against an increase in blood ammonia in an exercise intensity-dependent manner [23].

Many studies have indicated that ammonia is a useful physiological marker of prolonged intense exercise, and its appearance in blood is positively correlated with exercise intensity [1,32,40]. High ammonemia can be toxic to both muscles and the central nervous system (CNS). Such changes are believed to contribute to the disturbances in neuropsychological function and motor control deficits and are also observed in patients with cirrhosis and, therefore, could induce central and peripheral fatigue [27,64,70]. Therefore, measuring ammonia production during a sport session may represent an important tool to control exercise intensity and to understand the metabolic response of a given athlete. The canoe athletes experienced an increase in their blood ammonia levels during the exercise trial due to both stimuli, which remained up regulated even after the recovery period. This effect was followed by an increase in other measured nitrogenous compounds, such as urea, urate and creatinine. These responses may have

occurred as a result of an increased demand for ATP by muscle contraction, leading to adenosine monophosphate (AMP) deamination and, subsequently, the production of ammonia and urate [28–31]. Many studies have shown that ammonia production and release represents the exercise effort intensity, rapidly increasing in intensities greater than 50%–60% of $\text{VO}_{2\text{max}}$ up to maximal exhaustion [33,34]. Ammonia plasma concentration is also up regulated during prolonged (greater than one hour) submaximal exercise (60%–75% $\text{VO}_{2\text{max}}$). In these conditions, ammonia could be produced in increasing amounts through the breakdown of branched chain amino acid (BCAA) prior to oxidation for additional energy provision [35–37].

The response of the other nitrogen metabolites may shed light on understanding the protein and amino acid oxidation response during exercise. Urea and urate blood concentration is indirectly correlated with the myokinase (adenylate kinase, ADK) contribution to ATP synthesis. Under a resting physiological state, approximately 90% of the skeletal muscle adenosine monophosphate deaminase (AMPD) is in a sarcoplasmic position and in an inactive form. However, a significant change occurs as intense muscle contraction begin, when approximately 50%–60% of AMPD becomes bound to the myofibrils [30]. Binding of the enzyme increases its activity causing an increased rate of degradation of AMP to IMP. This is correlated with the appearance of urate in the blood which is a final metabolite of purine metabolism [32]. This increased breakdown of AMP will affect the equilibrium of the ADK reaction by creating additional ATP from ADP to increase the cellular energy charge and maintain contractions under conditions of increasing stress [31]. During intense exercise, when AMP production and deamination are high, ADP levels also increase as utilization of ATP exceeds re-phosphorylation [71]. Therefore, any strategies, such as diet adequacies and supplementations, to protect against hyperammonemia or an increment of any nitrogenous compound could enhance physical performance or prevent CNS injuries, as previously reported by our group [6,23,40].

During the canoe trial, glycemia rose significantly, which may be a result of the HPA axis activation. Exercise is known to be a potent activator of this endocrine system, resulting in the release of ACTH, as confirmed here. This ultimately culminates with glucocorticoids production and release into blood circulation, which may lead to gluconeogenesis activation and promotion of an adrenergic stimulus, providing glucose to blood from hepatic glycogenolysis [72–74]. Nevertheless, afferent neural feedback signals from contracting muscle and feedback signals mediated via the blood stream can stimulate glucose production to maintain glycemia. Therefore, central mechanisms coupled with the degree of motor center activity can be responsible for part of the increase in glucose mobilization, especially during intense exercise where hepatic glucose release exceeds peripheral glucose uptake, and plasma glucose rises [75]. Furthermore, cortisol is implicated in exercise induced lymphocyte apoptosis, via glucocorticoid dependent-pathways [76], which might affect immune function and protect the organism from an overreaction of the immune system in the face of exercise-induced muscle damage [77].

Hepatic glucose production increases during exercise, to cope with the augmented demand, as a product of liver glycogenolysis and gluconeogenesis. Whereas the former predominates during high intensity exercise, the latter contributes substantially with prolonged exercise and the concomitant decline in liver glycogen stores and with increased gluconeogenic precursor supply. In fact, it has been postulated that the increase in glucose production with exercise intensity in healthy subjects can be entirely attributed to increases in net hepatic glycogenolysis [78]. This pathway is also supported by our data. On the other hand, a decline in plasma insulin is important for the rise in glucose production during exercise [75], due to the fact that insulinemia tends to decrease in response to prolonged exercise, with a more pronounced effect on athletes than untrained individuals [79], which is in agreement with the results reported here.

5. Conclusion

The data presented here allow us to consider hormonal, metabolic, and signaling response together with the knowledge of nutrition and training environment. This combined information permits a better understanding of the individual responses of exercise and sport stress. Our group developed the concept of Sportomics with a focus on bridging the same existent gap between translational and personalized medicine [80]. As stated by Liebman et al. [81], the workflow bench to bedside approach is being refined in the face of a new bedside-bench-bedside approach. Sportomics is useful to evaluate the unprecedented kinetics of some metabolites [5,8,11,12,14,15,17,39,82] and to shed light on the importance of in-field metabolic analyses to the understanding of the inter-individual response to exercise. Besides an effective nutritional support, collecting physiological data during training and competition can provide important information about an athlete's clinical condition, bringing strategies to modify metabolism during exercise as well as supporting coaches to prescribe their sessions and recovery time. Therefore, and due to the uniqueness of this study, we believe Sportomics is a primal tool for training management and performance improvement, as well as for preserving health and increasing the quality of life of athletes.

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W.S.C., L.V.C., E.D., A.B. and L.C.C. conceived and designed the experiments; W.S.C., L.V.C., E.D. and A.B. performed the experiments; W.S.C., A.M.F. and L.C.C analyzed the data; A.B. contributed reagents/materials/analysis tools; W.S.C., A.M.F. and L.C.C wrote the paper.

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CAPÍTULO III

COMPARAÇÃO DA RESPOSTA METABÓLICA DE INDIVÍDUOS E MODALIDADES DIFERENTES A TESTES PADRÃO DE AVALIAÇÃO DE PERFORMANCE EM EXERCÍCIO

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**IMMUNE SYSTEM AND AMINO ACIDS BLOOD CONCENTRATION DURING
ULTRA ENDURANCE EXERCISE: A SPORTOMICS VIEW**

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**Immune system and amino acids blood concentration during ultra
endurance exercise: a Sportomics view**

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Abstract

Purpose. Ultra endurance exercise is a challenge for the Central Nervous System. It has been showed amino acid and ammonium metabolism related to neurotransmitters synthesis during prolonged exercise. Sportomics is a tool to study the individual metabolic changes during sports. Here we evaluated an ultramarathonist in respect of hormones, immune response, ammonium and amino acids metabolism during a simulated race.

Methods. Athlete participated at “The Badwater World Cup” and won two competitions. The athlete ran for eight hours (86 km) in an 800m synthetic track. Venous blood samples were collected 10 times: eight hourly and twice during recovery to measure approximately 75 cellular and biochemical parameters.

Results. Adrenocorticotrophic hormone reached 445% and stimulated cortisol (130%). In addition, adrenocorticotrophic hormone stimulated aldosterone (840%). Both leukocytes and neutrophils increased cooperatively and fit a sigmoid function with high correlation (Hill number = 3.0; $r = 0.97$) during the race, succeeded by an exponential decay. A raise of both urea (90%) and urate (20-30%) was also measured. Ammonia increased reached a peak of 290%, due to amino acid deamination (mainly Leu and Ile). Phe could be metabolized to Tyr fueling TCA. Trp catabolism (50%) might be linked to an immunoregulatory effect and reduction of serotonin synthesis.

Conclusion. The results showed the synergistic effect of leukocytes and neutrophils during the ultra endurance race. We also demonstrated the kinetics of amino acids consumption as energetic molecules, which in turn could privilege a

low ratio of 5-HT/dopamine (or catecholamine) in the brain. The findings bring information about the amino acids metabolism and lights up the discussion about the availability into the CNS during prolonged exercise.

Keywords: Ammonia, inflammation, muscle injury, glutamate, dopamine, serotonin.

Introduction

Personalized medicine (“n=1”) is defined by patient’s unique condition combined to optimization in drugs to manage the prevention, diagnosis and treatment of a disease. Inside this perspective, Sportomics is non-hypothesis-driven holistic top-down research and compromised in data generated by “-omics” science, as genomic, transcriptomic, proteomic and metabolomic with classical laboratories analyses, showing important information about the athlete’s unique condition in the field of play (BASSINI; CAMERON, 2014; RESENDE et al., 2011). This approach has been previously proposed by our group to study the athletes in real conditions faced during training or competition (BASSINI; CAMERON, 2014).

We have been using exercise as a model to study hyperammonia during exercise by manipulating diet and training itself (BASSINI; CAMERON, 2014). In this way, we showed that carbohydrate and glutamine (Gln) are able to reduce the ammonia during in a field endurance (nearly 34 Km for 120 minutes), protecting the CNS (CARVALHO-PEIXOTO et al., 2007). The source of ammonia production in endometabolism is to purine cycle nucleotide and amino acids breakdown during exercise (WILKINSON et al., 2010). Ammonia (here is defined as sum of $\text{NH}_3 + \text{NH}_4^+$) may cross the blood brain barrier (BBB) and be highly toxic to CNS (NYBO et al., 2005; WILKINSON et al., 2010). Inside it, ammonia can be detoxicated through glutamate (Glu) metabolism promoting neurotransmitters reduction during prolonged exercise in rats (GUEZENNEC et al., 1998). Depending the ammonia concentration and time of exposure, brain ammonia can

accumulate inside the synaptic cleft and promote seizures, convulsion, coma and death (FELIPO; BUTTERWORTH, 2002).

We also demonstrated that immune response during exercise is due to inflammation and muscle damage (GONCALVES et al., 2012; RESENDE et al., 2011). In this sense ultramarathon also presented leukocytosis subsets and systemic inflammation in response to micro tears during exercise (SHIN; LEE, 2013). These micro tears are underlying causes to acute phase proteins during prolonged exercise which have similar concentrations to chronic diseases (FALLON, 2001). It was showed that leukopenia might occur due to glutamine consumption by muscles after the exercise (CASTELL; NEWSHOLME, 1998), exposing the athlete to infection (CASTELL; POORTMANS; NEWSHOLME, 1996) This scenario referred the ultramarathon as a good model of extreme exercise condition to study modification on brain, hormones, immune system and muscle injuries (MILLET; MILLET, 2012). Therefore, we analyzed the inflammation, ammonia and amino acids blood response to an ultra endurance sport in an elite athlete male to comprehend the challenges faced by body and brain.

Methods

This study was approved by the Ethics Committee for Human Research at the Federal University of the State of Rio de Janeiro (CAAE:0053.0.313.000-07, 117/2007, renewed in 2011, 2013 and 2016) and meet the requirements on human research (Health National Council, Brazil, 1996). A written informed consent was read, discussed and signed by the athlete.

Subject:

An elite male ultramarathonist (46 yrs old) had a regular breakfast one hour before to start the ultra endurance race and was oriented to keep the routine of training, refraining of any medication or supplements prior to race. Then, the athlete started to warm up and was monitored by a Global Positioning System (GPS) watch. Water and food were intake *ad libitum* during the whole race (10% from protein; 10% from lipids; 80% from carbohydrates). The food was in creamy or liquid form. Blood samples were collected 10 times during the experiment: adding hourly (eight times) and after an hour (short recovery post – race) and 12 hours (long recovery post-race; Figure 1).

Collection and blood analyzes:

The venipunctures were drawn in antecubital vein, alternating arms on each sample collection. Total blood for hematological assays was collected into tubes (Vacuette, Greiner Bio-One Frickenhausen, Germany) containing EDTA, refrigerated at 8°C and analyzed in no more than three hours.

A blood aliquot was centrifuged 3000g for 10 min (RDE i model B-40) and serum/plasma was stored at -80°C for later biochemical analyses. The biochemical analyses included: alanine aminotransferase (ALT; EC 2.6.1.2), alkaline phosphatase (ALP; EC 3.1.3.1), aspartate aminotransferase (AST; EC 2.6.1.1), creatine kinase (CK; EC 2.7.3.2), lactate dehydrogenase (LDH; EC 1.1.1.27), gamma-glutamyltransferase (GGT; EC 2.3.2.2), glucose, lactate, ammonium, urate and urea were measured by enzymatic methods; chloride and creatinine by colorimetric methods; insulin, prolactin, cortisol, adrenocorticotrophic

hormone (ACTH) were measured by chemiluminescence; α -1-acid glycoprotein (α 1GP) was measured using nephelometry; aldosterone by radioimmunoassay method; sodium and potassium were measured using an ion selective electrode; C-reactive protein (CRP) by turbidimetric method; amino acids (alanine; arginine; asparagine; citrulline; glutamate; glutamine and Histidine; glycine; isoleucine; leucine; lysine; methionine; ornithine; phenylalanine; proline; serine; threonine; tryptophan; tyrosine and valine) were measured by high performance liquid chromatography.

The data were normalized to pre-race values to show the normalized variation during the experiment.

Curve fitting was performed using non-linear regression for leukocytes and neutrophils. The increase in leukocytes and neutrophil activities curve were fitted according to following Sigmoid function with four parameters:

$$y = y_0 + \frac{a}{1 + e^{-\left(\frac{x - x_0}{b}\right)}}$$

Calculated parameters for neutrophils were: $a = 178.4 \pm 22.9$; $b = 0.5 \pm 0.2$; $x_0 = 5.4 \pm 0.3$ and $y_0 = 131.3 \pm 10.5$. The calculated parameters leukocytes were: $a = 129.2088 \pm 24.2936$; $b = 0.8379 \pm 0.3981$; $x_0 = 5.1693 \pm 0.4421$ and $y_0 = 116.7994 \pm 9.8056$.

The decrease in leukocytes and neutrophil activities curve were fitted according to following Exponential decay function with two parameters:

$$y = ae^{-bx}$$

Calculated parameters for neutrophils were: a= 573.6854 and b= 0.0898; and leukocytes were: a = 316.3811 and b=0.0549.

Results

In this study we looked for the metabolic and signaling changes induced by a double marathon (~ 83 km) by the way of Sportomics. We followed up a world-class athlete during an eight hours running in an 800 m circuit (Fig. 1). He ran 83 Km with an average speed of 12 Km.h⁻¹.

To ensure that the changes were due to real metabolic and signaling modifications we evaluated the haematocrit alteration during the running. There was an increase (15%) in the haematocrit in the third hour with slight changes during the running (0 – 15%). We measure a decrease (15%) in the red blood cell volume during the recovery mainly in the first hour that remain increasing until the end of the protocol, reaching 85% of pre-running (Fig. 2).

To evaluate the electrolyte modifications caused by the running protocol we measured sodium, potassium and chloride concentrations in blood. Both sodium and chloride concentrations changed less than 7% during running and recovery. We measure a bigger decrease in kalemia (10%) from 3-6 hours of the protocol with another minor drop (5%) from 7-9 hours. The kalemia remained at 94% until the end of recovery (Fig. 3).

To understand muscle micro-injury during the protocol we measured classical biomarkers. Myoglobin blood presence rose almost four-fold in the first five hours (~45% of increase per hour) of running increasing in a greater velocity (95% of increase per hour) until the eighth hour. The protein in blood decreased to basal level during recovery. LDH in blood rose up to 60% during the running returning to 110% during recovery. Surprisingly CK presence in blood increased less than 25% in running decreasing to ~90% of pre-exercise in recovery, in a very similar pattern with AST (Fig. 4).

A meaningful increase in muscle micro-injuries markers can be followed by an inflammatory response. To evaluate it, we measured the presence of acute phase proteins CRP and α 1GP. The α 1GP presence in blood remained steady for the whole protocol while CRP kept constant during running increasing up to 180% during recovery (Fig. 5).

White blood cells response is a well-documented effect in exercise, following WBC presence allow us to observe a cooperative increase (Hill number = 3.0) in leukocytes beginning at five hours of exercise and remaining elevated until its ending. Leukocytes increased up to 230%, decreasing almost to basal levels during recovery (Fig. 6 A).

The cooperative increase in leukocytes is due to an additional effect of neutrophils increment (245% at seventh hour) and lymphocytes reduction (60% at seventh hour) in blood. The neutrophils-lymphocytes ratio (N/L) increased considerably during the last three hours of running. During the first five hours of exercise neutrophils account for ~75% and ~15% lymphocytes of WBC, this

relation dropped for ~90 and 5% respectively in the final three hours of running, being restored to initial proportion during recovery (Fig. 6 B).

To evaluate inflammation response we measured ACTH systemic signaling during the running. Cortisol and aldosterone increased in response to ACTH in the first seven hours (130% and 840%) of exercise, decreasing until the ninth hour and remained constant. Interestingly, aldosterone did not return to basal level during recovery (440%). Prolactin concentration decreased until the sixth hour (26%), increasing until recovery with a peak in seven hours (150%; Fig. 7).

In order to access amino acids metabolism during race we followed their concentration in blood (Supplementary Table 1; Figs 8-9) in association with other metabolites. Glucose rose during the first four hours of running reaching 60% of its initial concentration, decreasing to 120% until the end of the exercise. Raising again on short recovery (80%) and returning at the end of the experiment. We also measure a drop of both basal insulinemia (10-30%) and lactatemia (40-70%) during most of the running (Fig. 8).

During the first four hours of running, we measured a drop in Leu and Ile concentrations (up to 50% of pre-running concentrations), Ala in blood reached a similar concentration (40%) without total recovery. Ile and Leu from fourth to fifth hours rose nearly 60% and 40%. Ile and Leu had a dropped of around 25% from fifth to eighth hours, returning to 25% and 10% higher than pre-race values. Glu concentration pattern was similar to Ile and Leu. Glu kept its concentration constant at baseline until the first fourth hours. Glu from fourth to fifth hours rose

30%, decreasing 30% from sixth to eighth hour, followed by an increment of 30% at ninth hour and a reduction to baseline during the recovery (Fig. 8 and 9).

Val and Gln + His increased 10-20% since the beginning of race and remained constant until the seventh hour. An increase in blood BCAA concentration was measured during the recovery (Fig 8).

Aromatic amino acids (AAA) concentration dropped similarly (5-20%) during the first fourth hours, increasing until the sixth hour. We measure a 50% drop in Phe and Trp concentrations from sixth to eighth hour, associated with an increase (~100%) in Tyr concentration. The three AAA rose their concentrations during the fast recovery hour and reached the basal level at the end of the protocol (Fig. 8 and 9).

Thr concentration increased 75-100% from sixth to ninth hours with a peak at eight hours of protocol. Asp; Asn; Met and Orn remained nearly constant during the protocol. Lys and Tau concentrations decreased during fast recovery (Suppl. Table 1)

We evaluated the nitrogenous metabolism final products for the understanding of the amino acid metabolism. Both creatinine and urate increased 10-20% during the exercise. Urea increased almost 90% at eight hours and remained constant until the end of the experiment. Ammonium doubled its concentration at first hour, reaching a peak of 290% at the seventh hour, dropping to ~135% of basal levels at recovery (Fig. 10).

DISCUSSION

An ultramarathonist trains to run distances over the classical marathon of 42.195m. Our athlete is an experienced runner that participated at Badwater World Cup which consist of three races (Death Valley Desert; Brazil 135 and Arrowhead) of 135 miles each one. The athlete ran at this three ultra endurance races and won two. Recently, the athlete participated at Spartathlon Ultra Race at Greece.

We measured an increase in haematocrit levels (~10%) in our athlete that might be associated with a volemia decrease. This result is reinforced by a similar increase in blood creatinine concentration. It is well established that excessive sweating and inadequate liquid intake contribute to dehydration and electrolytes imbalance.(WASKIEWICZ et al., 2012). Our results showed small variations in both chloride and sodium.

During exercise ammonia is released from amino acids deamination for use as carbon skeleton donors providing either tricarboxilic acid cycle (TCA) intermediates or glucose synthesis. Ammonia is also released from AMP for further metabolism until urate (for a review see Wilkinson et al., 2010). Ammonia is metabolized mostly in hepatocytes to urea. This rational can give us an indirect way to figure out ammonia origin. Urea is the sum of all the ammonia produced by endo and exometabolism (microbia). Since that it is unlikely that exercise affect microbia, we can assume that the ammonia produced by exercise metabolism is excreted as urea. This means that an increase in free ammonia and uremia is due to both amino acid and AMP deamination, otherwise the

increase of urate show us the contribution of AMP deamination and further metabolism of IMP.

During exercise a decrease in kalemia can be related to a major risk of cardiac arrhythmia.(CLAUSEN, 2003) We have been showing in the past years that some athletes develop a major hypokalemia during exercise that can be corrected by proper intervention (Rezende et al, 2011). In this observation, the potassium concentration in blood dropped from the second to the fifth hour by 10% returning to basal level and impairing again. Kalemia changes are related to exercise intensity (Ole M. Sejersted & Gisela Sjøgaard, 2000). Different responses to changes in potassium concentration in blood are described. An increase in the AMP:ATP ratio is related with decrease in kalemia (Jang H. Youn & Alicia A. McDonough, 2009). We measure an increase in ammonia, urate and urea associated with kalemia decrease. Kalemia drop is associated with insulinemia increase. It is likely that the increase in AMP:ATP ratio indirectly measured by the way of an increase in ammonia and urate with an increase of glycemia leads to a decrease in kalemia. This hypothesis is reinforced by the one hour increase in the kalemia (returning it to the basal levels) from the 5-6 hours of training associated with a previous decay in glycemia 4-5 h. The following increase in glycemia was again associated with dropping in kalemia.

It was also described that potassium released from muscles might stimulates aldosterone (YAMAUCHI et al., 1998), but the observed reduction of potassium suggested that had little effect on aldosterone. The recovery of potassium to basal level might be induced by this hormone which is normally

stimulated by ACTH as previously reported during the ultra endurance.(YAMAUCHI et al., 1998) Additionally, ACTH also stimulated cortisol secretion during ultra endurance.(PESTELL; HURLEY; VANDONGEN, 1989) that can explain the increase of cortisol secretion detected at fourth hour herein.

Ultramarathon is a good model to analyse the current injury biomarkers in consequences of ultra endurance race. (MILLET; MILLET, 2012) We were surprised by the little increase of CK level during the running that could be explained in two ways: the running had a small impact on the release of CK in blood or it was due to the elevated basal levels since the beginning of exercise, when we lost sensitivity in the measure. Regarding it, we demonstrated that LDH and CK were mainly increased at eight hours of race while others injury biomarkers had slight changes. However, during the recovery period, reduction in AST and ALT achieved equally their initial values. We also investigated myoglobin and observed one slope with two distinct velocity of augment, reaching the higher increment at eight hours. Our myoglobin result is not in accordance with reported by others, probably due to difference among analytical methods, experimental design or health status of the athlete.(FALLON et al., 1999)

It is well known that exercise induce leukocytosis (BASSINI; CAMERON, 2014; BESSA et al., 2008). Here, we demonstrated that the response of neutrophils was synergistic easily seen by a sigmoidal behavior. In fact, the mobilization of leukocytes is stimulated by cortisol; catecholamines and different cytokines during endurance exercise.(STARKIE et al., 2005) After the end of

exercise neutrophils showed an exponential decay during the recovery of ultra endurance exercise. (NEUBAUER et al., 2013; WASKIEWICZ et al., 2012)

Part of these kinetics could be explained by observing acute phase proteins response profile. A slight increase of α 1GP during ultra endurance race was accompanied by a little contribution of lymphocytes raise to leukocytosis, which is understandable since at high concentration α 1GP mildly suppress the proliferation of lymphocytes, probably due to a subset of T-cells signalling.(CECILIANI; POCACQUA, 2007) Moreover, it was observed that either normal or inflamed serum (<300 mg/L) α 1GP inhibits neutrophils without affecting its spontaneous migration, including the chemotactic response to formyl-methionyl-leucyl-phenylalanine (fMLP). (FOURNIER; MEDJOUBI; PORQUET, 2000; HOCHÉPIED et al., 2003; MCCURDY et al., 2012) We were able to detect herein, hence the increased neutrophils level could be taken up PCR bound to its ligands, as previously demonstrated.(ABLIJ; MEINDERS, 2002) Only at recovery period with neutrophils decay we could observe PCR increase. However, accumulation of leukocytes in the muscle tissue was notify on human studies involving various types of 'muscle-damaging' exercise (PEAKE et al., 2017) and may play role at neutrophil reduction level during recovery. Also, PCR seems to inhibit neutrophils migration (ABLIJ; MEINDERS, 2002), possibly acting on feedback mechanism.

The ultra endurance sports require extreme physical preparation. In this way, it is important to understand peripheral biomarkers of CNS metabolism during an ultramarathon.(AGAWA et al., 2008) Carbohydrates and amino acids in

bloodstream are important to keep muscle and brain activities during the activity.(NOAKES et al., 1988; WILKINSON et al., 2010) The creamy and liquid food were consumed during the race provided adequate carbohydrate-loaded to maintain the glucose levels in the first fourth hours (NOAKES et al., 1988). The increase in glicemia was related to the decrease in kalemia during the protocol. These findings can be explained by earlier studies that showed that an increase in insulinemia can promote hipokalemia (MAGISTRETTI; ALLAMAN, 2015; NOAKES et al., 1988). For a good review in potassium homeostais please refer to Youn & McDonough (2009) (YOUN; MCDONOUGH, 2009). In addition it has been previously shown that an increase of AMP:ATP ratio can promote a significant decrease in kalemia (ZHENG et al., 2008)(Fig.11)

Ammonia/ammonium during long exercise has been discussed as central key to fatigue.(BANISTER; CAMERON, 1990) It was reported in endurance the ammonia accumulation in CNS after 3 hours of exercise in cycle ergometer at moderate intensity.(NYBO et al., 2005) Additionally, it might affect the transport system at BBB and could facilitated the amino acid entrance into the brain in rat models.(HILLMANN et al., 2008; JONUNG et al., 1985) We observed Ala and Tau reduction during the ultra endurance race probably playing a role as direct and indirect substrates to piruvate. Tau could influence the Thr, Gly and Ser metabolism as precursors to pyruvate(ISHIKURA et al., 2011) and be the source the first ammonium wave. In our observations from sixth and seventh hour of race, the second increased wave of ammoniun was accompanied by reduction of Asn, Asp, Phe, Trp, Tyr, Leu, Ileu, Arg and Glu due possibly to cataplerosis in trycarboxylic acid cycle (TCA) in muscles from fifth to eitght hour.

Differently, we detected that Gln levels remained constant above the basal levels. These amino acids are the main ammonium carriers in blood to liver in order to promote urea and neoglucogenesis.(BASSINI et al., 2013) We were able to observe an urea increased concentration after fourth hour reducing the toxic effects of ammonium to brain (BASSINI et al., 2013; CARVALHO-PEIXOTO et al., 2007). Additionally, Lys and Met augmented after the same period probably acting as precursors to both Acetyl-CoA and Succinyl CoA (TCA intermediates)(LYUBETSKAYA; RUBANOV; GELFAND, 2006).

Tyr is a precursor to dopamine and catecholamines synthesis.(FERNSTROM; FERNSTROM, 2007) It could be reflected by enzyme phenylalanine 4-hydroxylase activation inducing the pathway of noradrenergic, adrenergic and dopaminergic neurotransmitters to compensate the Trp catabolism. (STRASSER; GEIGER; et al., 2016) In fact, Tyr competes with Trp and BCAA to be carried by System L1 and enter CNS (HAWKINS et al., 1995; SMITH, 2000), it was demonstrated that exercise increased dopamine in various brain nucleus.(FOLEY; FLESHNER, 2008) Herein, we observed an increment of Tyr during exercise and—taking these considerations, we could suggest that dopamine/catecholamines synthesis was affected by exercise.

The data reported here may be not extrapolated to other athletes, but they show an important metabolic adaptation during ultra endurance race. These adaptations were observed mainly from fourth to eight hour. Thus, we could fit the sigmoid curve at leukocytes in response to tissue injury and signaling to acute phase proteins. Stress hormones were stimulated mainly after fourth hours

which affected the electrolytes balance and energetic metabolism. We also demonstrated the kinetics of amino acids consumption to TCA during ultra endurance race, being responsible to ammonium production and other pathways.

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Disclosure:

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Legends

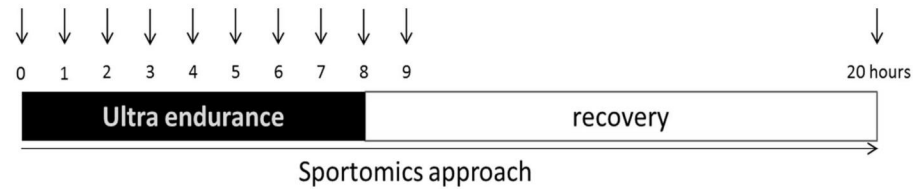


Figure 1. Ultra endurance race in Sportomics approach. The athlete ran during eight hours and had twelve hours of recovery period, ingested food and drank water *ad libitum*. Arrows indicate venous blood collection.

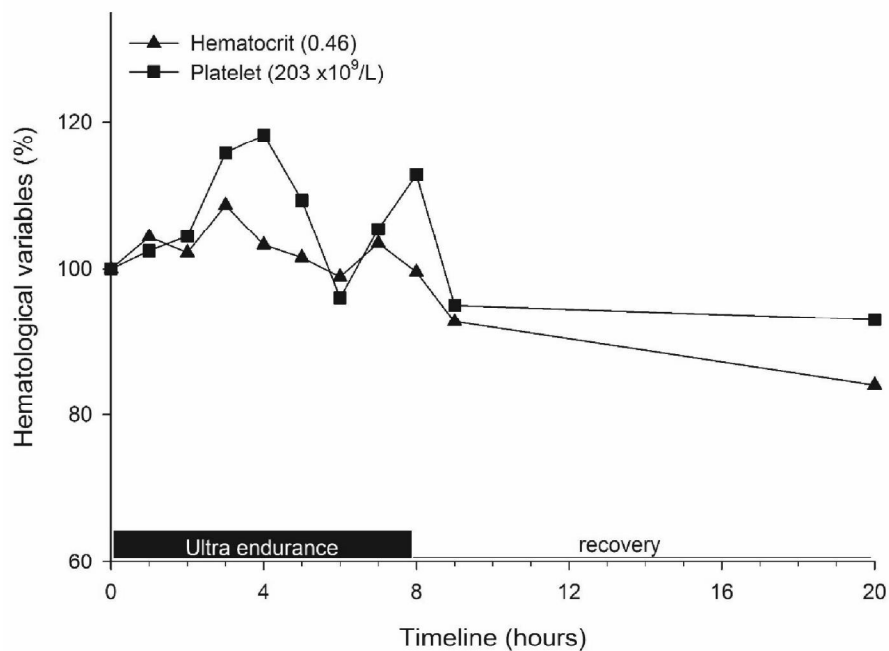


Figure 2. Haematological changes during ultra endurance race. There was a reduction at fourth to sixth hour of haematological parameters, haematocrit and platelet. Data are presented normalized and absolute values in label.

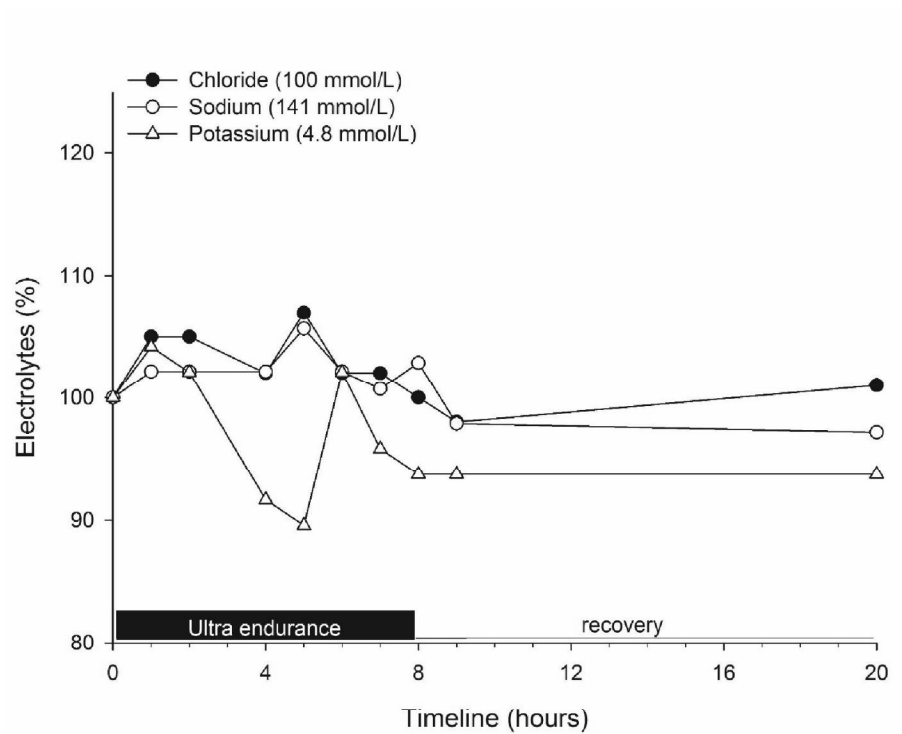


Figure 3. Electrolytes modifications during the race. It might be observed a small reduction at potassium from fourth to fifth hour during the race. Data are presented normalized and absolute values in label.

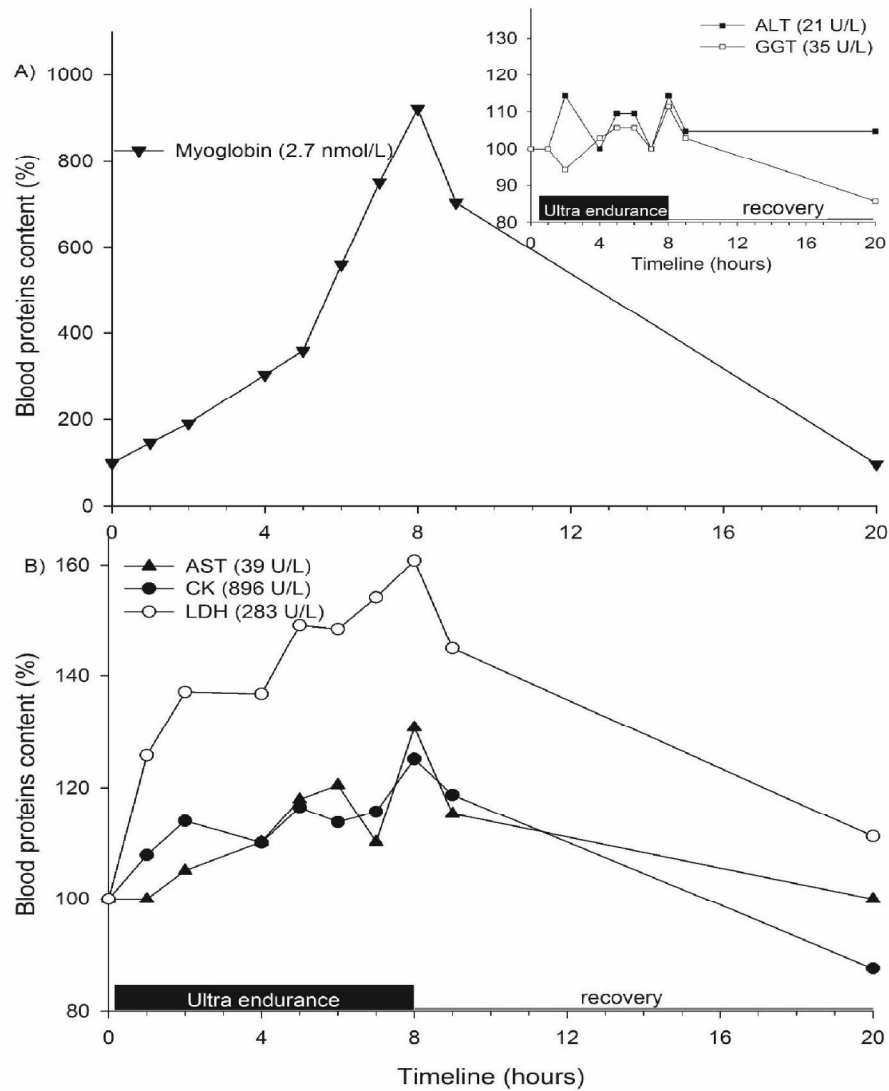


Figure 4. Blood proteins content in response to ultra endurance. (A) Myoglobin had two step of increment until eighth hour. Inset: ALT and GGT are the control to liver injury biomarkers. **(B)** AST and CK had similar kinetic pattern appearance in blood. LDH had higher variation compared to AST and CK. Data are presented normalized and absolute values in label.

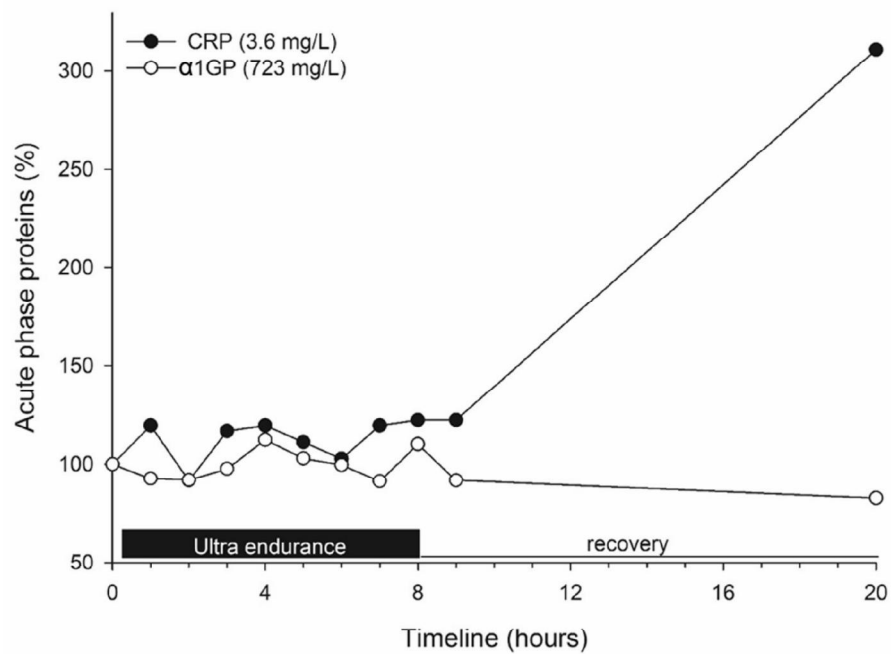


Figure 5. Acute phase proteins response to running. CRP increased in approximately twenty hours. α1GP kept constant throughout the running and recovery period. Data are presented normalized and absolute values in label.

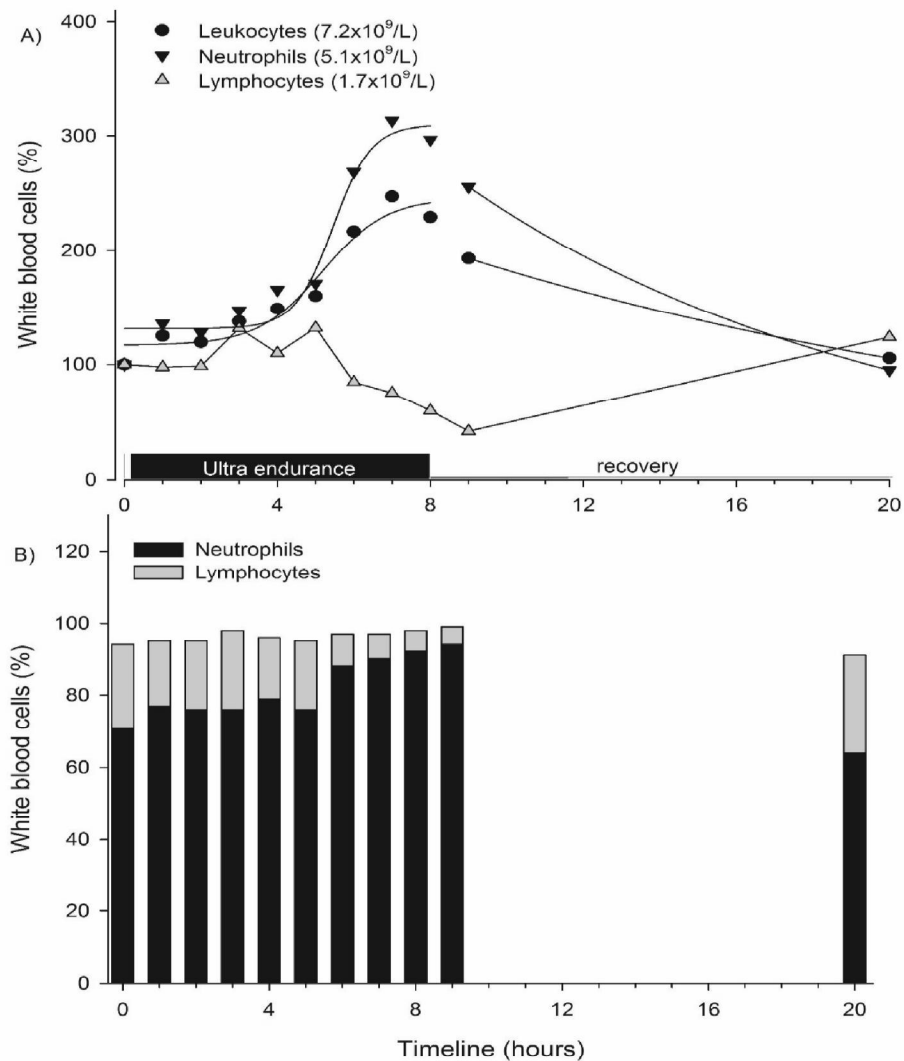


Figure 6. White blood cells mobilization during exercise and recovery. (A) Sigmoid function described leukocytes and neutrophils synergic effect on exercise. On the other hand, during recovery there was an exponential decay of neutrophils and leukocytes. **(B)** The differentiation of leukocytes in response to

exercise was main due to neutrophil mobilization and returned to basal values during recovery. Data are presented normalized and absolute values in label.

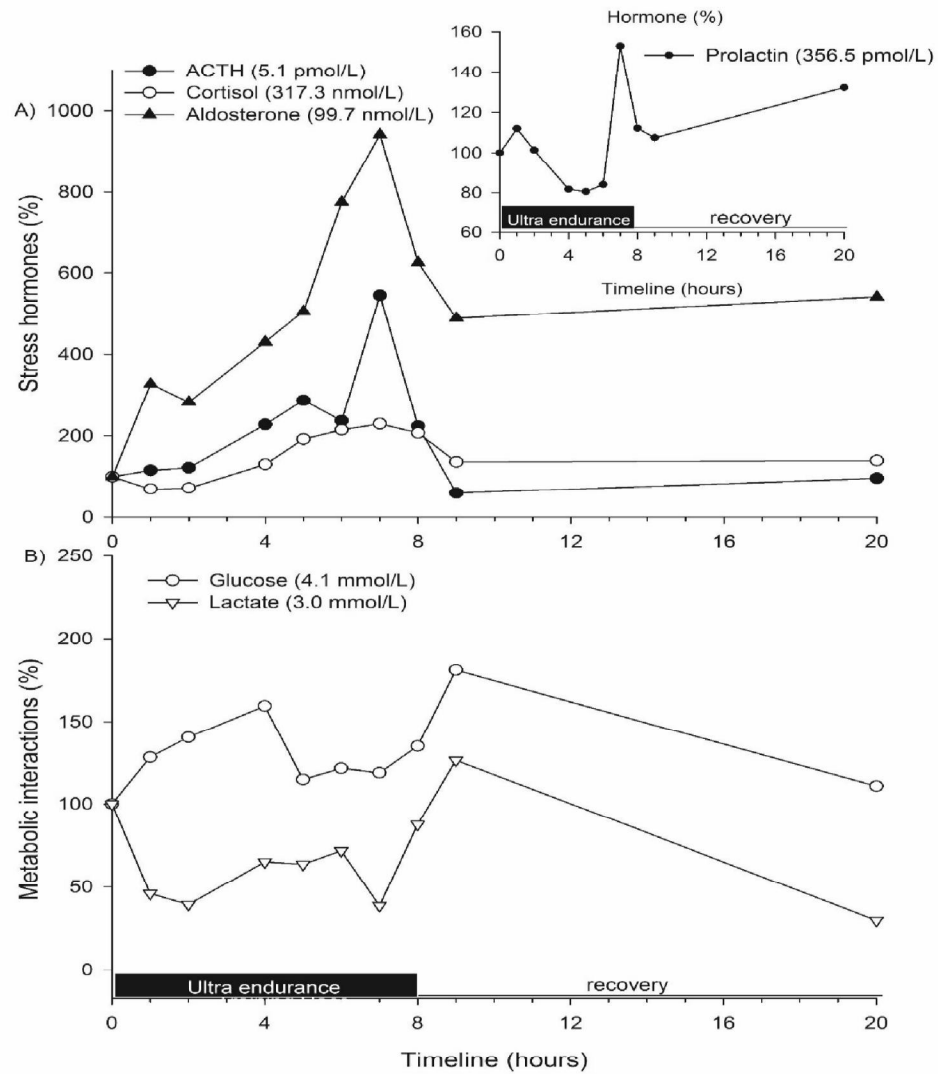


Figure 7. Neuroendocrine responsiveness and glycolytic pathway modulation during ultra endurance. (A) ACTH stimulated aldosterone and cortisol. Meanwhile, prolactin was reduced during exercise. **(B)** Glucose had two

peaks of increment. Lactate maintained reduced until seventh hour and started a similar pattern of glucose increment. Data are presented normalized and absolute values in label.

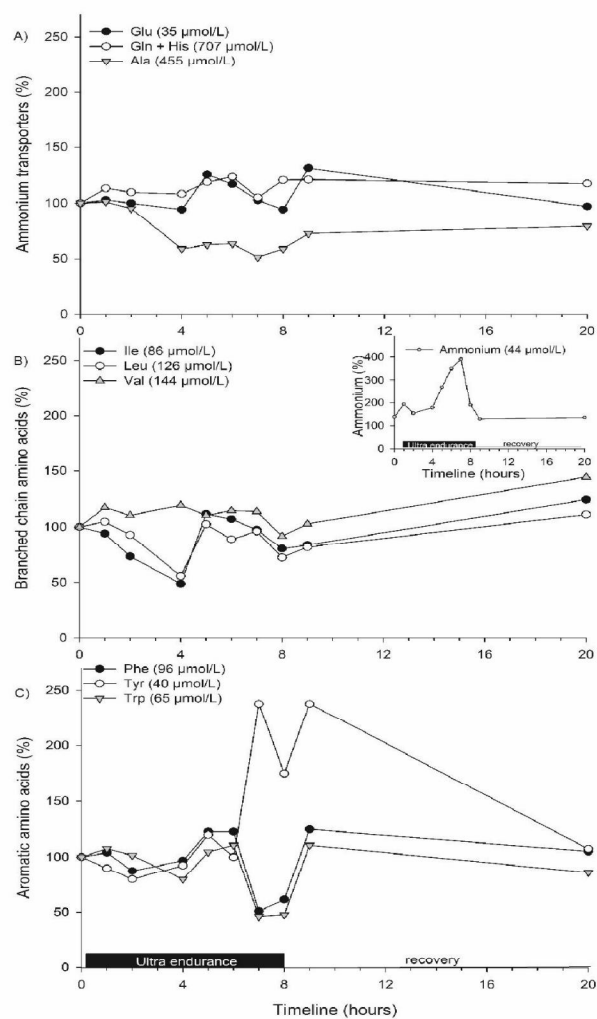


Figure 8. Amino acids metabolism during the ultra endurance. (A) Similar fluctuations of Gln and Glu levels were observed. Meantime, alanine decreased

over the same period. **(B)** Ile and Leu fluctuated and Val remained constant during exercise. Both, increased during recovery. Inset: Ammonia increased during running and returned to basal levels during recovery **(C)** Phe and Trp showed reduction from sixth to eighth hour, while Tyr increased.

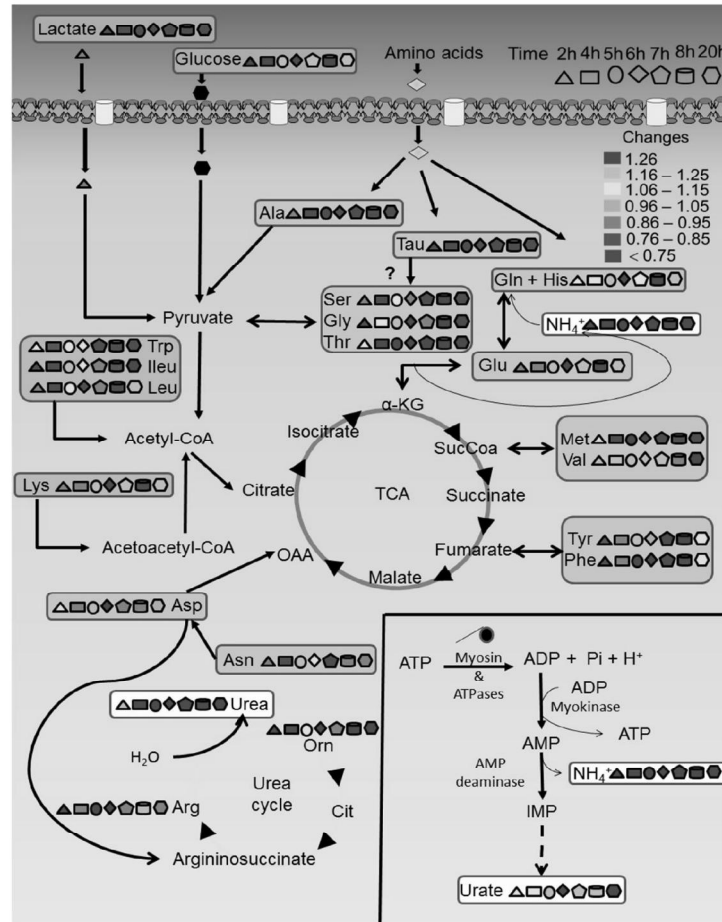


Figure 9. Tricarboxylic Cycle Acid intermediates, urea cycle and Purine Nucleotide Cycle (PNC) in response to race. Urea, urate and ammonium increased (above 26%) during the ultra endurance to race.

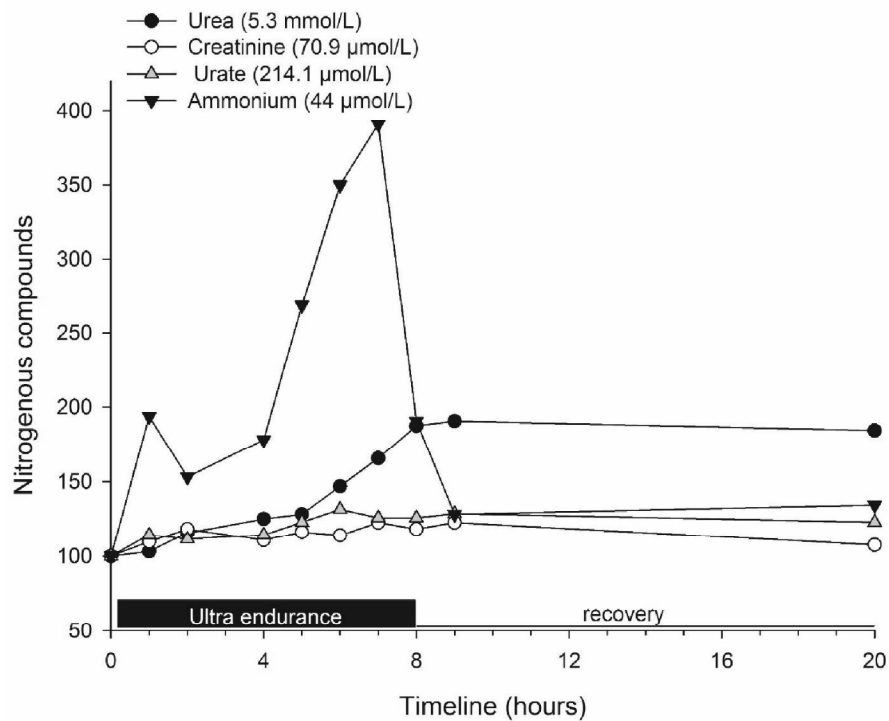


Figure 10. Ultra endurance effect on nitrogenous compounds. Ammonia increased in the first two hours and from fourth to seventh hour, returning to basal levels during recovery. Urea increased from fourth to eighth hour. Urate and creatinine had small increased during the protocol. Data are presented normalized and absolute values in label.

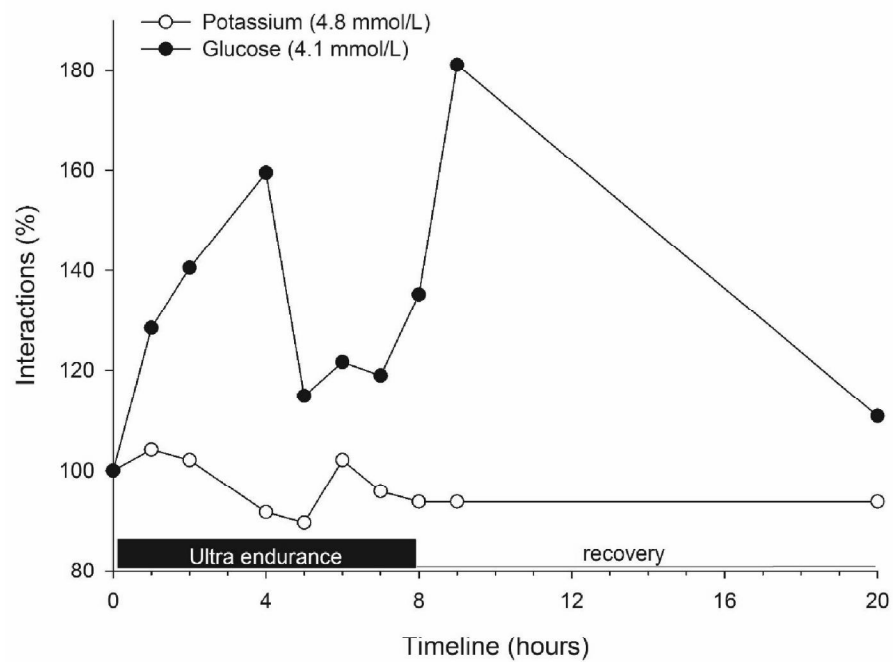


Figure 11. Relationship glicemia kalemia in response to ultra endurance.
 The increase in glicemia was related to the decrease in kalemia during the race.
 Data are presented normalized and absolute values in label.

Supplementary

Supplementary Table 1. Amino acids response to prolonged exercise and recovery.

Amino Acids ($\mu\text{mol/L}$)	Time (hours)									
	0	1	2	4	5	6	7	8	9	20
Ala	455	461	433	267	284	288	233	267	330	360
Arg	69	72	62	63	86	88	66	77	68	68
Asn	49	53	47	44	56	55	42	46	47	48
Asp	19	20	20	18	22	23	18	18	20	19
Gln + His	707	802	776	766	842	874	743	855	857	832
Glu	35	36	35	33	44	41	36	33	46	34
Gly	302	273	218	319	338	406	274	485	429	438
Ile	86	81	63	42	96	92	84	69	72	107
Leu	126	132	117	70	129	112	121	91	103	140
Lys	105	117	104	101	120	137	126	93	93	118
Met	23	26	25	19	28	41	18	17	14	22
Orn	47	31	60	32	51	61	44	60	66	62
Phe	96	100	84	93	118	118	49	59	120	101
Ser	106	122	101	81	111	100	74	158	159	82
Tau	120	104	107	82	95	83	77	93	96	104
Thr	79	97	86	109	138	146	130	167	132	105
Trp	65	70	66	52	68	72	30	31	72	56
Tyr	40	36	32	37	48	40	95	70	95	43
Val	144	169	159	172	159	165	164	132	148	208

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CAPÍTULO IV

ESTUDO DE RESPOSTAS ANÔMALAS A PROTOCOLOS ESPORTÔMICOS

**A SPORTOMIC FOLLOW-UP OF A MUSCLE INJURY SUCCEEDED BY
ACETAMINOPHEN HEPATOTOXICITY**

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**A Sportomic follow-up of a muscle injury succeeded by acetaminophen
hepatotoxicity**

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Running Head: A Sportomic, muscle injury and APAP hepatotoxicity

Brief communication

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ABSTRACT

Purpose: To communicate the diagnosis and evolution from a silent liver injury due to self-medication with large doses of acetaminophen (APAP).

Methods: Sportomic approach was used to analyze four elite cyclists, diagnosing and treating APAP abuse. **Results:** We evaluated both liver and muscle integrity using biomarkers that decreased after treatment (diet, rest and N-acetylcysteine).

Conclusion: We reported a diagnostic and successful treatment keeping both training and competition.

Keywords: N-acetyl-p-aminophenol, back pain, muscle damage, NAC, high-performance athlete.

INTRODUCTION

We previously proposed Sportomics as an approach to mimic both the real challenges and the conditions faced during sports situations (Resende et al. 2011). Sportomics is the use of "-omics" sciences together with classic clinical laboratory analyses to understand sport-induced modifications (Bassini-Cameron et al. 2008; Gonçalves et al. 2012) and can also be used for observational research. Generally speaking, our study is holistic and top-down, treating data in a systematic way and generating a large amount of data with a large computational effort (Porto et al. 2012).

Back pain is one of the prevalent injuries in high-level cyclists (Callaghan and Jarvis 1996; De Bernardo et al. 2012). It is a critical factor for athletes' performance because it renders them unable to effectively train or compete. Modern management of acute lower back pain emphasizes self-care, and the use of acetaminophen (N-acetyl-p-aminophenol, APAP) in relieving pain is widely accepted. APAP therapeutic doses are considered safe, but its hepatotoxicity is well known. Self-medication represents a risk to athletes due to the possibility of changes in metabolism that can modify the hepatotoxicity of the drug (for a review see McGill and Jaeschke 2013). In addition, APAP-induced hepatotoxicity can be exacerbated by vigorous exercise (Terneus et al. 2007), creating a synergism that leads to significant hepatocyte damage.

The use of different blood biomarkers to follow and prescribe exercise is well known, but the origin of classical liver injury biomarkers that increase, such as aspartate aminotransferase (AST) and alanine aminotransferase (ALT), during exercise is controversial (Pettersson et al. 2013). We have previously studied and proposed that it is possible to clinically differentiate AST and ALT tissue origin using γ -glutamyltransferase (γ GT), another liver injury biomarker, as a control (Bassini-Cameron et al. 2008). This approach allows us to follow and distinguish hepatocyte damage from muscle injury caused by exercise.

APAP-induced hepatotoxicity is proposed to be caused by mitochondrial damage, and reducing agents such as N-acetyl-cysteine (NAC) can be used as

an antidote (De Bernardo et al. 2012) to minimize hepatotoxicity and prevent liver failure and death (Larson 2007). In addition, carbohydrates and some amino acids intake, such as methionine (Met) or cysteine (Cys), have been proposed as a way to decrease and treat APAP poisoning (Shriner and Goetz 1992). Currently, the superiority of NAC treatment compared to Met as a way to decrease APAP-induced hepatotoxicity (Brok et al. 2006) is uncertain. In fact, the use of Met combined with APAP has been proposed and defended as “safe” APAP (Heptonstall 2006).

Here, we describe the use of the Sportomics methodology combined with a wide sports data bank (Porto et al. 2012) to diagnose, produce an intervention and follow the evolution of hepatotoxicity caused by a large APAP dose to treat back pain. This approach allowed us to prescribe and help an injured athlete in his recovery and return to training and competition within a two-week time frame.

MATERIAL AND METHODS

Subjects

Four national cycling elite men athletes participated in this case, following the same training and nutritional program in the previous eight months and with similar performances based on ergospirometry (Figure 1).

This study was conducted according to the guidelines dictated by the Declaration of Helsinki. All of the procedures involving human subjects were approved by the Ethics Committee for Human Research at the Federal University of the State of Rio de Janeiro (117/2007, renewed in 2011 and 2013) and met the requirements regulating research on human subjects (Health National Council, Brazil, 1996). The nature of the study and the procedures involved were described to all of the subjects, and written informed consent was obtained from all of the subjects.

Methodology

We performed a VO_{2max} (Bruce et al. 1973) and two sportomic evaluations (Resende et al. 2011) separated by 15 days. The whole blood for hematological and plasma biochemical profiles were determined using commercial kits (Labtest, Belo Horizonte, Brazil).

Data Analysis

The collected data were analyzed and compared injured and healthy athletes, which were expressed as the mean with standard deviation.

Intervention

We suggested that the athlete decrease both training intensity and volume by 30-40% of current levels and increase resting and sleeping time. We suspended the use of APAP and included a dietary plan that called for high carbohydrates (≥ 6 g of carbohydrates $kg^{-1} \cdot day^{-1}$) and high protein (≥ 2 g of proteins $kg^{-1} \cdot day^{-1}$). We recommended an increased intake of dietary Met and Cys and a daily dose of 3600-4200 mg of NAC.

RESULTS

No difference could be found in the athlete's VO_{2max} compared with the team's average (Figure 1).

The athlete's creatine kinase (CK), lactate dehydrogenase (LDH) and C-reactive protein (CRP) levels were higher than the average value of the three teammates (375%, 25% and 140%, respectively). In addition to the muscle injury markers, we measured an increase in AST (35%), γ GT (185%) and 350% in total bilirubin level in the injured athlete. We did not observe an increase in the levels of alkaline phosphatase (ALP) and ALT compared to the other teammates. An analysis revealed that both the direct and indirect bilirubin increased in the injured athlete. The indirect bilirubin increased almost 4.6-fold when compared to the direct bilirubin (direct:indirect ratio; ~1:4) (Table 1).

After two weeks of following our intervention, we re-evaluate the injured athlete and teammates using the same Sportomics protocol. The amounts of CK and γ GT in the blood were less than 20% and 70% of pre-treatment levels, respectively. We did not detect a change in the level of total bilirubin in the blood; the injured athlete's bilirubin level following treatment was still higher than the average value of his teammates. Nevertheless, we did detect an improvement in bilirubin conjugation by performing both direct and indirect bilirubin measurements (direct:indirect ratio; ~1:1) (Table 1).

DISCUSSION

Here, we report a Sportomic analysis as a personalized medicine approach from a team of four athletes during their preparation for an 800 km non-stop race. During the first Sportomic analysis, one athlete showed unexpected levels of muscle injury biomarkers and presented with much higher levels of inflammation markers than his teammates.

After a careful anamnesis, we found that the injured athlete had experienced back pain in the 96 h prior to the first evaluation. He attributed this pain to a

previous training session in that a bike drift occurred, followed by the onset of severe lower back pain. We suspected that the muscle injury was the principal cause of the high CK values. Consistent with this hypothesis, the athlete had higher levels of classical injury markers CK and LDH than his teammates, and these increases are consistent with our recent report of its kinetics appearance in the blood (Bessa et al. 2008). Our hypothesis of muscle injury is further supported by the elevated CRP value, which indicates an acute phase inflammatory response.

It has been proposed that endurance training can cause chronic liver injury (Wu et al. 2004). Given the high CK values in this athlete, we initially thought that he might be suffering from overtraining syndrome (OTS) (Margonis et al. 2007). However, analysis of the athlete's VO_{2max} test showed him to have similar performance to his teammates. Because the VO_{2max} test is widely accepted as the gold standard for OTS diagnosis, we excluded this possibility.

Following our Sportomics approach, we re-interviewed the athlete and discovered that he started a large daily dose of APAP to reduce back pain. The athlete indicated that he has been using 7000-9000 mg of APAP per day to relieve muscle soreness. Different studies have linked excessive doses of APAP to hepatotoxicity (Larson 2007; Silva et al. 2008). We have previously shown that it is possible to separate muscle and liver injuries using ALP and γ GT as hepatocyte integrity markers (Bassini-Cameron et al. 2008;). In this study, the athlete had a γ GT level nearly twice as high as his teammates while following the same training schedule, and his AST level showed a slightly smaller increase. These findings support the hypothesis of a liver injury. After ruling out diseases such as hepatitis and cirrhosis, we hypothesized that the athlete had suffered a muscle injury, after which his use of APAP resulted in hepatocyte toxicity.

Muscle injury diagnosis can be made through the variations of enzyme levels detected in the blood, especially CK and LDH (Bessa et al. 2008). However, ALT and ALP elevation are also observed in non-liver injury conditions and in apparently healthy athletes (Spiropoulos and Trakada 2003). γ GT is an abundant

enzyme in the liver, and abnormal γ GT levels can be used to diagnose various diseases. In exercise biochemistry, γ GT is used as a specific marker of liver injury to differentiate the location of damage (Silva et al 2008).

Although we cannot ignore the reported influence of endurance training on chronic liver injury (McGill and Jaeschke 2013; Terneus et al. 2007), we believe that the hepatotoxic effect of APAP was responsible in this case. Therefore, we concluded that the athlete was suffering from acute back pain caused by muscle injury and that subsequent APAP overdose resulted in hepatotoxicity exacerbated by exercise.

Based on these analyses, we suggested that the athlete decrease both training intensity and volume by 30-40% of current levels and increase resting and sleeping time. We suspended the use of APAP and included a dietary plan that included high amounts of carbohydrates (≥ 6 g of carbohydrates $\text{kg}^{-1} \cdot \text{day}^{-1}$) and high amounts of protein (≥ 2 g of proteins $\text{kg}^{-1} \cdot \text{day}^{-1}$).

NAC is a popular antioxidant because of its ability to minimize oxidative stress. The mechanism of NAC involves the synthesis of hepatic glutathione, preventing oxidative damage (Saito et al. 2010). NAC and other antioxidants have been used as APAP antidotes. To reinforce the dietary protection, we recommended a high intake of dietary Met, Cys and a daily dose of 3600-4200 mg of NAC, an APAP antidote widely used in clinical treatment.

After 15 days of following dietary and training recommendations, a second evaluation was conducted. CK and CRP levels in the athlete had fallen within the range observed in his teammates. The effectiveness of treatment was corroborated by biochemical analysis, which showed that the amounts of CK and γ GT in the blood were less than 20% and 70% of pre-treatment levels, respectively. The level of γ GT had decreased by 38% from the level measured at the first evaluation, but it was still higher than the levels of his teammates. We did not, however, detect a change in the level of total bilirubin in the blood; the injured athlete's level following treatment was still higher than the average value of his teammates. Nevertheless, we did detect an improvement in bilirubin

conjugation by performing both direct and indirect bilirubin measurements (Table 1). These results suggest that our diagnosis was correct and that the intervention that we developed may be effective in treating mild APAP toxicity in athletes. After two weeks of following our training and dietary recommendations, the athlete reported that the pain had been relieved. Moreover, the team was able to perform the race in 23 h and won third place.

In conclusion, this study has shown that collecting physiological data during training can provide important information about an athlete's clinical condition as well as the degrees of OTS and performance. Here, we describe the use of the Sportomics to diagnose, produce an intervention and follow the evolution of hepatotoxicity caused by a large APAP dose to treat back pain. This approach allowed us to prescribe and help an injured athlete in his recovery and return to training and competition within a two-week time frame.

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The authors declare that they do not have conflict of interest.

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Table Caption

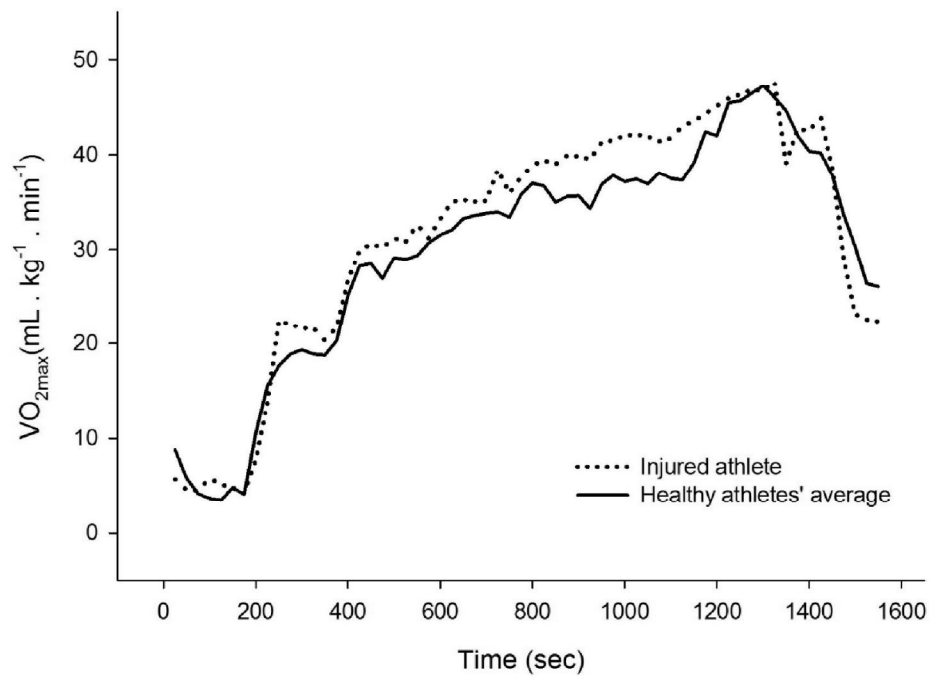
Table 1. Muscle and liver injury markers measured before and after treatment.

	Injured athlete	Healthy athletes	Injured athlete	Healthy athletes
Parameter	(1 st Analysis)		(2 nd Analysis)	
CK (U/L)	868.0	182.7 ± 19.8	147.0	190.3 ± 20.8
LDH (U/L)	383.0	308.0 ± 50.6	408.0	296.3 ± 53.0
AST (U/L)	57.3	42.0 ± 3.1	55.6	46.9 ± 11.4
ALT (U/L)	27.7	25.3 ± 3.1	19.8	35.2 ± 14.4
γGT (U/L)	47.1	16.6 ± 2.7	33.9	15.3 ± 2.3
ALP (U/L)	54.0	75.3 ± 11.3	44.0	81.3 ± 16.8
CRP (mmol/L)	62.5	25.9 ± 10.8	3.4	12.7 ± 5.1
Total bilirubin (umol/L)	44.3	9.7 ± 1.6	40.0	18.1 ± 2.3
Directbilirubin(umol/L)	9.6	4.4 ± 0.7	18.8	13.7 ± 4.8
Indirect bilirubin (umol/L)	34.7	5.3 ± 1.6	21.0	4.3 ± 2.5

Liver and muscle injury parameters were measured before and after team counseling and athlete treatment. CK, creatine kinase; LDH, lactate dehydrogenase; AST, aspartate aminotransferase; ALT, alanine aminotransferase; γGT, γ-glutamyltransferase; ALP, alkaline phosphatase; CRP, C-reactive protein. Data for healthy athletes are presented as the average ± SD.

Figure Caption

Figure 1. No difference was observed in the injured athlete's VO_{2max} compared with his team's average. Dashed line, injured athlete; straight line, healthy athletes' average.



CONCLUSÕES

A medicina personalizada, ou como mais recentemente vem sendo denominada a medicina de precisão, tem ampliado seu espectro de atuação nos últimos anos. O uso de biomarcadores para buscar características particulares de indivíduos para auxiliar no tratamento de doenças é uma realidade no panorama atual, na medida em que as entidades mórbidas e terapêuticas podem se apresentar de maneira diferente em cada indivíduo. A complementaridade de exames clínicos de quaisquer áreas e sua conexão com as diversas ciências “-ômicas” têm contribuído fortemente para o avanço de diagnósticos precisos e eficazes em diversas áreas da medicina e com maior ênfase nos estudos ligados à oncologia.

O uso da bioinformática em atividades ligadas à *data mining* em extensos bancos de informações tem permitido a integração de dados nunca antes confrontados, trazendo à luz da ciência cotidianamente elementos que nos permitem conhecer melhor o indivíduo, a doença e suas condições de vida.

É capital que se compreenda que os dados aqui relatados devem não ser extrapolados para outros atletas ou indivíduos, mas mostram importantes adaptações durante o exercício. Embora estejamos lidando com atletas de elite que pertencem a mesma equipe, submetidos às mesmas sessões de treinamento e rotinas de competição, suas respostas metabólicas são bem diferentes em alguns dos parâmetros que descrevemos. Tais análises nos permitem inferir que a diferença nas respostas metabólicas de atletas devam ser individualmente estudadas.

Nossos achados demonstram que o uso de testes de VO_{2Max} , que são bastante úteis para a compreensão das respostas cardiopulmonares ao exercício, não são a melhor opção quando investigamos o metabolismo durante a realização de diferentes tipos de exercício. Propusemos aqui que os protocolos esportômicos devam ser usados para melhor entender o metabolismo em situações de campo, já que a abordagem esportômica reproduz situações reais de treinamento e

competição. Em nossos estudos foram apresentadas novas possibilidades para o gerenciamento do treinamento, na medida em que foram associados marcadores de injúria, respostas inflamatórias, carga de treinamento e cinética de recuperação.

Os dados aqui apresentados nos permitem considerar a resposta hormonal, metabólica e de sinalização, juntamente com o conhecimento do ambiente de nutrição e treinamento. Essa informação combinada permite uma melhor compreensão das respostas individuais do exercício e do estresse esportivo. Nosso grupo desenvolveu o conceito de esportômica com foco em superar a distância existente entre os achados científicos e medicina translacional e personalizada. A esportômica é útil para avaliar a cinética sem precedentes de alguns metabólitos e ressaltar a importância das análises metabólicas no campo para a compreensão da resposta individual ao exercício. A coleta de dados fisiológicos durante treinamento e competição pode fornecer informações importantes sobre a condição clínica de um atleta, trazendo estratégias para modificar o metabolismo durante o exercício, além de apoiar os treinadores para prescrever suas sessões e tempo de recuperação. Devido a singularidade deste estudo, acreditamos que a esportômica é uma ferramenta primordial para o gerenciamento de treinamento e a melhoria do desempenho, bem como para preservar a saúde e aumentar a qualidade de vida dos atletas. Acreditamos, sobretudo, que o conhecimento gerado pelas avaliações esportômicas nos auxiliará na compreensão da resposta celular e sistêmica durante estados hipermetabólicos.

Em conclusão, este estudo mostrou que a coleta de dados fisiológicos durante o treinamento ou competição pode fornecer informações importantes sobre a condição clínica e de desempenho de um atleta. Descrevemos o uso da esportômica para diagnosticar, produzir intervenções e melhorar a performance de atletas. Demonstramos que a esportômica permite a compreensão individualizada do metabolismo sistêmico e celular e pode servir como uma ligação entre a ciência e terapêutica.

MATERIAL COMPLEMENTAR

SEÇÃO A

**SPORTOMICS AND PERSONALISED TRAINING: SOME FINDINGS IN
MICROINJURY MARKERS AND INFLAMMATORY RESPONSE OF OLYMPIC
CANOEISTS**

Dados apresentados na International Biochemistry of Exercise Conference 2015
(São Paulo, BRA)

Magno-França, A.; Magalhães-Neto, AM., Bachini, F; Bassini, A., Cameron, LC.

Material e Métodos

O desenho do protocolo esportômico aqui apresentado está ilustrado na figura 01. Foram testados quatro canoístas de calibre olímpico, incluindo campeões mundiais, no experimento 1 (E1) e cinco atletas de alto rendimento com níveis de performance semelhantes no experimento 2 (E2). O sangue foi coletado em jejum (T1); antes e após uma sessão de canoagem (T2 e T3); depois de uma sessão de musculação (T4) e após recuperação (T5) de 70 min em E1 e 45 min em E2. O tempo gasto para cada experimento foi de aproximadamente 350 min. O volume geral remado em cada sessão foi de 16km, percorridos em cerca de 50 min.

Os protocolos de treinamento utilizados foram testados por um período de quatro anos e acompanhados por diferentes treinadores durante a fase de pré-temporada de treinamento.

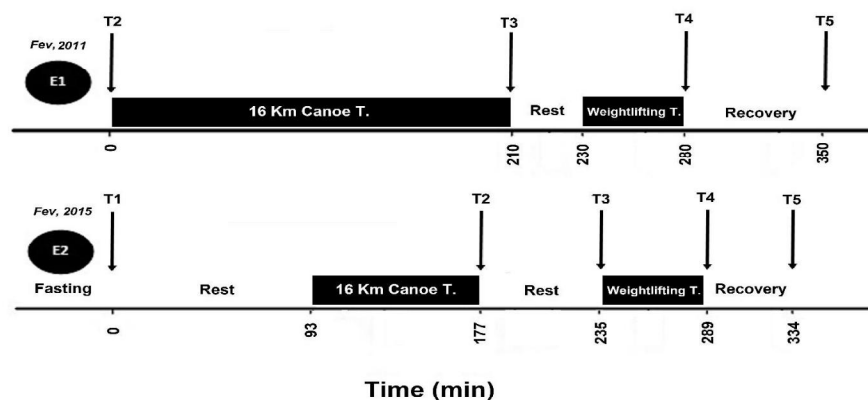


Figura 01. Desenho experimental. Avaliações Esportômicas em E1 e E2. Amostras coletas em jejum (T1); antes e após o treinamento em canoa (T2 e T3); após o treinamento de musculação (T4); e recuperação (T5).

Resultados

Os resultados apresentados no presente experimento sugerem que os analitos observados possuem perfis individuais, gerando diferentes respostas metabólicas de acordo com o atleta e modalidade de exercício avaliado.

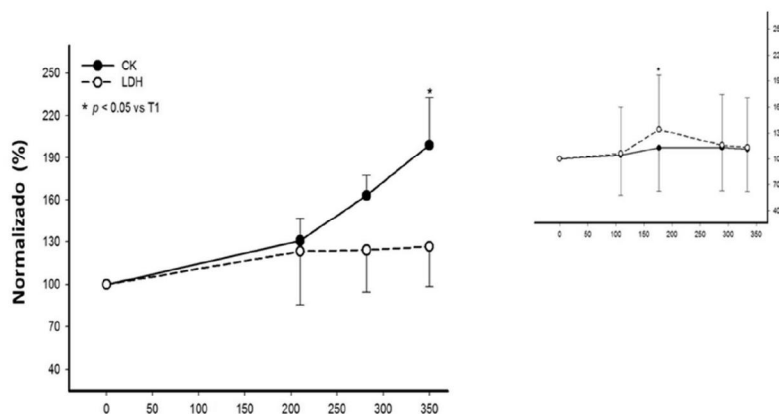


Figura 02. Contraste nas concentrações de CK e LDH. Enquanto em E1 (esq) observa-se um platô na concentração de LDH e uma grande elevação na concentração de CK, durante E2 (dir), a concentração de CK permaneceu inalterada e LDH atingiu um aumento de 35% em T3.

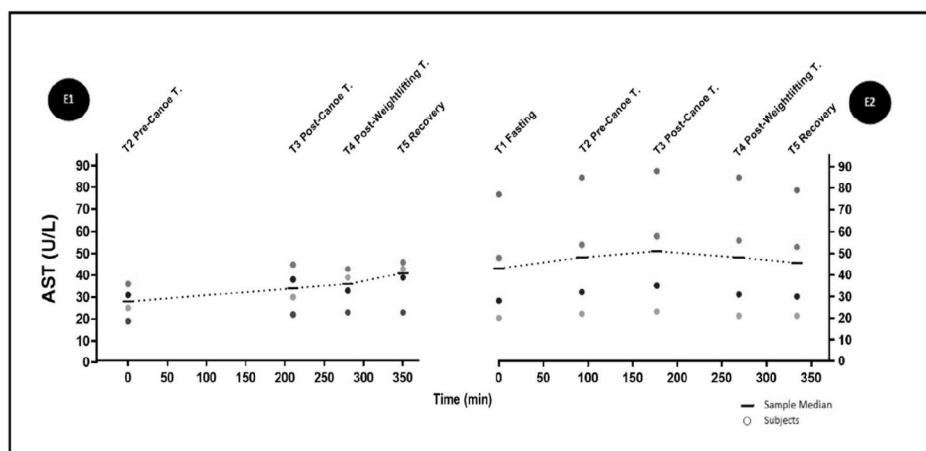


Figura 03. Valores absolutos das concentrações de AST durante E1 e E2. Observa-se um aumento de 40% na concentração de AST ao longo do primeiro

experimento, com uma elevação de 10% entre T4 e T5. Em E2, a maior concentração desse analito foi de aproximadamente 20% em T3, retornando aos parâmetros iniciais em T5.

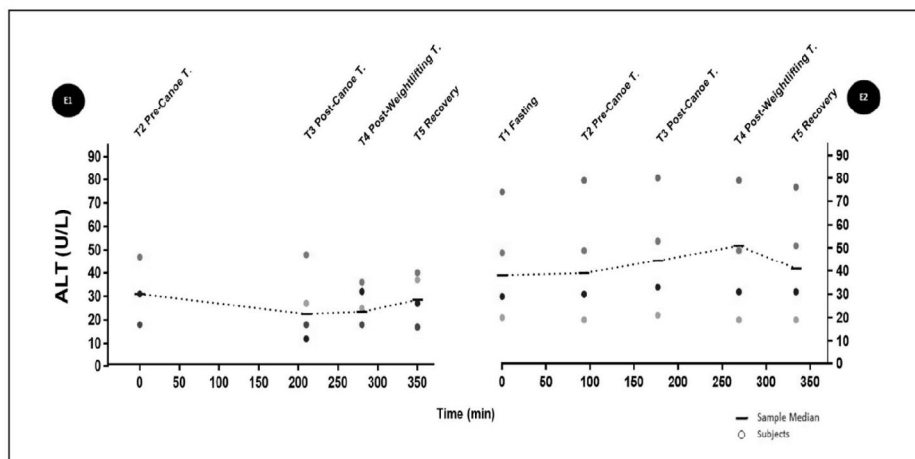


Figura 04. Concentrações de ALT ao início e ao final dos experimentos. Mesmo apresentando uma cinética diferente, a concentração deste metabólito durante E1 e E2, atingiu em T5 aproximadamente os mesmos níveis.

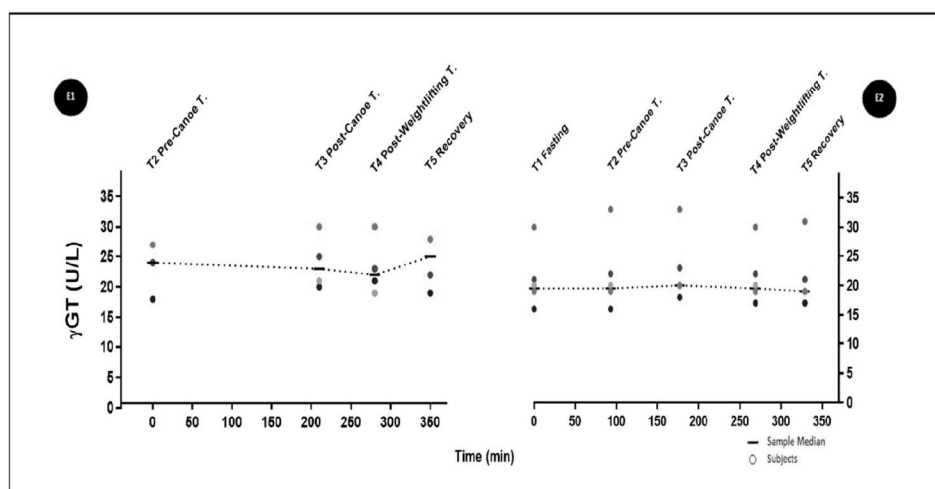


Figura 05. Concentrações inalteradas de γ GT durante o protocolo. A concentração de γ GT permaneceu inalterada durante todo o desenho experimental, demonstrando uma cinética similar para todos os sujeitos.

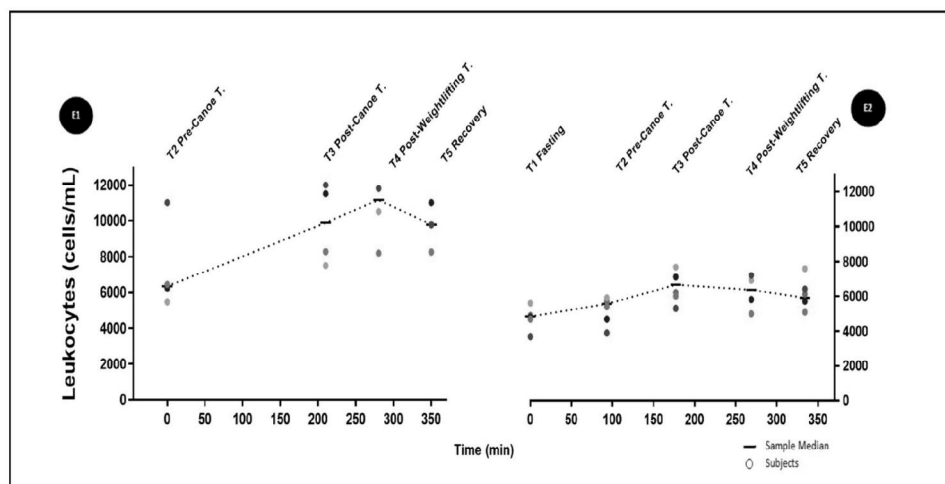


Figura 06. Variações nas respostas leucocitárias. Apesar da maior intensidade de exercício, durante E2 a concentração de leucócitos apresentou diminuição contínua a partir de T3, enquanto que em E1 foi possível observar um aumento de 10% no mesmo ponto, seguido de uma diminuição de T4 para T5.

Resultados

Foram escolhidos aqui dados ligados à micro injúria muscular relacionados às respostas imune e inflamatória.

Durante E1, os níveis de CK se elevaram ~60% em T4 e 100% em T5 em resposta ao exercício (concentrações entre 480.6 ± 201.8 a 884.5 ± 244.7 U/L). As concentrações de CK em E2 foi similar em todos os sujeitos, sendo distribuída em quatro ranges de variação: 200-300 U/L; 600-800 U/L; 1400-1700 U/L; e 1400-2000 U/L.

A atividade da lactato desidrogenase (LDH) no sangue não se alterou em E1, elevando-se até 35% em E2. A concentração de aspartato aminotransferase (AST) sofreu um incremento de 30% após a sessão de musculação em E1,

atingindo 40% de incremento em T5. Durante E2, a variação dos níveis de AST apresentou aumento inferior a E1, chegando a um crescimento de aproximadamente 10-18% ao longo do protocolo.

Não foram observadas alterações na alanino aminotransferase (ALT) e γ -glutamyltransferase (γ GT) em ambos protocolos.

Todas as mudanças ocorridas nos clássicos marcadores de injúria apresentados estão correlacionadas a modificações no setor branco do sangue, que em E1 revelou um aumento dos leucócitos da ordem de 40% a partir de T2, enquanto em E2 essa variação atingiu 40% dentro do mesmo período.

Discussão

Ao compara-se os experimentos, a resposta de leucócitos e as concentrações de γ GT, AST and ALT apresentam resultados correspondentes. Por outro lado, as cinéticas de CK e LDH apresentaram-se bastante diferentes. As concentrações dos marcadores de injúria com exceção de γ GT indicam que a elevação dessas enzimas deve originar-se primariamente em razão de danos a nível muscular e não hepático, como já proposto anteriormente por nosso grupo. As diferenças entre CK e LDH podem estar ligadas à resposta individual assim como à adaptação a uma especial etapa da temporada.

Apesar dos programas de treinamentos apresentarem semelhanças, os atletas em E1 perfizeram 16 km em aproximadamente 210 minutos, enquanto em E7 o mesmo percurso foi finalizado em 85 minutos. Durante o primeiro experimento, o treinamento foi interrompido muitas vezes por correções técnicas e discursos feitos pelo treinador. Assim, a intensidade do segundo protocolo é crucial para analisar os resultados e concluir, também na concentração dos metabólitos apresentados na presente análise, que os indivíduos em E1 foram mais adaptados ao exercício do que em E2. Em adição, três dos quatro atletas envolvidos no E1 também estavam presentes em E2.

Conclusão

Os resultados apresentados no presente experimento sugerem que os analitos observados possuem perfis individuais, gerando diferentes respostas

metabólicas de acordo com o atleta e modalidade de exercício avaliado. Assim como na medicina personalizada, a esportômica foca em evitar o método da tentativa-erro, considerando o papel da intervariabilidade das respostas metabólicas. Os resultados aqui apresentados focam em uma abordagem individualizada, de maneira a estabelecer padrões de intensidade de treinamento e estratégias de recuperação apropriadas.

**A SPORTOMIC STUDY OF AMMONIA AND AMINOACIDS METABOLISM IN
COMPETING WORLD-CLASS JUDO ATHLETES**

Dados apresentados no Congresso Anual do American College of Sports
Medicine 2013 (Indianapolis, EUA)

Elizabeth Cataldi; Adriana Bassini; **Alexandre Magno-França**; Luis Viveiros;
André N. Monteiro. L.C. Cameron

Material e Métodos

Dois atletas de alto rendimento da modalidade de Judô, ambos do sexo masculino, foram avaliados em dois experimentos esportômicos com diferentes desenhos: competição (E1) e pré-competição (E2).

No primeiro experimento (E1) foi utilizado um modelo de competição internacional, com cinco combates realizados tomando diferentes intervalos de descanso entre eles. Nesse experimento participaram os dois atletas, sendo um da categoria peso pesado (J1) e outro da categoria até 73 quilos (J2).

No segundo experimento (E2), somente o atleta (J2) foi avaliado durante um dia de treino de alta intensidade. As análises dos perfis de amônia, urato e ureia foram mensuradas utilizando kits comerciais (LEAC® e Labtest®, respectivamente) e os aminoácidos (AAs) foram quantificados por cromatografia líquida de alta eficiência (HPLC), em Laboratório de Análises Clínicas.

Resultados

Ao observarmos a Figura 01 verificou-se, com a análise das áreas sob as curvas, que o atleta J2 apresentou aumento de 55% na concentração de amônia quando comparado ao atleta J1. Nos combates isolados, o atleta J2 apresentou 217% de elevação na amonemia, ao passo que o atleta J1 teve aumento da ordem de apenas 67%. Os perfis de amônia obtidos pelos atletas reforçam que os momentos de maior estresse metabólico ocorreram durante o primeiro e segundo combates em ambos os lutadores. Em contrapartida, foi possível constatar uma diminuição da amoniogênese e excreção durante e após o terceiro combate (Figura 01).

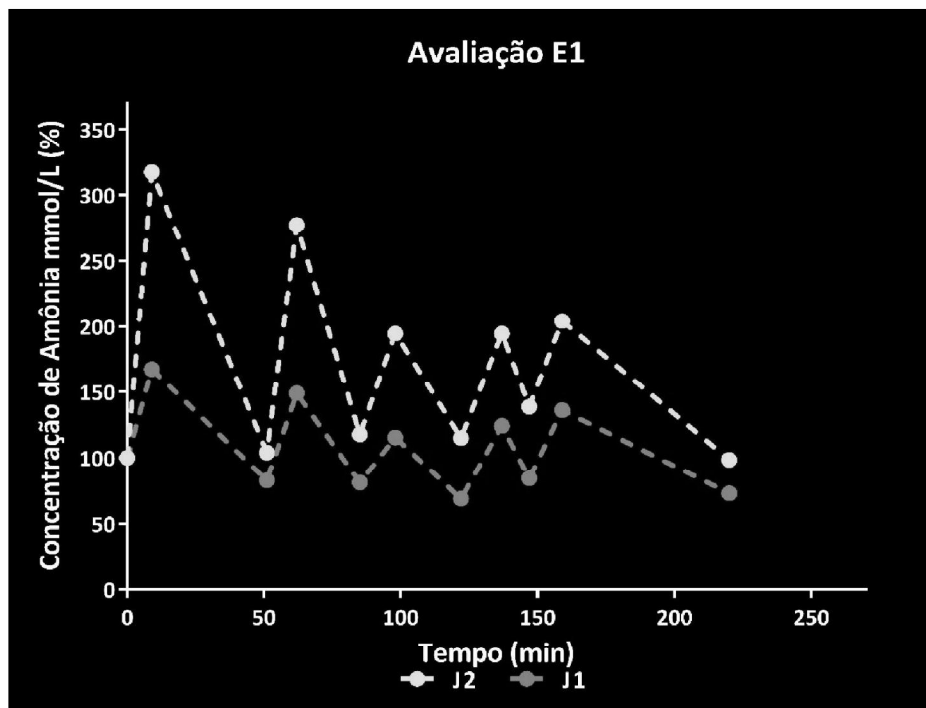


Figura 01: Alteração das concentrações de Amônia durante a Competição Internacional de Judô.

Em E2, a amonemia líquida medida no atleta J2 apresentou diminuição de 21% quando comparado com o resultado obtido durante E1 (Figura 02).

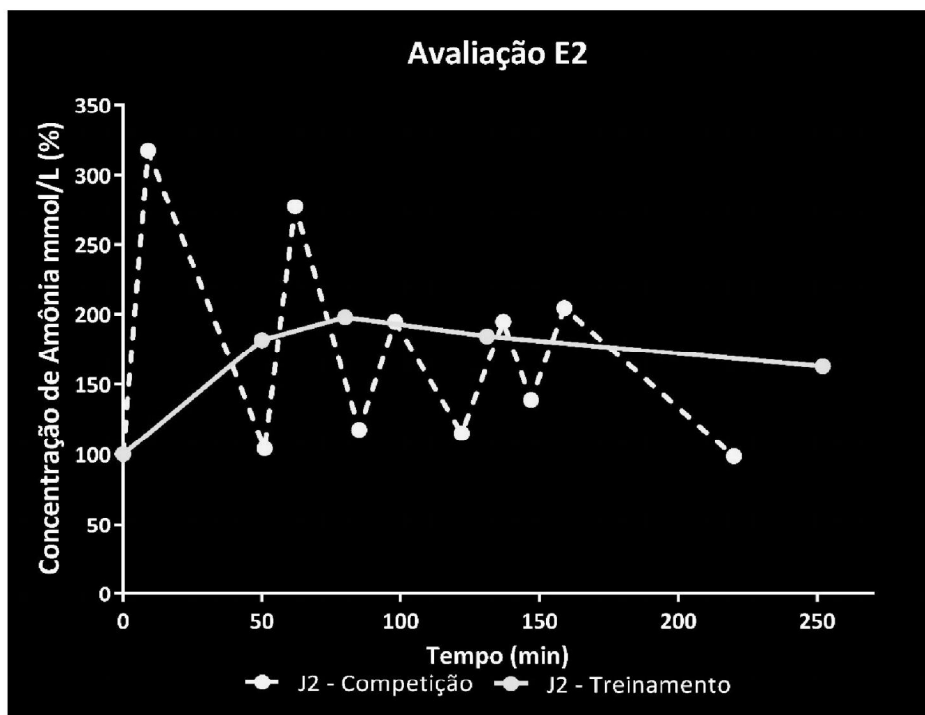


Figura 02: Variação da concentração de amônia no sangue durante treinamento de alta intensidade.

A análise das concentrações de urato demonstra que em E1 a uratemia do judoca J1 elevou-se 26% enquanto a do atleta J2 teve um incremento de 15% (e depois 18% em E2). Por outro lado, a uremia teve sua concentração aumentada em 6% no atleta J1 enquanto que no atleta J2 foi possível observar um aumento de 10% (e depois 3% em E2) (Figura 03).

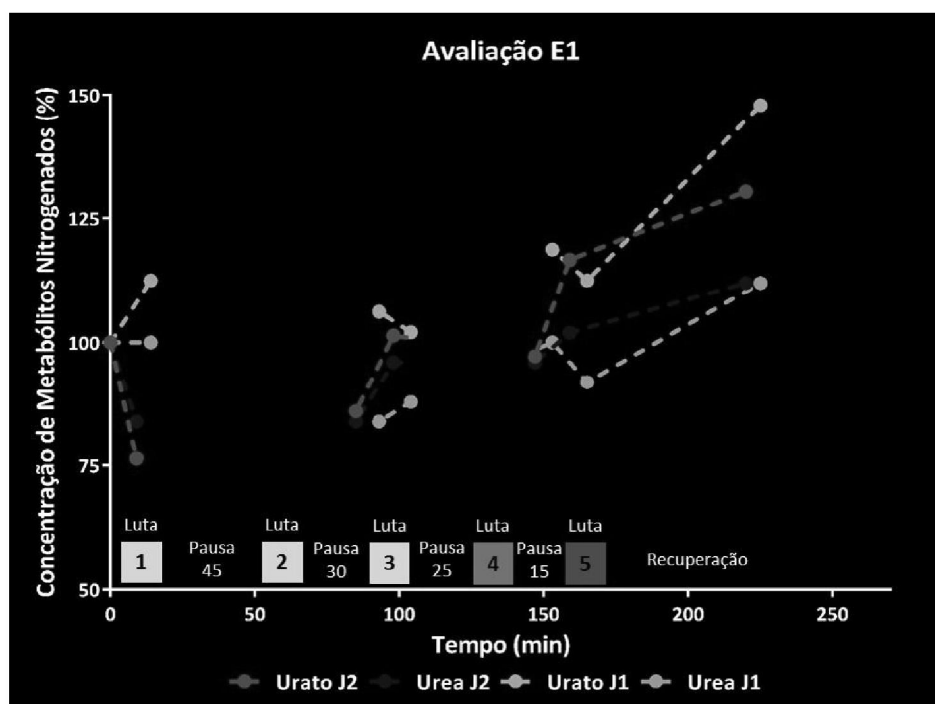


Figura 03: Variação da concentração de metabólitos nitrogenados quantificados no sangue durante um modelo internacional de competição de judô.

Nos três primeiros combates em E1, os aminoácidos (AA) gliconeogênicos: alanina (Ala), glutamato (Glu) e glutamina (Gln) apresentaram aumento em suas concentrações em ambos lutadores. Da mesma maneira, o tempo de recuperação às concentrações basais de AAs demonstrou tendência semelhante em ambos os lutadores (Figura 04).

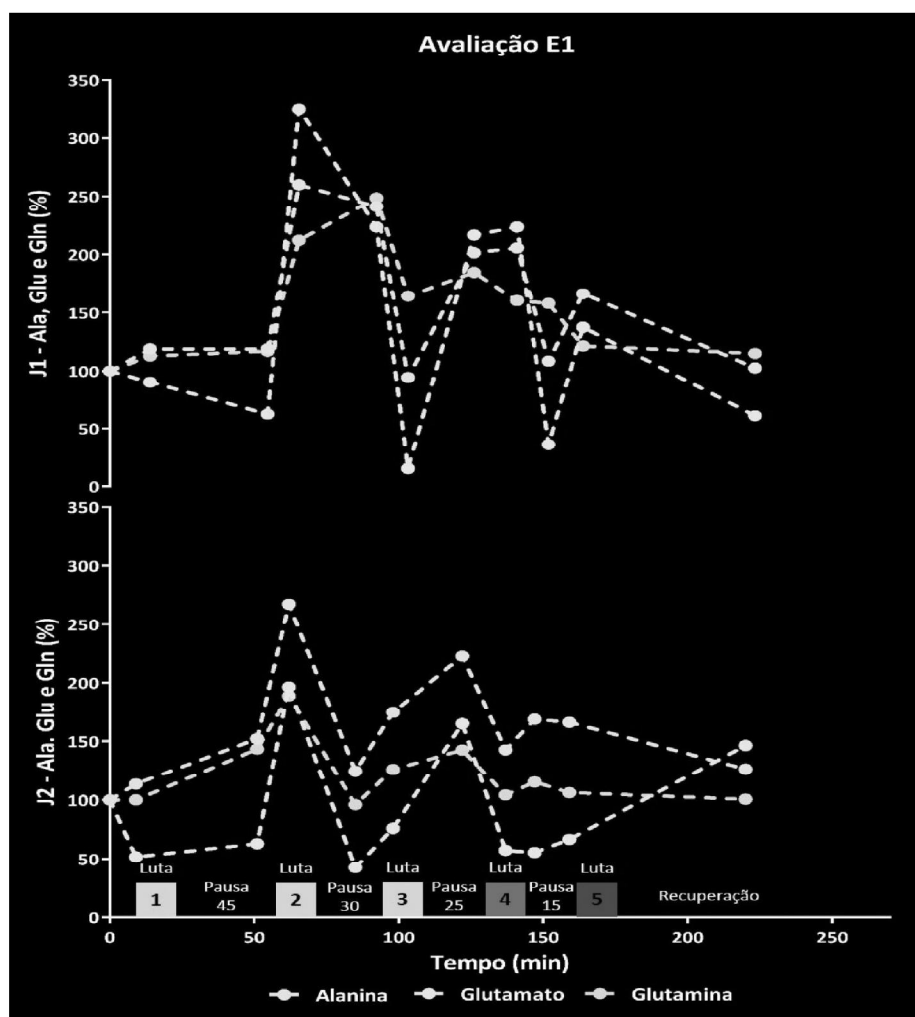


Figura 04: Alteração dos perfis de aminoácidos Gliconeogênicos: Ala, Glu, Gln analisados nos atletas J1 e J2 durante uma competição internacional de judô.

A tendência metabólica apresentada na Figura 04 para o atleta J2 foi semelhante também durante a avaliação em E2. Entretanto, os AAs Ala e Gln elevaram suas concentrações em até 43% em diferentes velocidades (Figura 05).

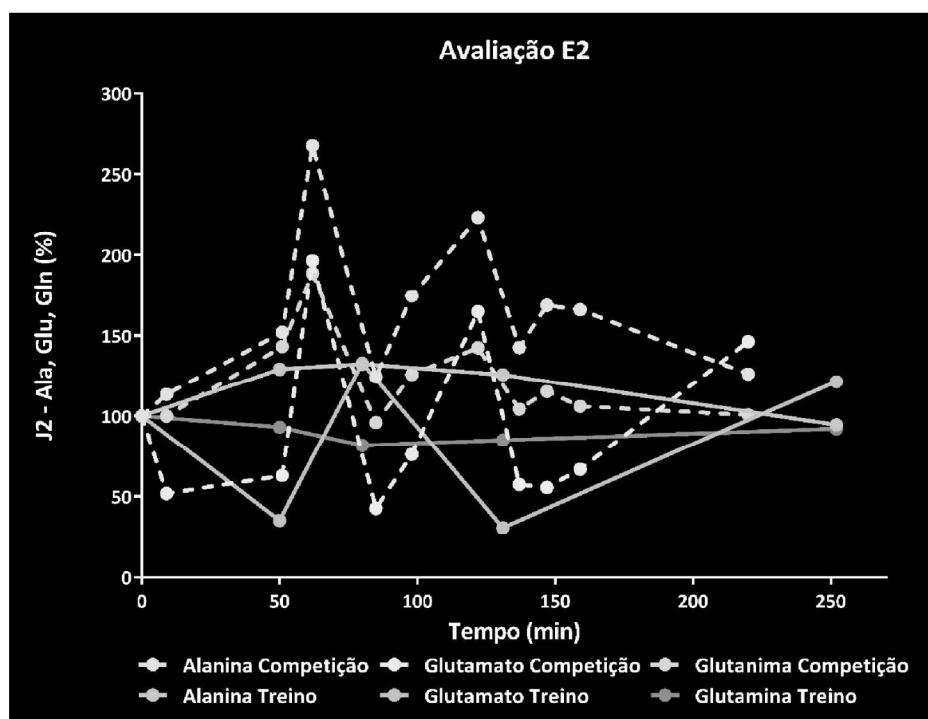


Figura 05: Concentrações sanguíneas dos aminoácidos Ala, Glu, Gln obtidos para o lutador J2 durante o treinamento de alta intensidade.

Com base nos resultados obtidos, é possível sugerir que o aumento de amônia no atleta J2 pode ter sido atribuído ao maior envolvimento da atividade da “myokinase”, já que o atleta J1 conseguiu manter os substratos de gliconeogênese por mais tempo e em concentrações mais elevadas

Conclusão

Os achados aqui descritos podem indicar a importância das reservas de aminoácidos no controle e diminuição da amonemia durante o exercício e sua possível utilidade no entendimento do metabolismo dos aminoácidos e da amônia durante competições de judô.

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**SPORTOMICS ANALYSIS OF METABOLIC RESPONSES IN TOP ELITE
ATHLETES USING A 100M DASH RACE AS A MODEL**

Dados apresentados no Congresso Anual do European College of Sports
Science 2015 (Malmö, SWE)

Magno-França, A.; Magalhães-Neto, AM., Bachini, F., Cataldi, E; Bassini, A.,
Cameron, LC.

Material e Métodos

Duas velocistas de alto rendimento foram avaliadas durante um treinamento de 100m. Neste experimento foram coletadas seis amostras de sangue em diferentes tempos: jejum (T0); pré-corrida (T1); imediatamente pós-corrida (T2); e após recuperação de 2, 5 e 10 minutos (T3, T4 e T5, respectivamente), como demonstrado na figura 01. Neste desenho experimental, foram analisados aproximadamente 40 analitos presentes no sangue para entender as diferentes respostas individuais ao exercício.

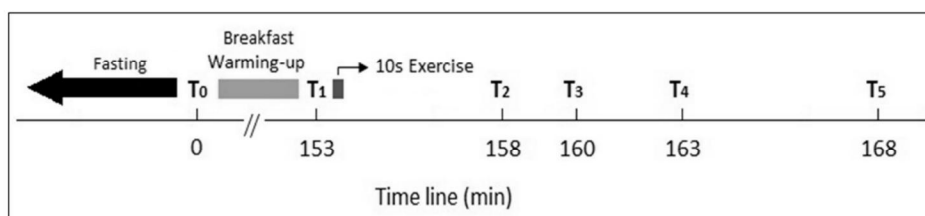


Figura 01. Desenho experimental da avaliação. Linha do tempo das coletas sanguíneas nos diferentes tempos: jejum (T0), pré-exercício (T1) e pós-exercício (T2, T3, T4, T5).

Resultados

As observações realizadas no presente trabalho, após a análise dos resultados obtidos, sugerem que as alterações de amonemia na atleta 1 apresentanduas grandes elevações (T1 - T2 e T3 - T4), atingindo picos de aproximadamente 130 $\mu\text{mol/L}$ (250%) e 148 $\mu\text{mol/L}$ (315%), e decrescendo para 90 $\mu\text{mol/L}$ em T5. Em contraste, a segunda atleta apresentou apenas um pico de aproximadamente 205 $\mu\text{mol/L}$ (540%);, em T2, mantendo um platô entre os tempos T3 a T5 em torno de 135 $\mu\text{mol/L}$ (320%). AUC 1: aumento de 164,4 %.min; AUC 2: aumento de 192,4 %.min (Figura 02).

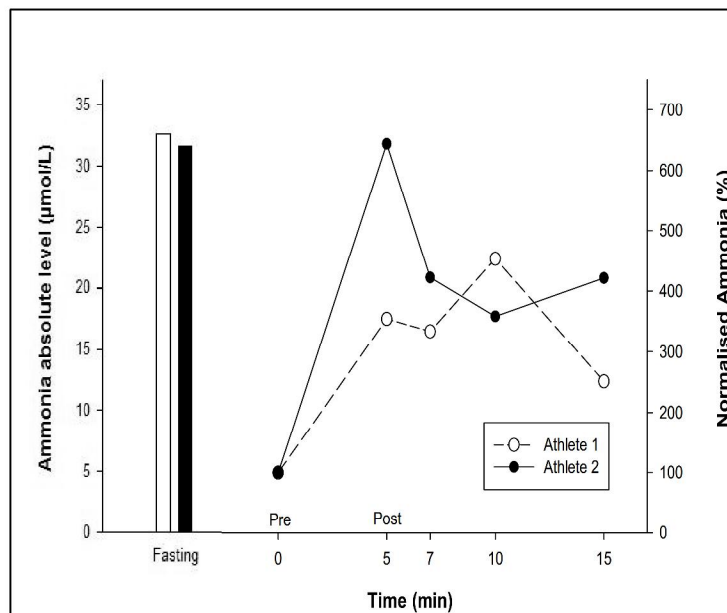


Figura 02. Diferença do clearance da amonemia entre as atletas.

A figura 03 demonstra a curva da cinética da lactatemia das duas atletas avaliadas. Os resultados sugerem que as duas curvas apresentam aspectos similares para ambas as atletas. No entanto, a atleta 1 apresentou um aumento na concentração de lactato de aproximadamente 275% (3,9 - 14,9 mmol/L), enquanto que a atleta 2 apresentou um aumento da ordem de 800% (1,8 - 16,0 mmol/L) na concentração de lactato (Figura 03).

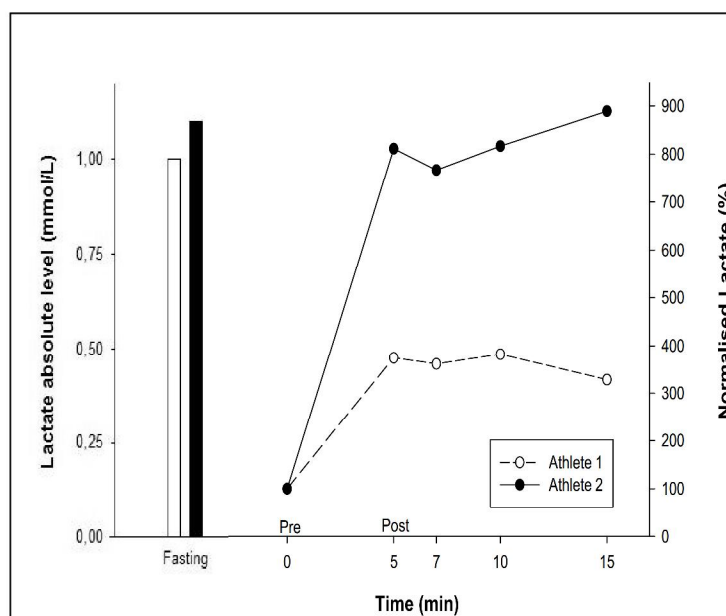


Figura 3. Curva cinética de lactato (mmol/L, %) nas duas atletas avaliadas.

Por fim, as análises das concentrações sanguíneas de creatinina nas duas atletas avaliadas não apresentaram mudanças durante o exercício. O analito quantificado apresentou uma cinética regular nos tempos T1 a T3, em ambas as atletas (Figura 04).

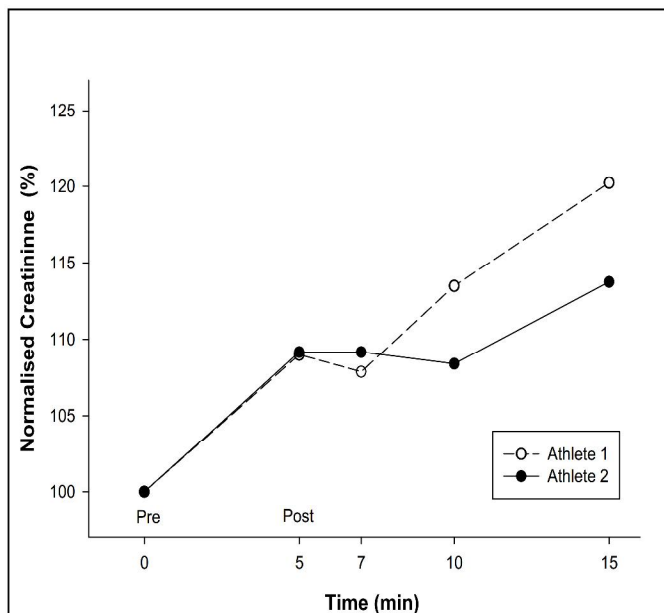


Figura 04: Curva cinética de creatinina (%) nas atletas 1 e 2.

Conclusão

Embora estejamos lidando com dois atletas de elite da mesma equipe, com as mesmas sessões de treinamento e rotinas de competição, suas respostas metabólicas são bem diferentes em alguns dos parâmetros metabólicos que descrevemos. Esta análise nos permite inferir que a diferença nas respostas metabólicas de ambos atletas se deve a uma maior reserva de ATP na musculatura do Atleta 1 ou a uma menor eficiência bioenergética do Atleta 2.

SEÇÃO B

**A COMPARISON OF AMINO ACID AND AMMONIA METABOLISM DURING
DIFFERENT SPORTOMICS PROTOCOLS AND VO₂MAX ANALYSIS**

Dados apresentados no Congresso Anual do American College of Sports
Medicine 2013 (Indianapolis, EUA)

Autores: André N. Monteiro; Adriana Bassini; **Alexandre Magno-França**; Luis
Viveiros; Elizabeth Cataldi; L.C. Cameron

Material e Métodos

Foram realizados três experimentos esportômicos com duas atletas medalhistas olímpicas das modalidades pentatlo moderno (MP) e taekwondo. As atletas foram avaliadas metabolicamente durante competições ou sessões de treinamento, além de um teste de VO_{2Max} .

No primeiro experimento (E1), a atleta da modalidade taekwondo (F1) foi avaliada durante um treinamento de combate; no segundo experimento (E2), a atleta de pentatlo moderno (MP1) foi avaliada durante o campeonato brasileiro de sua modalidade. Por fim, no terceiro experimento (E3), ambas atletas foram avaliadas em um teste de VO_{2Max} .

As análises dos perfis de amônia, urato e ureia foram quantificadas utilizando kits comerciais (LEAC® e Labtest®, respectivamente) e os aminoácidos (AAs) por cromatografia líquida de alta eficiência (HPLC), em Laboratório de Análises Clínicas.

Resultados

Os resultados obtidos após as análises de E1 e E2 revelaram que os valores de concentração de amônia aumentaram 371% na atleta F1 e 331% na atleta PM1, quando comparados com os respectivos valores atingidos durante o protocolo de avaliação de VO_{2max} .

Os picos de uratemia durante E1 e E2 foram aproximadamente cinco vezes maiores quando comparados aos níveis alcançados em E3. Por outro lado, os níveis da uremia apresentaram-se semelhantes quando a análise de VO_{2Max} e o protocolo esportômico foram comparados. Os aminogramas da atleta de taekwondo demonstraram aumento nas quantificações de valina (Val), isoleucina (Ile), fenilalanina (Phe), tirosina (Tyr), alanina (Ala) e glutamato (Glu) em E1 quando comparados com E3. No entanto, as concentrações de Phe e Tyr da atleta PM1 foram maiores em E3 ao comparar-se com E2.

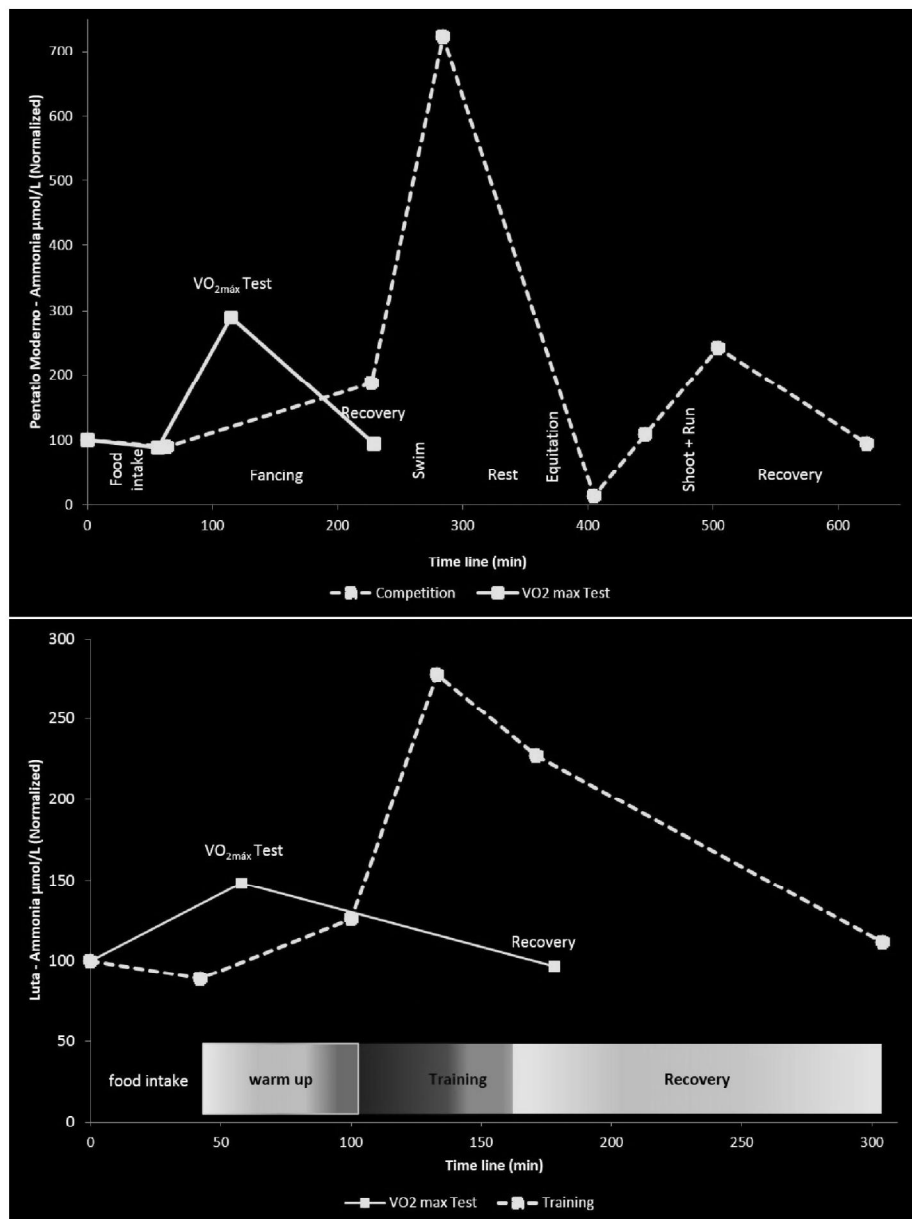


Figura 01: Comparação entre as avaliações E1, E2 e E3. As comparações demonstraram diferença na elevação da amonemia em E1 e E2 quando comparados aos testes de VO₂Máx.

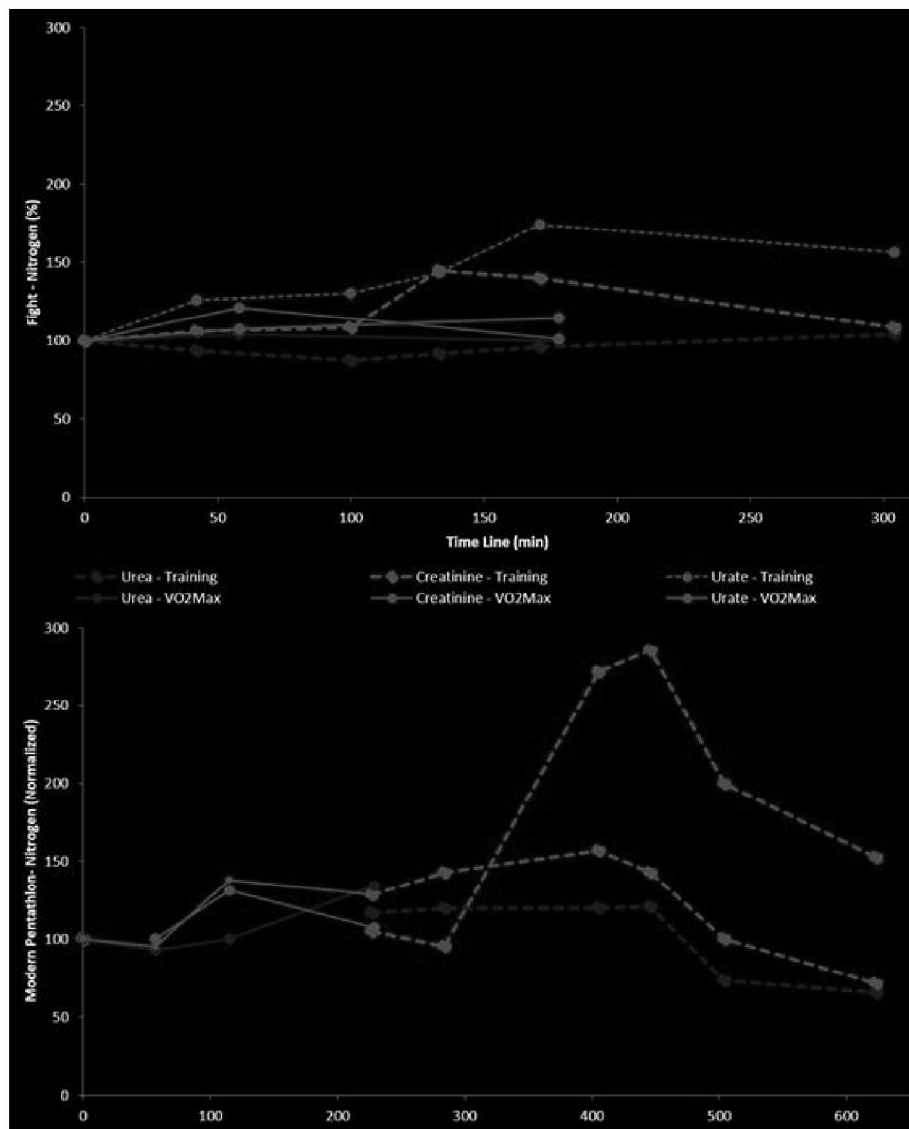


Figura 02: Comparação dos metabólitos nitrogenados respectivamente das atletas de taekwondo e pentatlo moderno em E1 e E2

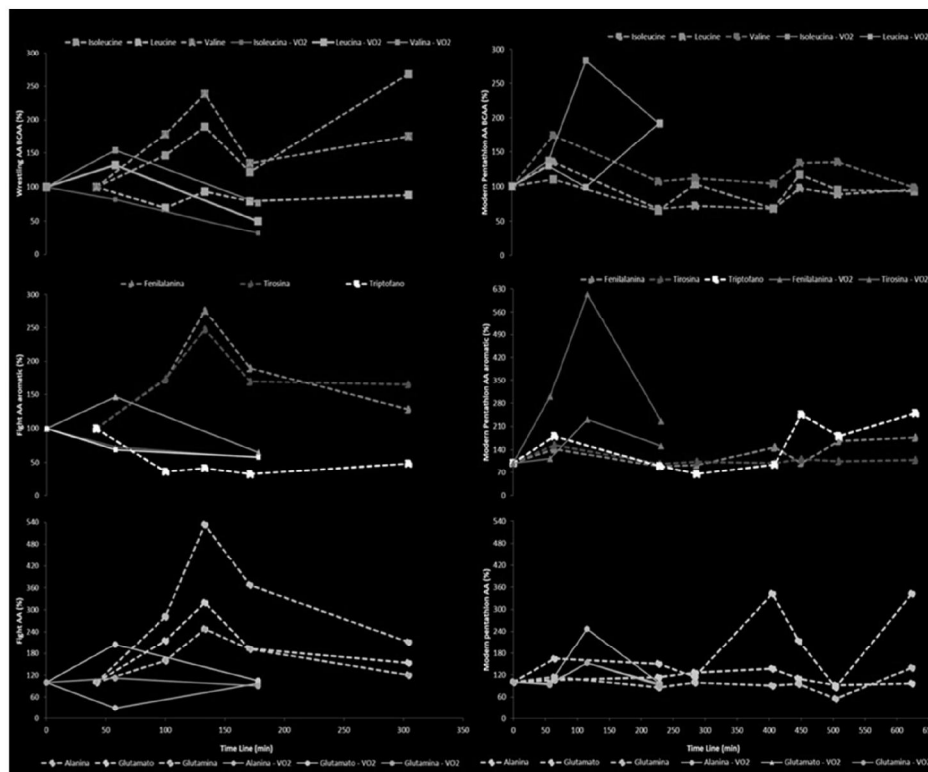


Figura 03: Aminograma dos experimentos E1 (esq) e E2 (dir). Há maior mobilização dos aminoácidos em razão do exercício como demonstrado pela cinética dos aminoácidos cetoglicogênicos, glicogênicos e aromáticos durante os experimentos esportômicos.

Conclusão

Nossos achados demonstram que o uso de testes de VO_{2Max} , que são bastante úteis para a compreensão das respostas cardiopulmonares ao exercício, não são a melhor opção quando investigamos o metabolismo da amônia e de aminoácidos durante a realização de diferentes tipos de exercício. Propomos que os protocolos esportômicos devem ser usados para melhor entender o metabolismo em situações de campo.

SEÇÃO C

**THE WINNING OF AN OLYMPIC MEDAL IN MODERN PENTATHLON: NEW
BORDERS OF SPORTS SCIENCES USING AN SPORTOMICS APPROACH**

Dados apresentados no Congresso Anual do European College of Sports
Science 2014 (Amsterdam, NED)

Magno-França, A.; Magalhães-Neto, AM., Bachini, F., Bassini, A., Cameron, LC.

Material e Métodos

Neste experimento, uma atleta de alto rendimento de pentatlo moderno (MP) foi monitorada durante aproximadamente 34 meses (2010 a 2013), durante dez diferentes coletas semanais (3-6 dias cada). Os períodos de coletas foram: jejum, pré e pós treinamento das diferentes disciplinas do MP, e recuperação de 90 minutos. Foram mensurados aproximadamente 300 metabólitos presentes em amostras de sangue e urina.

Resultados

Em trabalhos anteriores desenvolvidos pelo nosso grupo, a atividade da enzima creatina quinase (CK) foi analisada para estimar a intensidade do exercício (Bessa et al., 2008). Durante o primeiro ano de experimentos, as concentrações de CK apresentaram um aumento da ordem de 300% (190-677 U/L) na maior parte do período de treinamento da atleta. No segundo ano, este aumento foi cerca de 400% (135-550 U/L) e atingiu quase 800% (119-928 U/L) no terceiro ano de treinamento. Durante esse período foi possível estabelecer um padrão que permitisse retornar-se aos níveis basais de CK medidos nos inícios de cada semana de experimento. No último ano, a evolução no treinamento demonstrou que o aumento dos neutrófilos diminuiu quase dez vezes mais em exercícios de maior intensidade. Em adição, a resposta dos linfócitos foi um terço menor do que a dos neutrófilos.

A concentração de alanina aminotransferase apresentou elevação de concentração em resposta ao aumento da intensidade do exercício (pico de aproximadamente 350%), substancialmente maior quando comparada com a concentração de aspartato aminotransferase (pico em 180%).

Em contraste, não foram observadas alterações na gama-glutamilttransferase e as modificações no treinamento associadas à dieta e a recuperação achataram a resposta da proteína C, abolindo seu aumento mesmo em intensidades maiores de exercício.

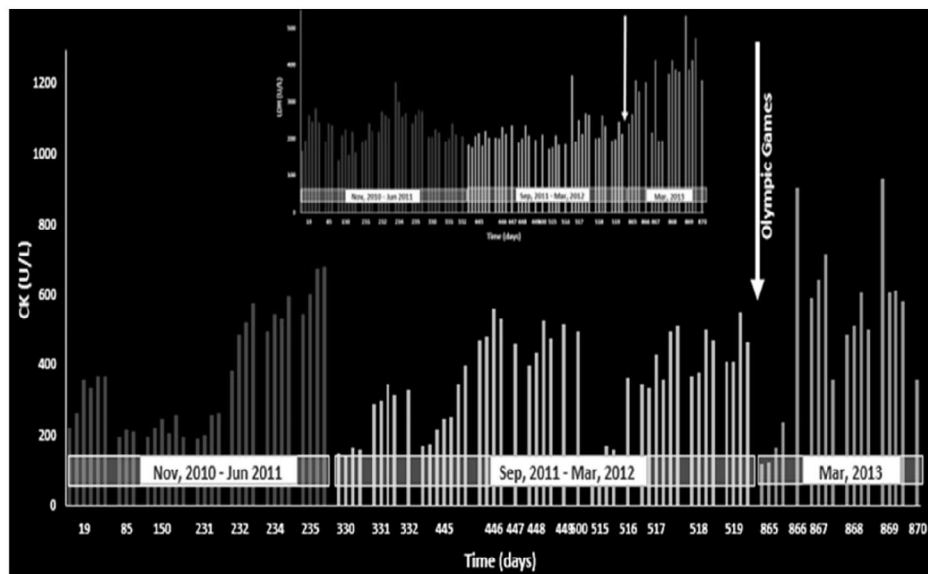


Figura 1. Atividade da enzima creatina quinase (CK) e lactato desidrogenase (LDH) durante três anos consecutivos de treinamento (2010-2013).

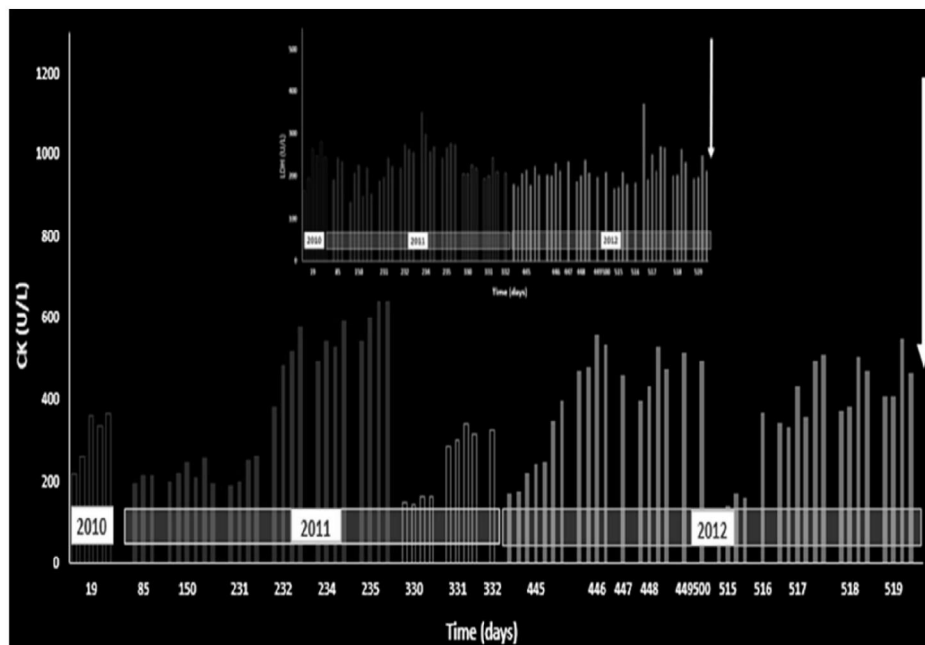


Figura 2. Atividade da enzima Creatina quinase (CK) e lactato desidrogenase (LDH) durante diferentes temporadas de treinamento antes dos Jogos Olímpicos. O uso das concentrações de CK atuou para auxiliar na determinação dos níveis ótimos de treinamentos.

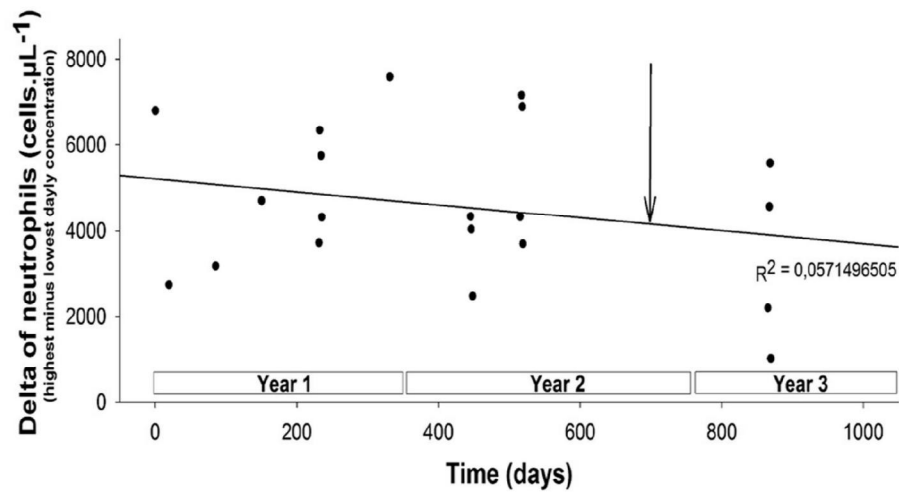


Figura 3. Range de concentração (mais elevadas subtraídas das menos elevadas) de linfócitos presentes no sangue em cada dia de experimento, medido durante três anos consecutivos de treinamento (2010-2013).

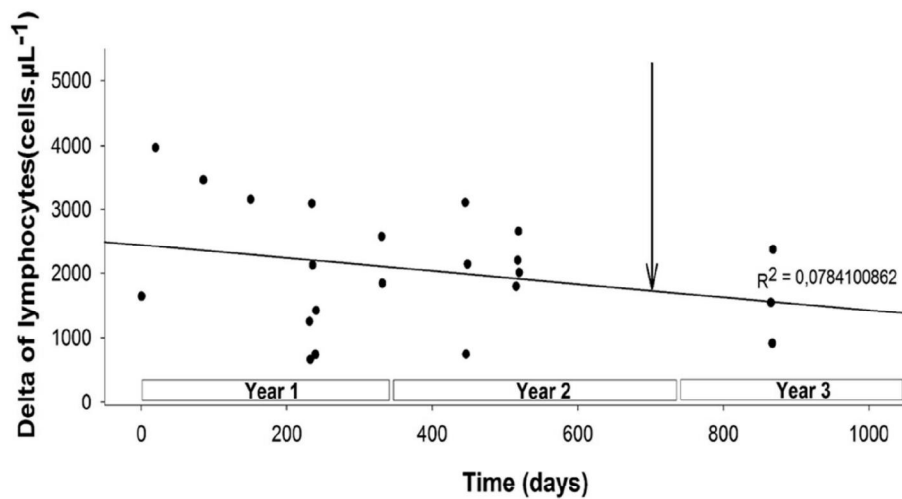


Figura 4. Range de concentração (mais elevadas subtraídas das menos elevadas) de neutrófilos no sangue em cada dia de experimento, medido durante três anos consecutivos de treinamento (2010-2013).

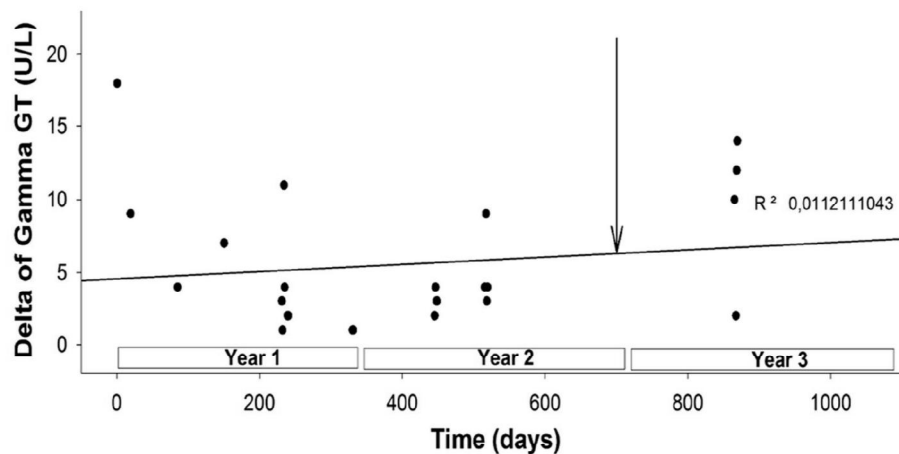


Figura 5. Range de concentração (mais elevadas subtraídas das menos elevadas) de gamaglutamiltransferase (Gamma GT) presentes no sangue em cada dia de experimento, medido durante três anos consecutivos de treinamento (2010-2013).

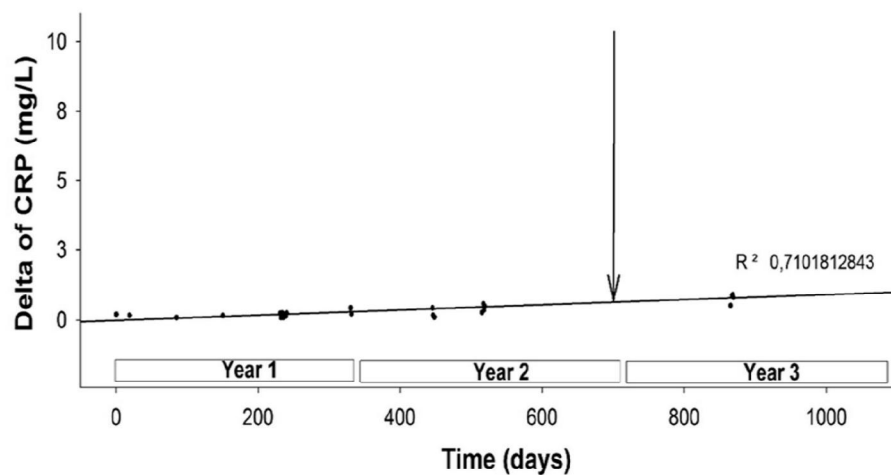


Figura 6. Range de concentração (mais elevadas subtraídas das menos elevadas) de proteína C-Reativa (CRP) presentes no sangue em cada dia de experimento, medidos durante três anos consecutivos de treinamento (2010-2013).

Discussão

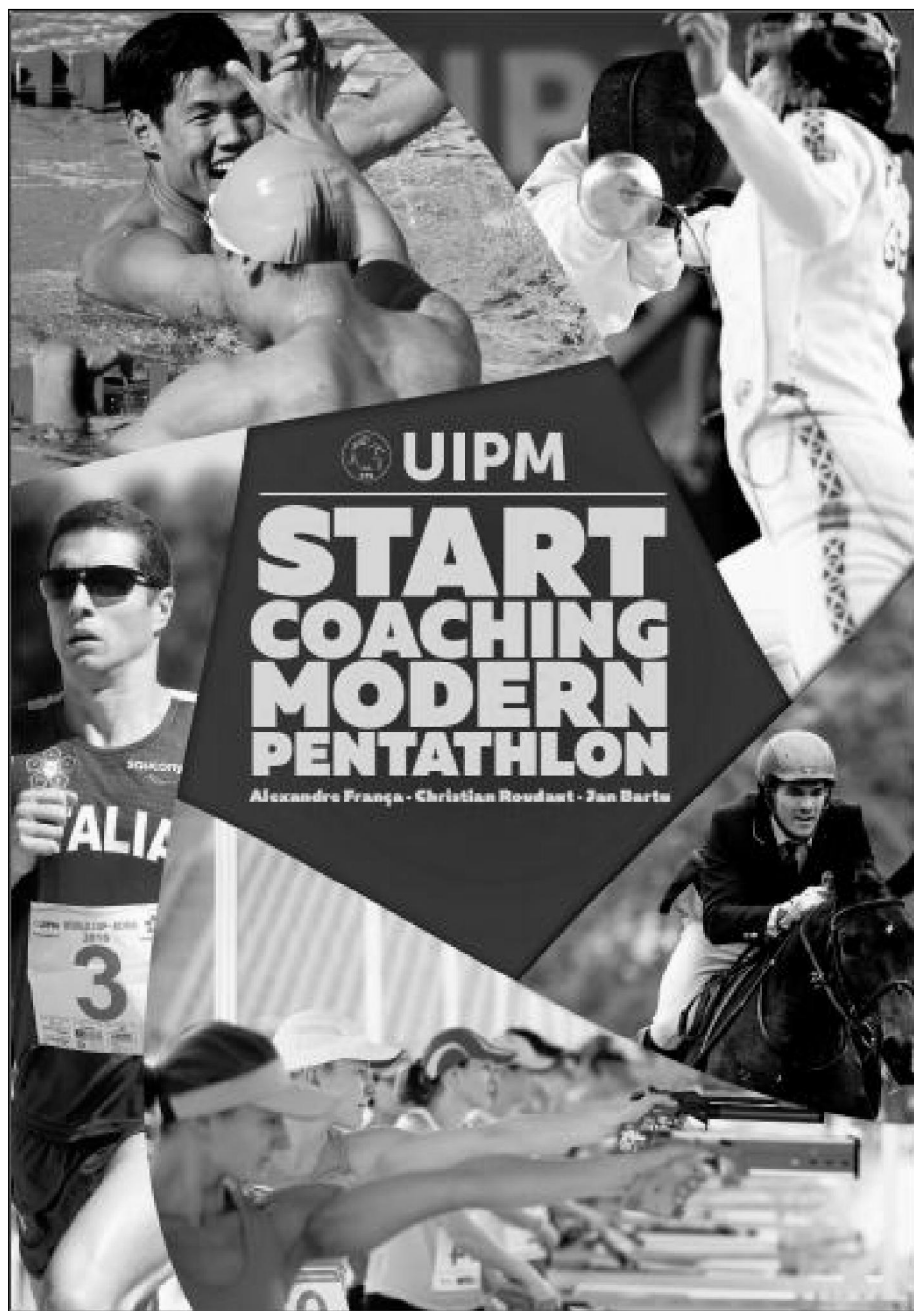
Marcadores de injúria muscular e resposta inflamatória indicaram a melhor cinética de recuperação para diversas fases do treinamento. Em adição, mostramos que o aumento na intensidade do treinamento seguiu-se por uma diminuição da resposta inflamatória sem a perda de imunidade. Ao monitorar essas análises é possível adotar programas de treinamentos mais intensos diminuindo a chance de lesão muscular. Dentro de nosso conhecimento, essa é a primeira vez que se realizam análises bioquímicas e de sinalização para uma atleta de pentatlo moderno corroboradas pela conquista de uma medalha olímpica.

Conclusão

A abordagem esportômica reproduziu situações reais de treinamento e competição. Nesse estudo, foram apresentadas novas possibilidades para o gerenciamento do treinamento, na medida em que foram associados marcadores de injúria, respostas inflamatórias, carga de treinamento e cinética de recuperação.

SEÇÃO D

LIVRO PUBLICADO:
“START COACHING MODERN PENTATHLON”



Acknowledgments:

The UIPM would like to thank Maître Michel Sicaud and Dr Genadijua Sokolovus
for their substantial contribution in the preparation of this book.

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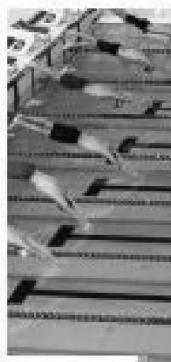
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Swimming



Laser-Run



Fencing

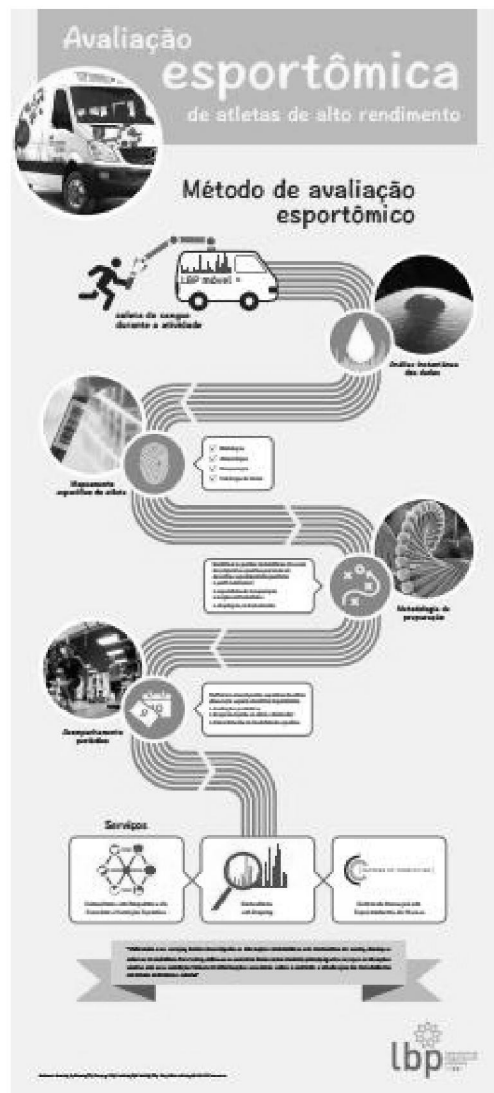


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RT

INFOGRÁFICOS PUBLICADOS



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- Integração com técnicas e técnicas modernas;
- Experimentos.

MISSÃO

Investigar proteínas como macromoléculas em sistemas biológicos, identificando suas funções e estruturas em células e organismos, para a produção de biotecnologias.

VISÃO

Ter um papel de liderança em pesquisas de ponta em biotecnologia e inovação científica.

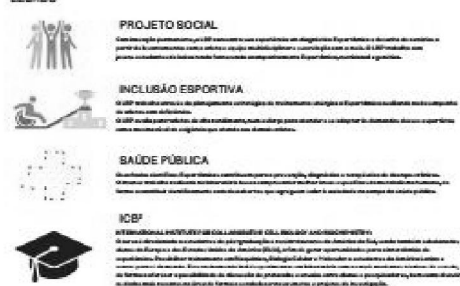
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BASSINI, ADRIANA; GARCIA, T. ; MAGALHÃES NETO, A. M. ; **MAGNO-FRANCA, A.** ; BACHINI, F. ; CATALDI, E. ; SANTOS, R. ; Silva, C V ; CABRAL, L. ; CAMERON, L. C. . Esportômica e hidratação. 2015. (Desenvolvimento de material didático ou instrucional - Infográfico).

Registrado sob o nº 715028 junto ao Escritório de Direitos Autorias da Fundação Biblioteca Nacional

MANUSCRITO EM PREPARAÇÃO:
“SPORTOMICS FINDINGS BASED ON METABOLIC ANALYSIS OF
WORLD-CLASS CANOEISTS DURING A TWO-DAY TRAINING
PROTOCOL”.

Sportomics findings based on metabolic analysis of world-class canoeists during a two-day training protocol

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2. Material and Methods

2.1 Subject

We followed and evaluated four professional men canoeists (xx-xx years old), during two different training protocols in two successive days. The study was approved by the Ethics Committee for Human Research of the Federal University of State of Rio de Janeiro and conformed to the requirements and guidance for carrying out research in human subjects (Health National Council, Brazil, 2010). Throughout the entire period the athletes did not have health problems or use ergogenic substances or any other drugs. Laboratory tests included hematological and biochemical analyses allowing us to be aware of possible metabolic variations.

2.2 Experimental design

As showed in Fig. 1 we collected 15 blood samples, as follows: on the first day, at fasting (T1); pre and post (T3) morning paddling; after a gym session (T4) and a 45 min recovery (T5); pre (T6) and post (T7) afternoon paddling; after a running training (T8) and a recovery of 60 minutes (T9). On the second day, at fasting (T10); pre (T11), during (T12) and post (T13) paddling; after a gym session (T14) and a recovery of 60 minutes (T15). For this study we analyzed nearly 40 blood parameters in order to understand the different personal responses to exercise. All subjects underwent the same conditions of feeding and resting, remaining confined throughout the test period.

2.3 Laboratory analysis

Samples for biochemical assays were collected from the antecubital vein and centrifuged (3,000g; 10min at 4 °C). All of the blood sampling procedures, centrifugation and blood storage were carried out immediately after collection to

avoid the loss of volatile compounds. Serum and plasma were aliquoted, frozen in liquid nitrogen, and stored at -80 °C for later analysis. The blood, serum, and plasma were analyzed in duplicate or triplicate, when necessary.

Biochemical analyses were conducted using an automatic analyzer (ADVIA 1200 Chemistry System, Siemens, USA). Alanine aminotransferase (ALT), alkaline phosphatase, aspartate aminotransferase (AST) creatine phosphokinase (CK), lactate dehydrogenase (LDH) and γ -glutamyltransferase (γ GT) were quantified by colorimetric methods. Samples for hematological assays were collected into a tube with K3-EDTA (Vacurette, Greiner Bio-One, Germany) and stored at 4 °C for later automated analysis (COULTER® STKS™ Hematology Flow Cytometer, Beckman Coulter, Fullerton, CA) as well as white cell counts (total and differential) were measured.

2.4 Mass Spectrometry

We also collected Dried Blood Spots (DBS) of the samples to a deeper study using tandem mass spectrometry (MS/MS), as described bellow.

- Enzyme assay using 3.2-mm DBS

Reagents. Samples were extracted in 20 mmol/L sodium-phosphate (Sigma) solution (pH 7.1). GAA assay buffer contained 0.34 mol/L sodium phosphate and 0.17 mol/L citrate buffer (Fluka), pH 4.0. We prepared GAA assay cocktail by the sequential addition of 1.8 mL of 100 g/L CHAPS (Sigma), 15.9 mL GAA assay buffer, and 0.3 mL of 0.8 mmol/L acarbose in water (Toronto Research Chemicals) to a vial containing 0.12 mol internal standard [7-d5-benzoylamino-heptyl]-[2-(4- hydroxy-phenylcarbamoyl)-ethyl]-carbamic acid tertbutyl ester] and 12 mol GAA substrate [7-benzo ylamino-heptyl)-[17]-carbamic acid tert-butyl ester]. Substrate and internal standard were given to us by Genzyme. The vial was vortex-mixed after each addition. The final assay cocktail contained 0.67 mmol/L substrate, 6.6 mol/L internal standard, 0.013 mmol/L acarbose, and 10 g/L CHAPS. We used Brand pipettors and Eppendorf single-channel pipettes for reagent preparation. Sample preparation. We punched 3.2-mm DBS

into 96-well plates (Greiner Bio-One) using a Wallac DBS Puncher (PerkinElmer). The first and last well of each plate contained blank filter paper. We added 70 L extraction buffer to each well and sealed the plates with a silicone plate-sealer (Pall). Samples were extracted at 37 °C and 750 rpm with shaking for 1 h in aWallac NCS Incubator (PerkinElmer).We transferred 10L extract to a new plate with 15 L GAA assay cocktail in each well. The plates were sealed and incubated for 22 h at 37 °C. Enzyme reactions were quenched by adding 100 L of 1:1 ethylacetate:methanol (Merck). After mixing, we transferred samples to a 96-well deep-well plate (Brand). To prepare the liquid-liquid extraction, we added 400L of 1:1 ethylacetate:methanol to each well, followed by 400 L ethylacetate and 400 L distilled water (B. Braun, Melsungen, Germany). Samples were mixed by pipetting up and down and centrifuged at 1400g for 4 min. We transferred 300 L of the upper phase to a new plate using the Quadra3 pipetting machine (Tomtec) and dried it using the Minivap (Porvair Sciences). Samples were reconstituted in 100 L of 80:20 acetonitrile:water (Fischer Scientific; Merck). All transfers were made using a Transferpette 12-channel pipette (Brand).

Tandem mass spectrometry. Analyses used a API 2000 triple-quadrupole mass spectrometer (PE Sciex), Neogram (PerkinElmer Wallac), and Analyst v1.1 software (MDS Sciex) in positive ion mode. Instrument settings were as follows: curtain gas pressure 20 Torr, collision cell pressure 4 Torr; ion spray voltage 5500V, source temperature 2500 °C; gas 1 10 psi; gas 2 70 psi; declustering potential 16V, focusing potential 380V; entrance potential 5V, collision energy 21V; collision exit potential 10V. Samples (10 L) were injected into the electrospray source with a Gilson 819 Injection Module (Gilson) using acetonitrile/water (80/20 vol/vol) as solvent at a flow rate of 250L/m (PerkinElmer Series 200 MicroPump). Mass spectrometer parameters were optimized to give highest sensitivity for transitions m/z 503 403 (GAA internal standard) and m/z 498 398 (GAA product).

2.5 Data analysis

The data collected were analyzed and compared for identifying the differences between the athletes themselves along the whole period of collection.

3. Results

3.1 Muscle Stress Biomarkers

The two-day protocol involved diverse types of exercises and intensities in a typical canoeist routine. The experiment was used to measure the response of several metabolites in blood concentration. Regarding injury markers we analyze graphics on an individual basis, focusing at the enzymes creatine kinase (EC 2.7.3.2; CK), lactate dehydrogenase (EC 1.1.1.27; LDH), aspartate aminotransferase (EC 2.6.1.1; AST), alanine aminotransferase (EC 2.6.1.2; ALT) and γ -glutamyltransferase (EC 2.3.2.2; γ GT).

3.1.1 CK

During the first day of experiment, the four athletes presented approximately the same CK kinetics but in different concentration levels: 300, 700, and 1700 U/L. Three out of the four reached 5-10% increase after the second paddling session, while Athlete 4 (grey dot) reached 20% at the same point. In the second day, all of them returned to values between 5-15% difference in comparison to baseline levels of the first day (T1). On this day, CK concentration increased in all subjects by 15%-25% in comparison to T10 (baseline of day 2).

3.1.2 LDH

Likewise, CK, Athletes had the same order of magnitude at their initial LDH concentration (T1). All of them followed the same pattern on day 1, reaching

two plateaus: the first one from T2 to T5, raising 10-20% as compared to T1; the second from T6 to T9, with a 10% additional increase. Coincidentally, these patterns are related to samples collected at the first (paddling/gym) and second (paddling/running) set of exercises. During the second day all athletes fluctuated 15-25% at T12 and T13, if compared to the baseline of the second day (T10).

3.1.3 AST

Athlete 1 (green dot) kept a concentration pattern of 80-90 U/L along the whole experiment. In the first day, Athletes 2 (red dot) and 3 (blue dot) followed a similar kinetics of LDH, rising plateaus of ~10 and 20% increase at T2 to T5 and T6 to T9, respectively. During day 2, while Athlete 4 (grey dot) AST concentration remained stable at 20 U/L, Athletes 2 (red dot) and 3 (blue dot) rose consistently 20% as of the paddling session (T12), keeping this level until the end of the experiment.

3.1.4 ALT

When analyzing ALT concentration in both days, we found out that in three out of four athletes it varied from 10 to 20% increase in the samples collected during and immediately after exercise. However, Athlete 4 (grey dot) did not show changes in his plasma ALT concentration.

3.1.5 γ GT

In the first day there were less of 10% changes in the concentrations of each athlete along the collections. Athletes 2, 3 and 4 were distributed along the concentration range 15-20 U/L, while Athlete 1 (green dot) was stable at 30 U/L. During the second day we measured the same range pattern, but Athletes 2 (red dot) and 4 (grey dot) elevated by 20% their response during and immediately after the canoe paddling session.

3.2 Blood cells

3.2.1 Hematocrit

Generally speaking, we observed hemodilution during the whole experiment comparing recovery samples (T9 and T15) to T1. On the first day, athletes hemoconcentrated after exercise 1-2%. During the second day all subjects decrease their concentration near 2% at T15 in comparison to the baseline of the day. We underline that we note hemoconcentration after the paddling sessions (T7 and T13) as described, but not after the gym (T4 and T14) either the running (T9) sessions.

3.2.2 Erythrocytes

Considering the first day of experiment, all athletes presented slightly changes in response to exercise. Athletes 1 (green dot) and 4 (gray dot) showed 3-4% cell count decrease by the end of the day (T9); contrastingly, Athlete 3 (blue dot) was 7% below the initial values and Athlete 2 (red dot) did not show any variation. On the following day, we note approximately the same kinetics with all athletes downregulated by 3-5%.

3.2.3 Leukocytes

Total White Blood Cells (WBC) presented two increase patterns on the first day, raising all subjects 20-30% from T2-T5 and from T6-T9 we measured another extra 30% increase to Athletes 1 (grey dot) and 2 (red dot); 90% to Athlete 3 (blue dot) and 60% to Athlete 4 (grey dot). Even after recovery (T10), Leukocytes concentration remained one third higher than the baseline (T1) in Athlete 4 and 10% to the others. By the successive day, variations emerged only from the sample during the exercise (T12) when athletes augmented by 50-70% their leukocytes concentration. At the two last samples of the

experiment (T14 and T15) total WBC decrease consistently until a point 10-20% higher than the baseline excepting Athlete 3 (40%).

3.2.4 Neutrophils

Neutrophils raised progressively along the whole first day, staying on a plateau in between the end of the gym training (T4) and the beginning of the second paddling session (T6). Athlete 2 (red dot) is the one who raised less after recovery (T9 – 100%) while Athlete 4 (grey dot) was the highest (230%) regarding the baseline values. On the following day magnitude levels were like in day 1, but at T15 Athlete 3 (blue dot) leukocytes were ~45% higher and Athlete 1 (green dot) 40% lower than the Athletes 4 and 2.

3.2.5 Lymphocytes

Comparing to baseline values, lymphocytes showed increases after exercise at T3 (30-40%); T7 and T12 (70-150%). Acute responses of Athletes 3 (blue dot) and 4 (grey dot) to exercise were higher than the other subjects during the experiment. All concentration returned to the previous baseline values at T15.

3.2.6 Eosinophils

Concerning Eosinophils concentration, we can join Athletes 1 (green dot) and 2 (red dot) to one side and Athletes 3 (blue dot) and 4 (grey dot) to another. The first group displayed a descendent trajectory (35-50% in comparison to the baseline) to the point of the first recovery time (T5). Therefore, we can note a substantial increase after the second canoeing session of the day (T7), when Athlete 2 reached 200% increase versus T5. Both showed a decrease close to initial values at T9 (Recovery). On the other hand, Athletes 3 (blue dot) and 4 (grey dot) presented similar kinetics being up or down regulated from 30 to 60% against T1. On the second day of experiment eosinophils performed close to the same values.

3.3 Metabolic pathways substrates, intermediates and products

3.3.1 *Glucose*

We measured the glycemia along day 1 and it fluctuated on a 10-20% basis when compared pre-and post training samples, except Athlete 2 (red dot) who reached 38% after T3. Differently, recovery values at T9 increased by 45% in comparison to T1, except Athlete 2 (red dot) with a 70% increment. On the next day subjects presented similar kinetics, but recovery values (T15) were only 10-20% higher than the baseline (T10).

3.3.2 *Ammonia*

Results showed similar patterns of concentration been upregulated in the samples collected after paddling (T3 and T7, 20-30%↑) and after running (T8 and T9, 40-50%↑) The exception was Athlete 2 (red dot) who presented almost two-fold increase at recovery time (T9). On the following day, baseline values changed from 80 to 95μmol/L. Athletes 2 (red dot) and 4 (grey dot) incremented their concentration 150% at T13 (post paddling) when compared to T11 (pre-paddling), contrasting to Athlete 1 (green dot) with only 20% increase. The four athletes returned to the previous day baseline values at T15 (recovery), which was the last sample collected in the experiment.

3.3.3 *Lactate*

Athletes' lactate blood levels increased in response to exercise mainly at T7 and T12 (post paddling) as well as at T14 (post gym training). Considering these points, Athlete 2 (red dot) showed six to 10-fold lactatemia increment in comparison to baseline values. In its turn, Athletes 3 (blue dot) and 4 (grey dot) raised 250-1500% lactate concentration in the same period. All subjects turned

to values close to the initial concentration parameters by the last sample of each day of experiment (T9 and T15), reaching 1-3 mmol/L.

3.3.4 Urea

Urea concentration increased in all subjects progressively during the first day. While Athletes 1 (green dot) and 4 (grey dot) presented results close to median values the whole period, Athlete 2 (red dot) was down regulated Athlete 3 (blue dot) was up regulated by 5 to 20% in comparison to that statistic parameter. On the following day results show increases of less than 10% in all canoeists.

3.3.5 Uric Acid

Urate kinetics was very similar to Urea on day 1, with a slightly concentration increase in the second half of the day. Even if Athlete's 1 (green dot) curve was alike the others, his absolute values were 40-50% higher, variating around 6 to 7 mg/dL. Whereas Athlete 1 concentration did not change on day 2, the others keep stable until the end of the padding session (T13), rising nearly 30% at the recovery punction (T15).

3.4 Amino acids

3.4.1 Alanine

All athletes showed similar patterns of Alanine concentration, demonstrated by higher values after exercise when compared to Fasting and Recovery samples. Recovery presented approximately the same concentration in both days, and Fasting values in the second day were nearly 30% lower than the first. Athlete 3 (blue dot) produced the widest concentration range with a two-fold increase in between T1 and T3.

3.4.2 Arginine

Concerning Arginine blood concentration, we highlight Athlete 1 (green dot) fluctuating always above 20 nmol/mL with peaks after exercise around 40 nmol/mL, while Athlete 4 (grey dot) was below 9 nmol/mL during the whole experiment.

3.4.3 Asparagine

Each athlete presented different patterns of Asparagine concentration during the two days being the common situation the progressive decrease in T6 (pre-paddling), T7 (post-paddling) and T8 (post-running). Except this progressive decrease, Athlete 2 (red dot) showed the tiniest variation in a range from 45 to 51 nmol/mL.

3.4.4 Aspartic Acid

Unlike the amino acids already shown, concentration of Aspartic Acid in both days was higher at Fasting in comparison to the other samples. The lowest values were reached by Athlete 2 (red dot – 47 to 70 nmol/mL) and the highest concentration range by Athlete 1 (green dot – 91 to 152 nmol/mL).

3.4.5 Citruline

The highest increases in Citruline concentration were observed at T9, which was the last recovery sample of the first collection day. Comparing to the samples at T6 (pre-paddling) 200 min before, Athletes 1 (green dot) and 3 (blue dot) augmented their blood concentration by two-fold and Athlete 2 (red dot) by two and a half folds while Athlete 4 (grey dot) did not show any changes.

3.4.6 Glutamine

Glutamine blood concentration increased in all athletes at the two first post-paddling collections in comparison to the pre-paddling, raising 15-30% at T3 and 7-17% at T7. The exception was Athlete 4 (grey dot), who diminished 17% at the same section. Interestingly, all athletes decreased their values at T13 in comparison to T12.

3.4.7 Glutamic Acid

Athlete 1 (green dot) showed blood concentration much higher than the other canoeists did. In comparison to median values he reached 30-75%↑ from T1-T5, 10 to 25%↑ from T6 to T9 and 35-120% from T10-T15.

3.4.8 Glycine

Response of athletes to exercise was widely different. We identified a similar pattern of Glycine blood concentration, increasing from fasting (T1) to post-paddling (T3), slightly decreasing at T4 (post-gym) and T5 (recovery). The broadest concentration range was presented by Athlete 3 (blue dot) going from 150 to 202 nmol/mL.

3.4.9 Histidine

Histidine present approximately the same kinetics of Glutamine, but the increment of the concentration was quite higher (48-82% at T3; 18-38% at T7). At that time, Athlete 4 (grey dot) decreased around 8%.

3.4.10 Isoleucine

During the first day we noted similar Isoleucine blood concentration at T1 (fasting) and at the last sample collected (T9, Recovery) with the highest peaks at post exercise, reaching 106 nmol/ml at T3 by Athlete 4 (grey dot) and 110

nmol/mL at T7 by Athlete 1 (green dot). Contrastingly, during the second day values at the end of the experiment (T15) were around 30% lower than at fasting (T10).

3.4.11 Leucine

The leucine kinetics was quite similar to isoleucine excepting the peak of Athlete 1 (green dot).

3.4.12 Lysine

Considering three blocks of exercise (T1-T5; T6-9; T10-T15) we observe a slightly progressive decrease in all athletes. While the difference between the highest and lowest concentration during the whole experiment fluctuated around 37-45 nmol/mL in three athletes, Athlete 4 (grey dot) presented a 62 nmol/mL difference.

3.4.13 Methionine

Unlike the other canoeists, Athlete 1 (green dot) decreased his Methionine blood concentration from T1 to T5 (80%↓) and from T6 to T9 (38%↓) as well. On the second day (T10-T15), all athletes got a step up the Met concentration staying from 6,4-9,7 nmol/mL.

3.4.14 Ornithine

Athletes 1 and 3 (green and blue dot) revealed lower Ornithine concentration after exercise when compared to all samples collected before exercise. Contrastingly, the only increase post exercise was reached by Athlete 4 (grey dot) at T3 (26%↑ in comparison to T2).

3.4.15 Phenilalanine

While three out of four athletes kept the same pattern of Phenilalanine concentration staying in between 40-60 nmol/mL along the two days, Athlete 1 (green dot) showed concentration from 63 to 112 nmol/mL peaking post exercise at T7 and T 12.

3.4.16 Proline

Regarding Proline blood concentration, Athletes exhibit such diverse patterns along the experiment. In general, when analyzing the three blocks of exercise (T1-T5; T6-9; T10-T15) we observe a slightly progressive increase in all canoeists. Peaks of Proline concentration were found at T15 by Athlete 1 (green dot -220 nmol/mL) and T13 by Athlete 4 (grey dot – 210nmol/mL).

3.4.17 Serine

Athletes 2 and 3 (red and blue dot) displayed similar kinetics on both days, fluctuating their blood Serine concentration from 43 to 80 nmol/mL. In contrast to the other canoeists, Athlete 4 (grey dot) showed a big increase in response to the first paddling session (T3, 33%↑ in serine blood concentration). The other three athletes did not show raises after the different exercise session and Athlete 1 (green dot) exhibited de lower serine concentration, coming down to 45nmol/mL after the last paddling session (T13).

3.4.18 Threonine

Considering three blocks of exercise (T1-T5; T6-9; T10-T15) we observe a Gaussian distribution (lower-higher-lower) in Threonine blood concentration of all athletes. Especially Athletes 1 and 3 (green and blue dots) had very similar patterns with a mild increase in response to exercise, reaching nearly 15-30% less values at the end of the block in contrast to the beginning.

3.4.19 Tryptophan

Athletes 1 and 2 (green and red dots) increased slightly and progressively their Tryptophan blood concentration along the experiment. We highlight the acute responses to exercise in comparison to pre-exercise samples, when these same athletes reached around 20% increase at T3 and 10% at T7.

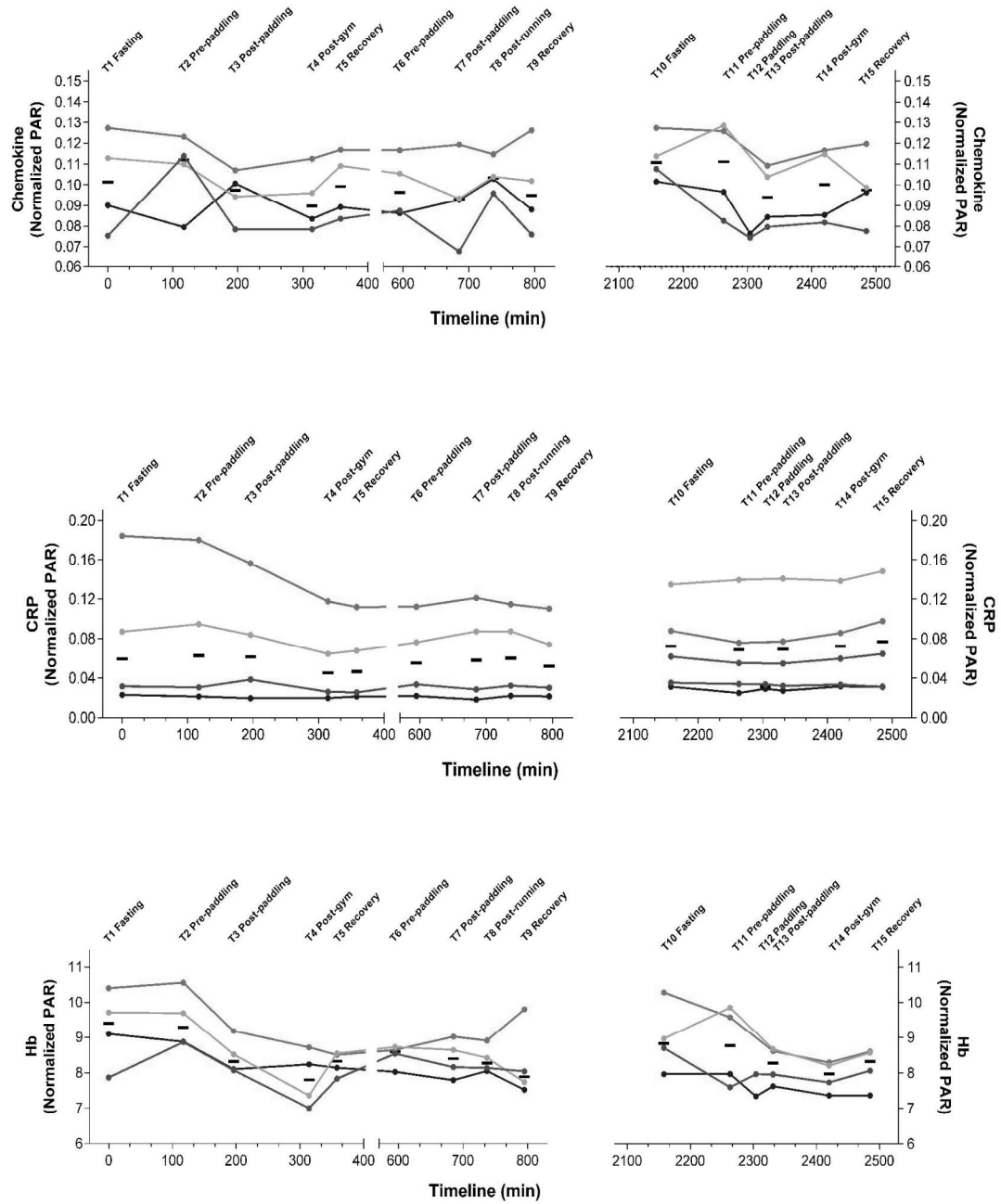
3.4.20 Tyrosine

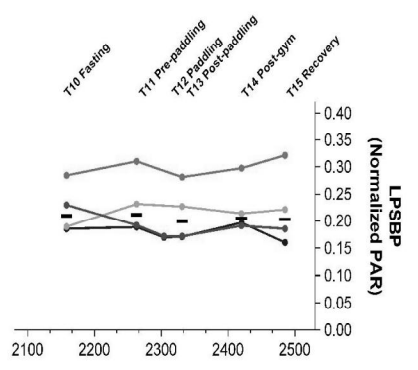
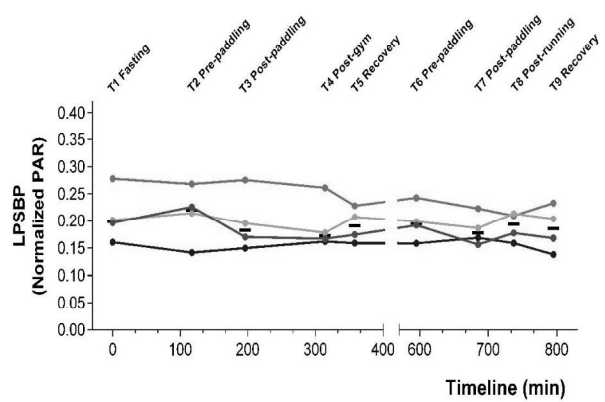
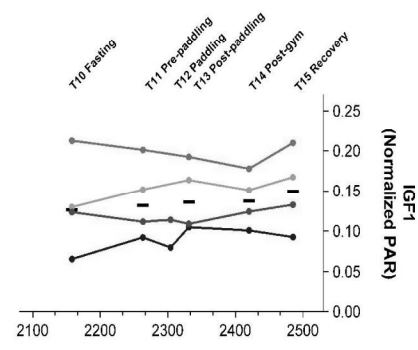
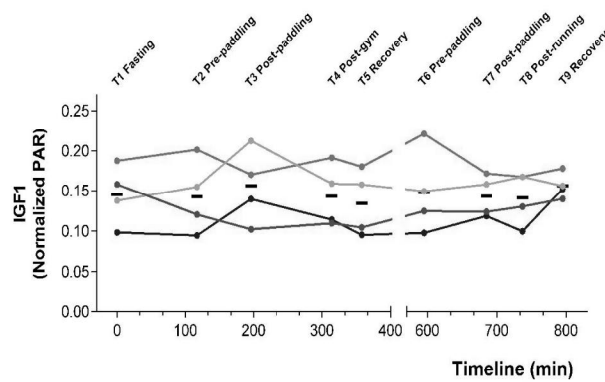
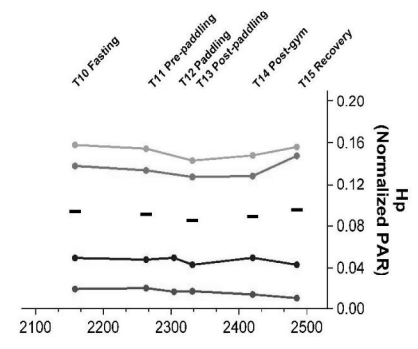
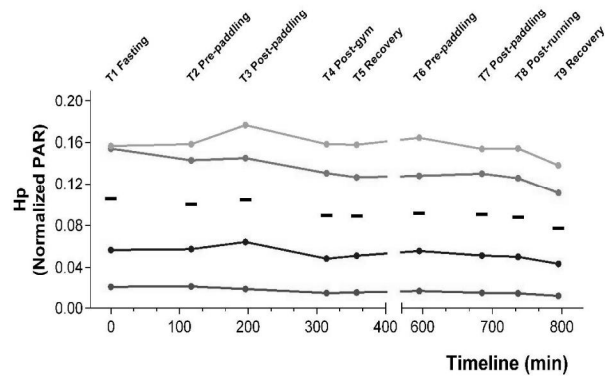
While the same Tyrosine concentration patterns of all Athletes were kept from T1 to T9, Athlete 1 (green dot) presented an odd kinetics from T10 to T 15. Concentration raised by one third in all samples after fasting, including the recovery collection.

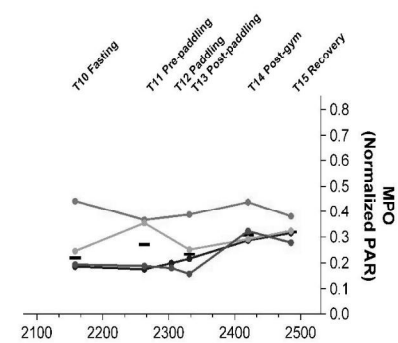
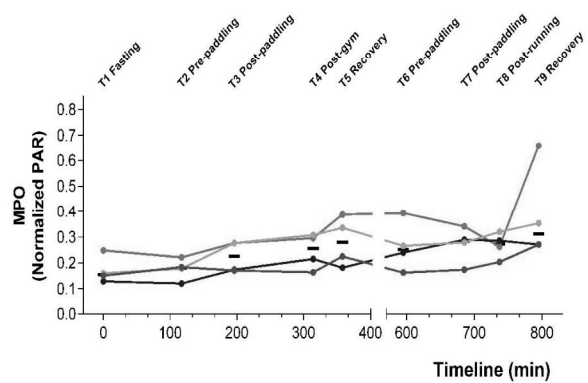
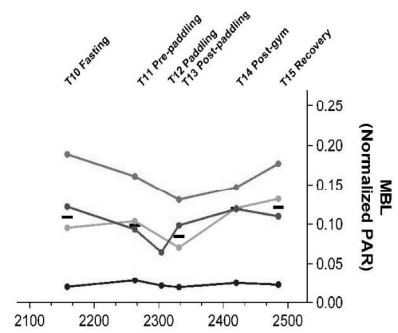
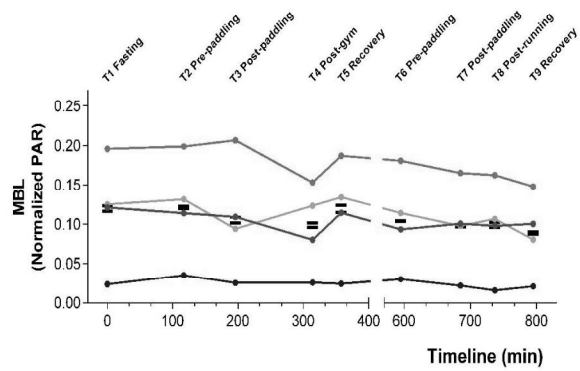
3.4.21 Valine

Valine kinetics was pretty similar to Tyrosine. Athlete 4 (grey dot) showed the highest (287 nmol/mL, T3) and lowest (113 nmol/mL) concentration, both collected immediately after exercise.

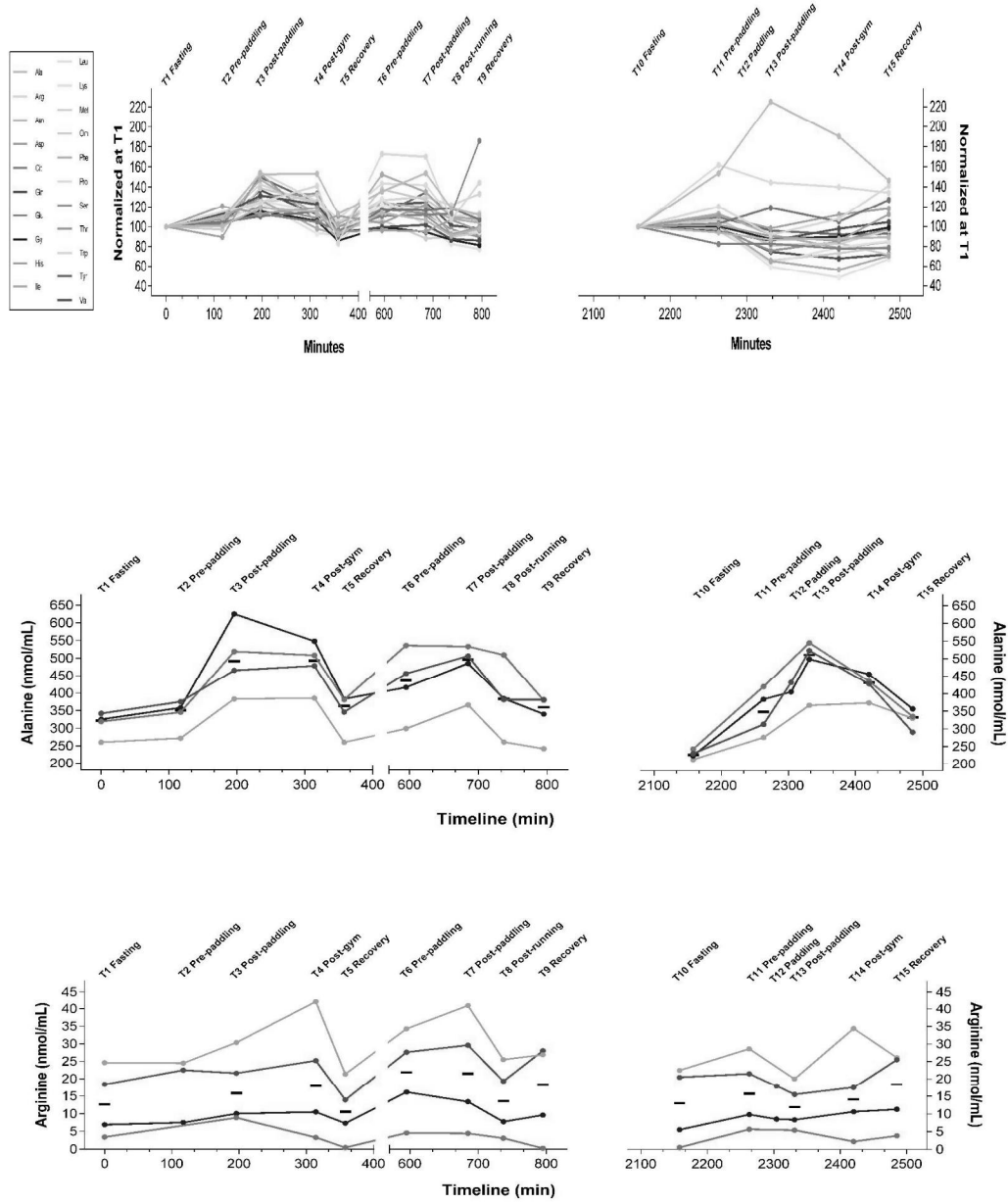
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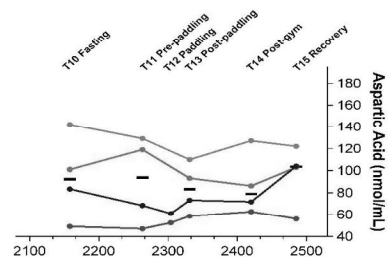
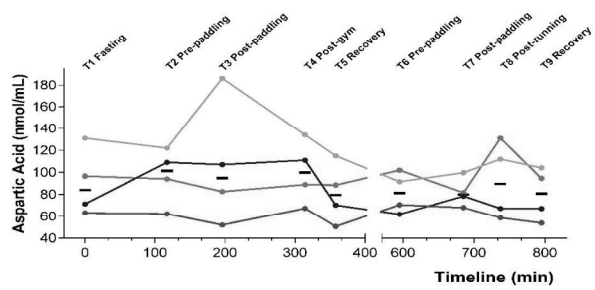
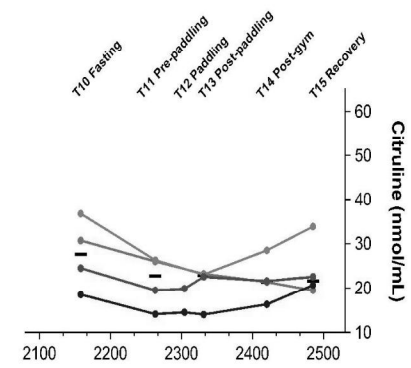
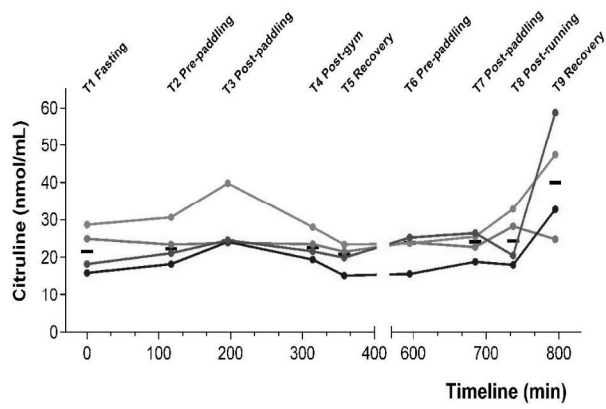
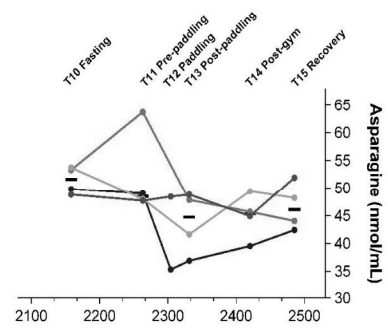
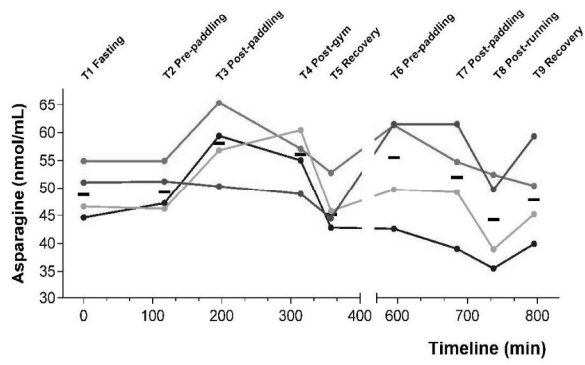


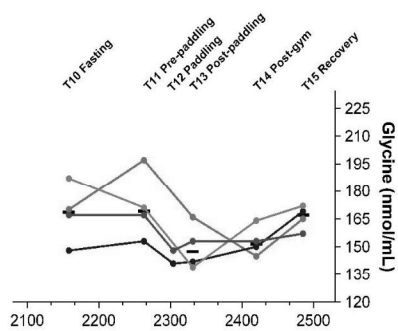
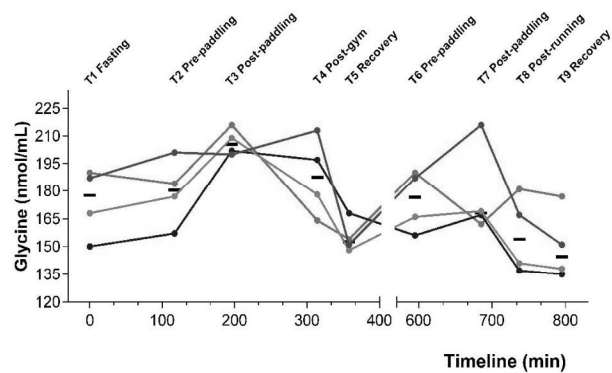
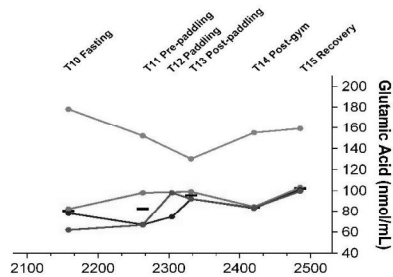
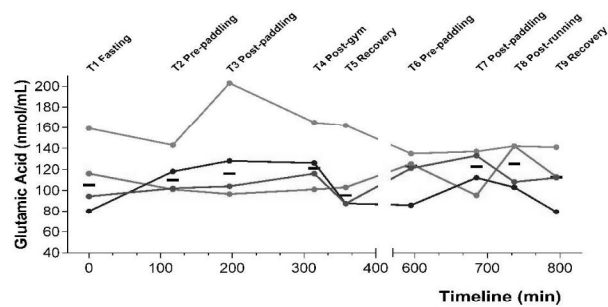
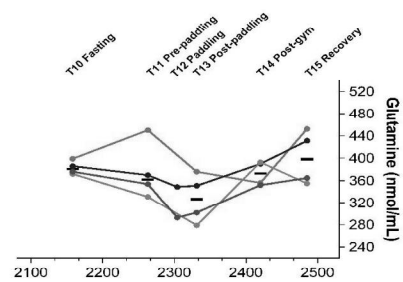
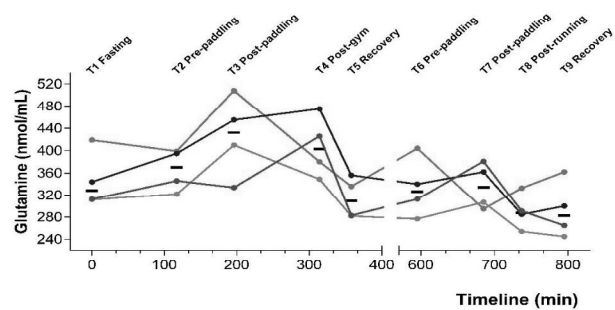


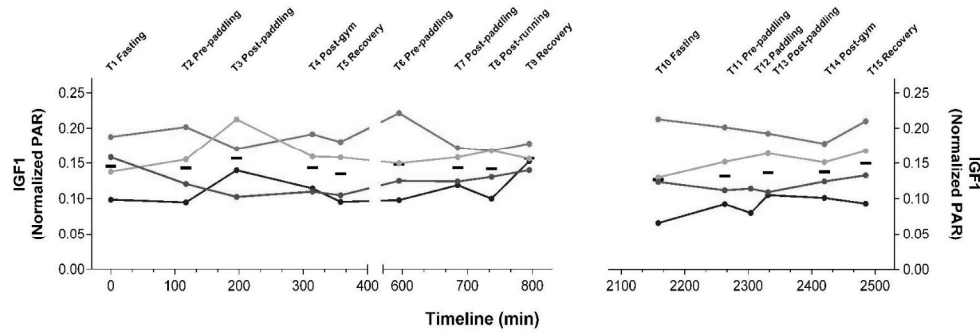
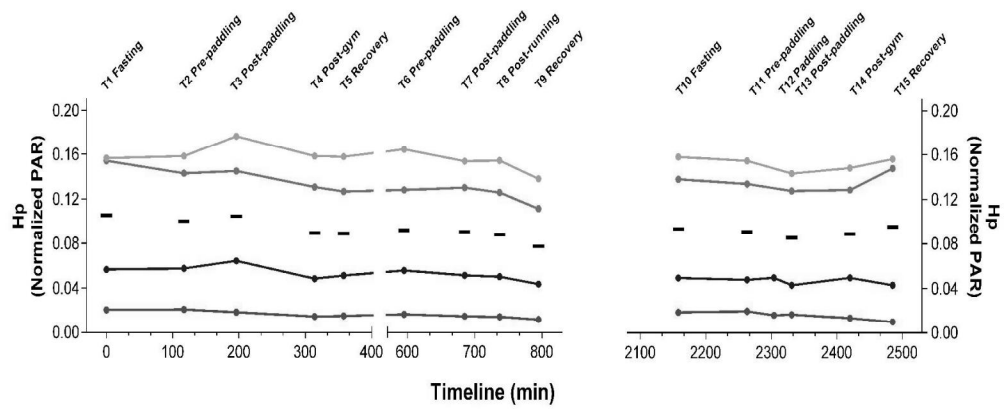
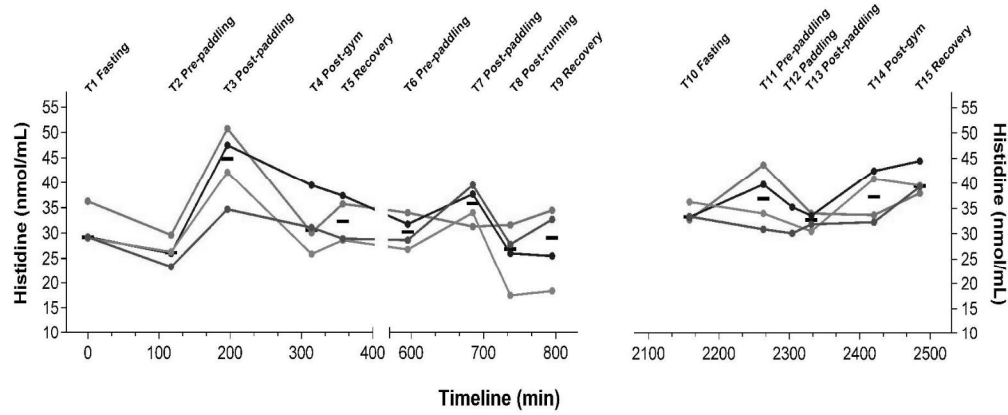


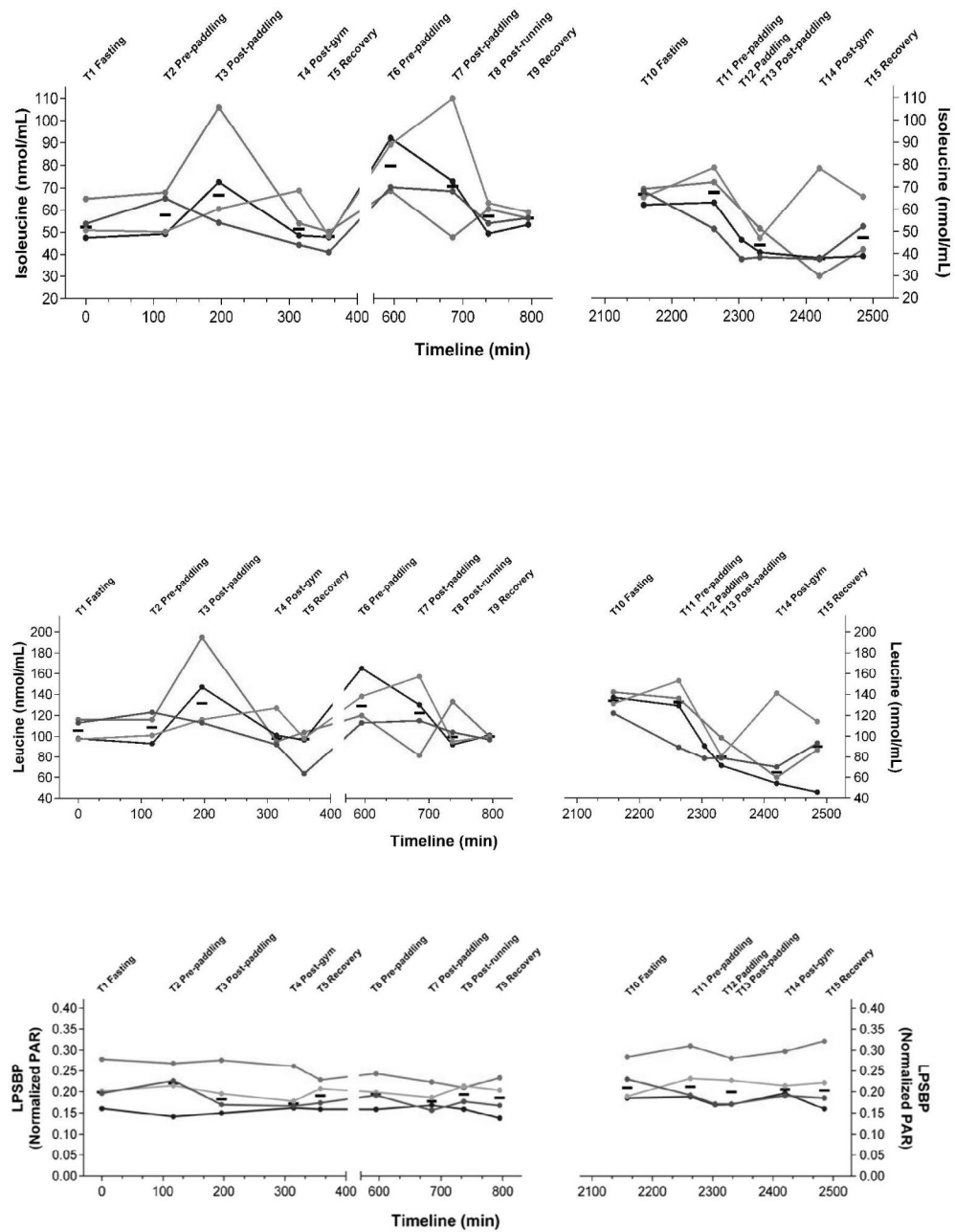
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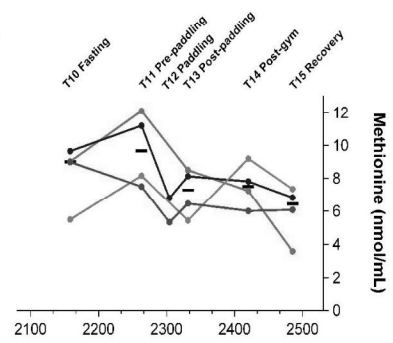
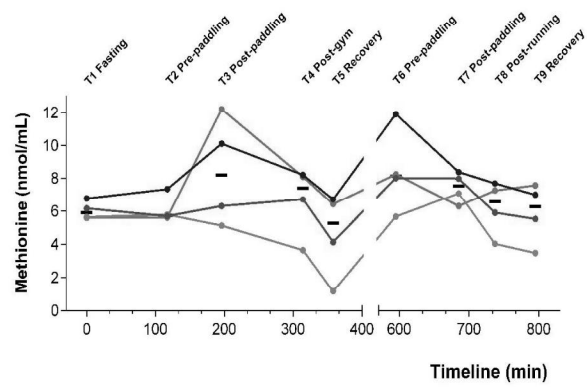
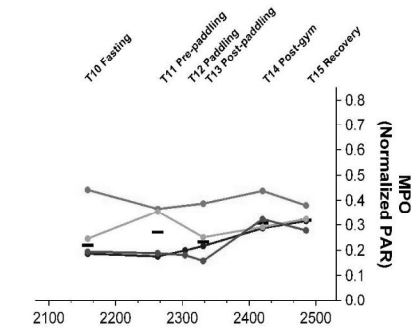
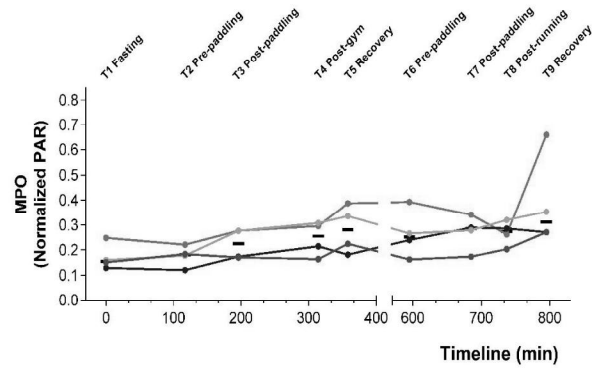
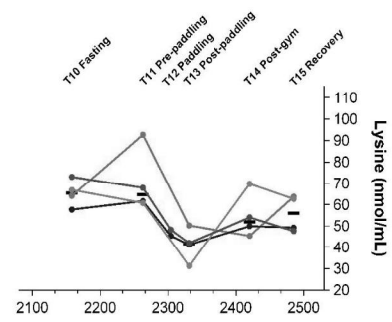
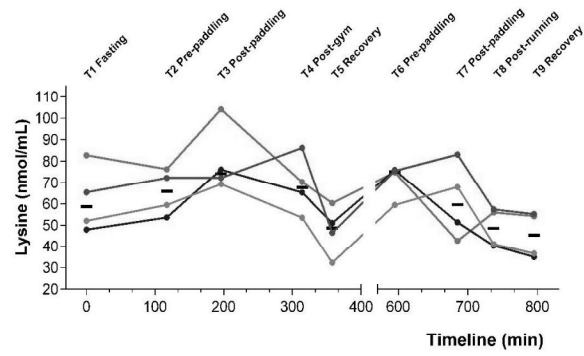


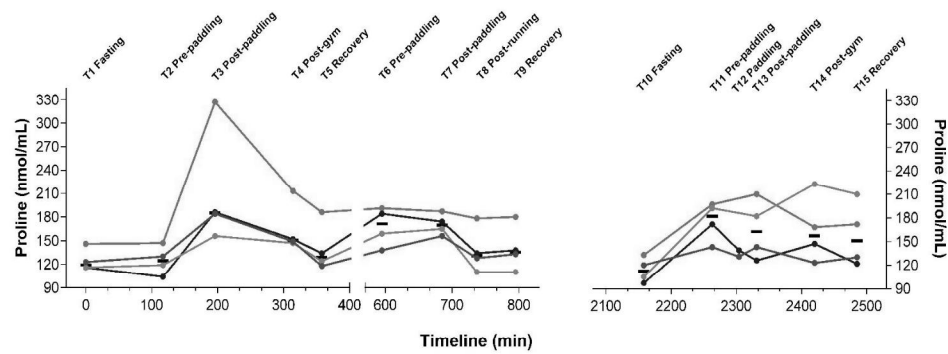
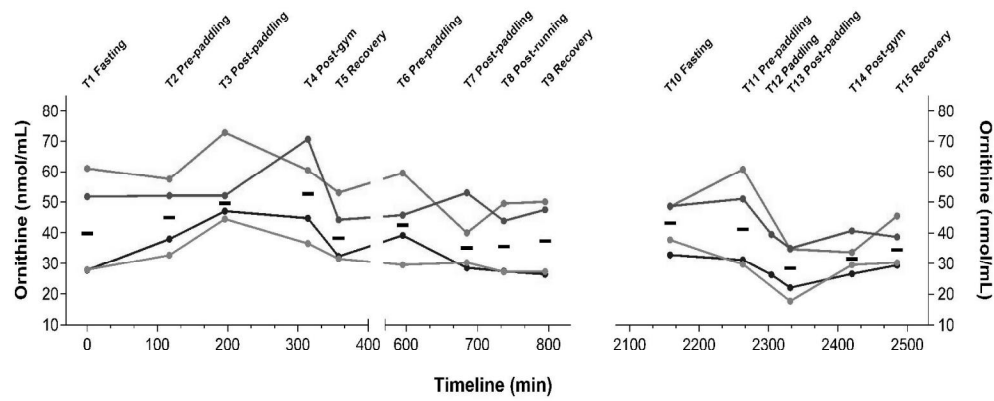
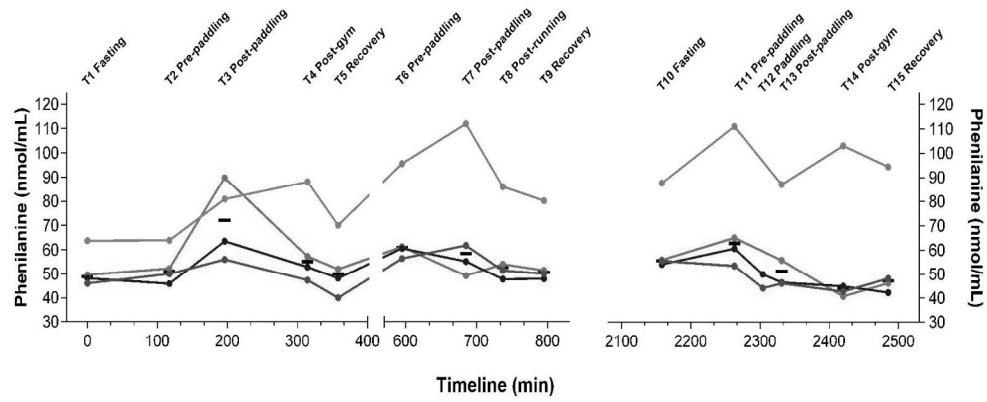


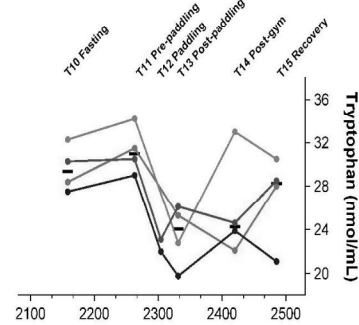
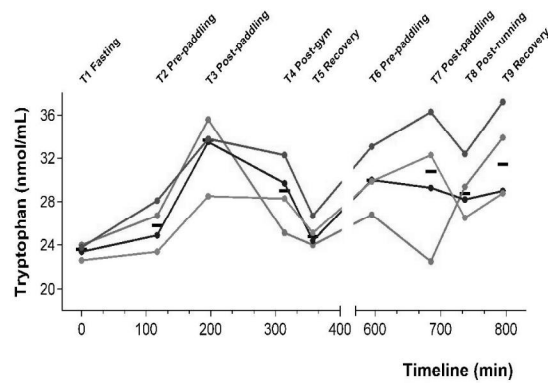
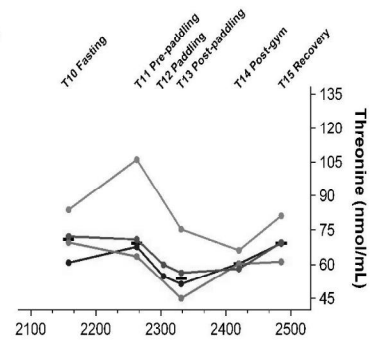
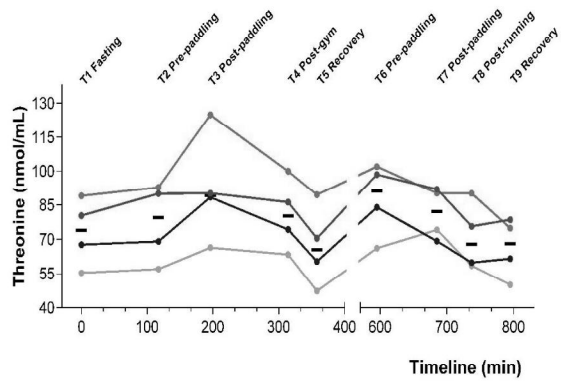
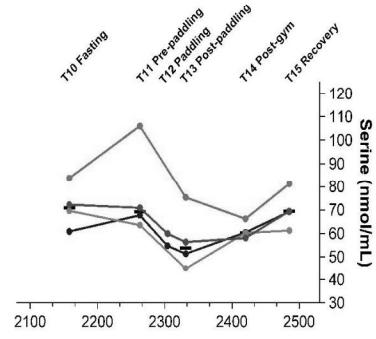
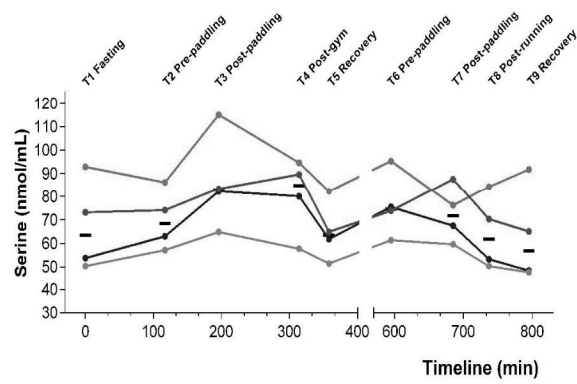


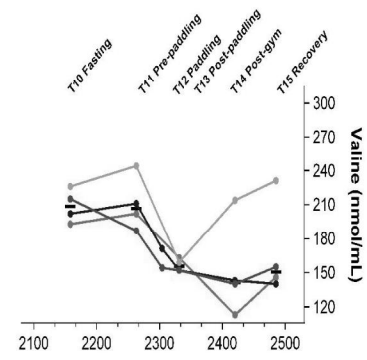
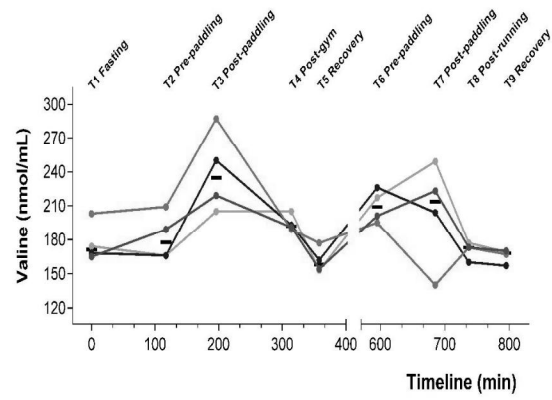
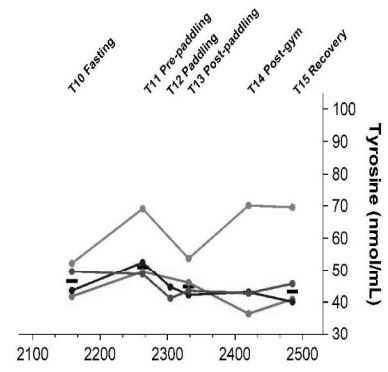
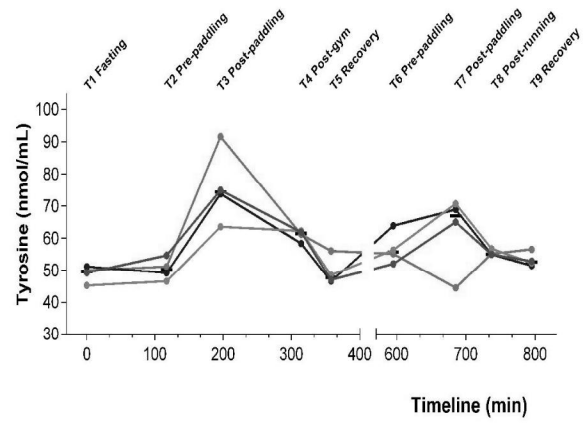




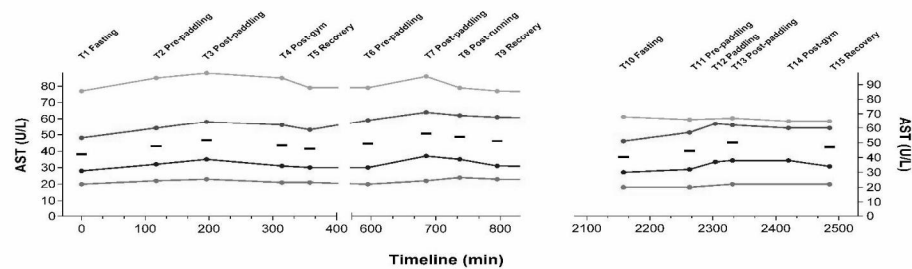
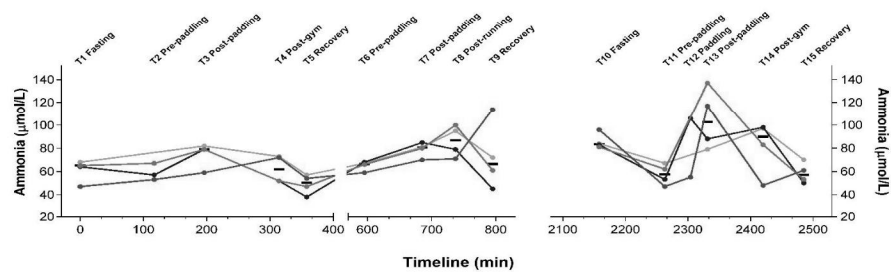
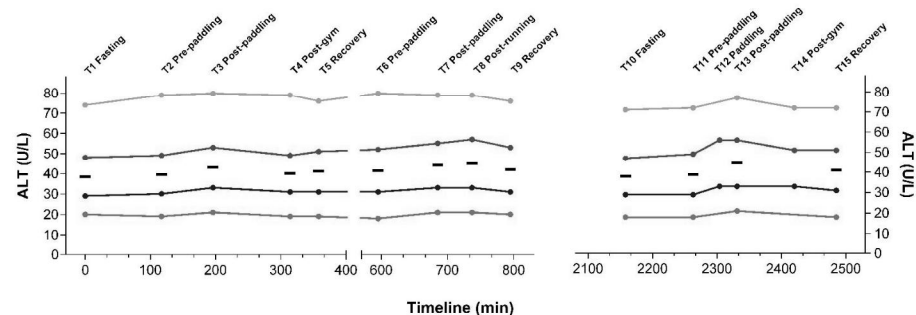


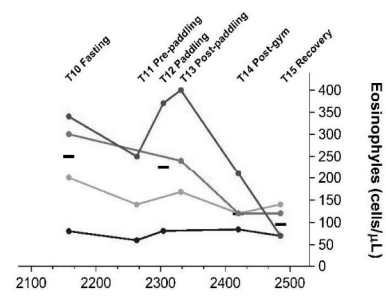
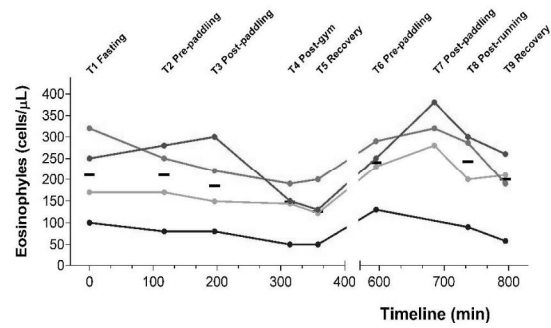
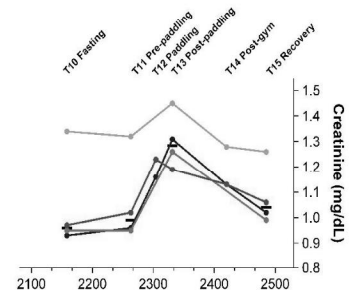
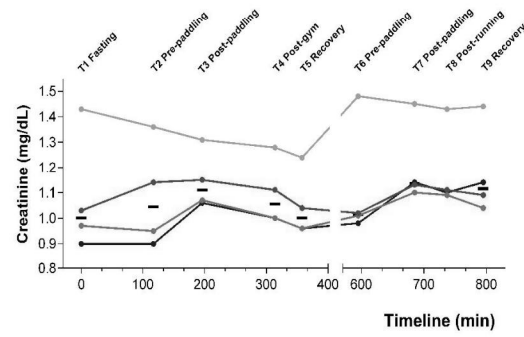
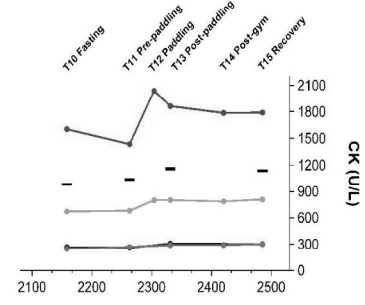
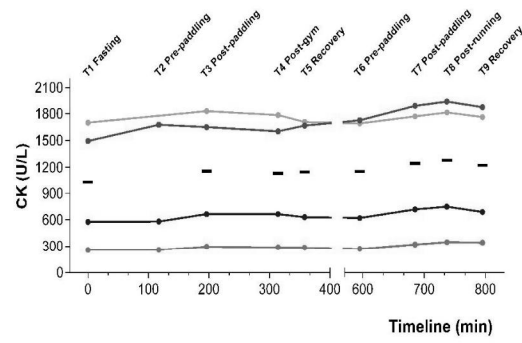


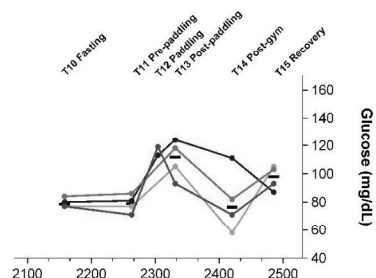
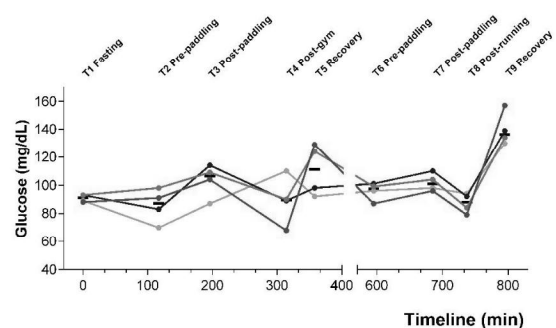
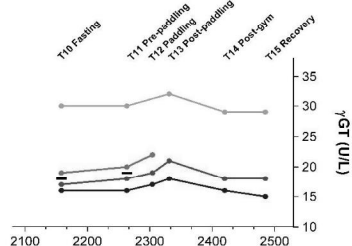
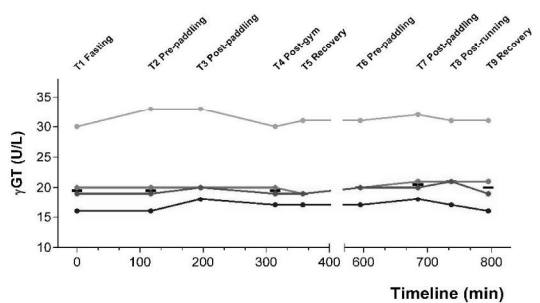
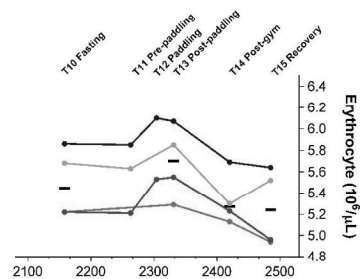
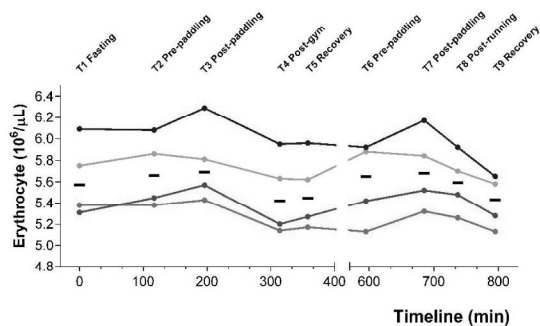


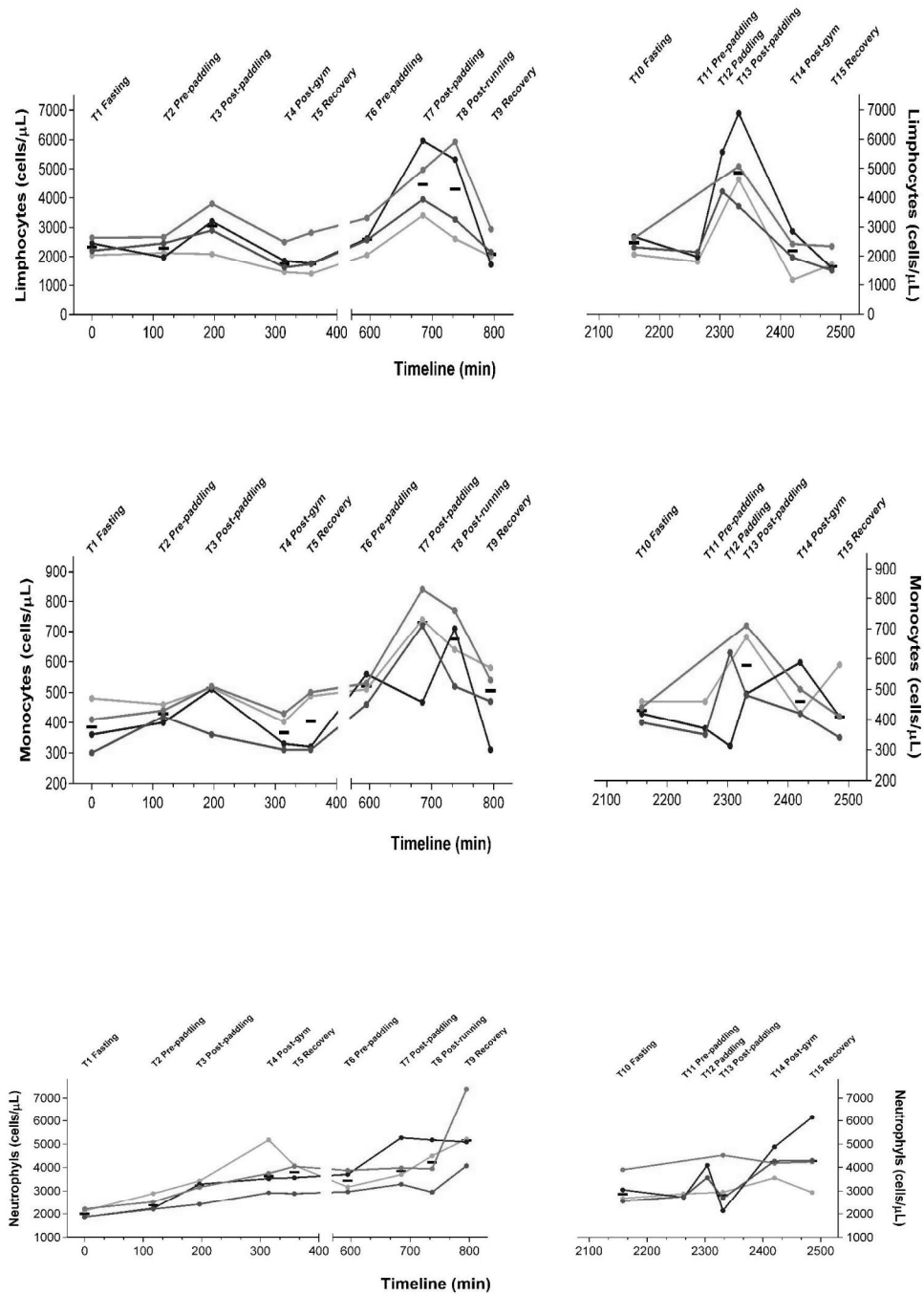


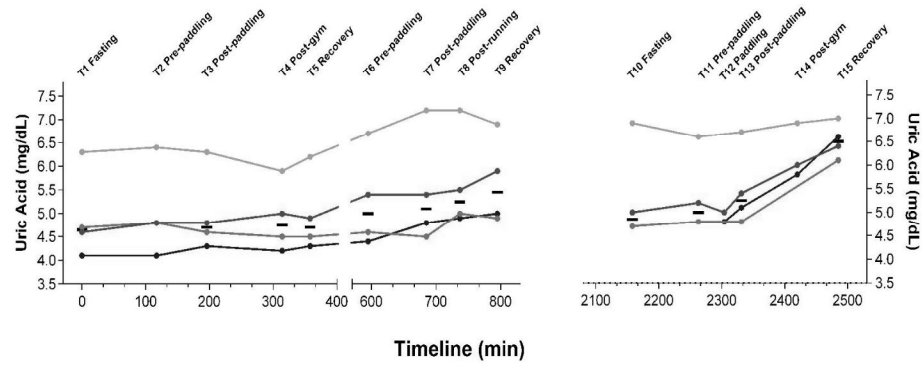
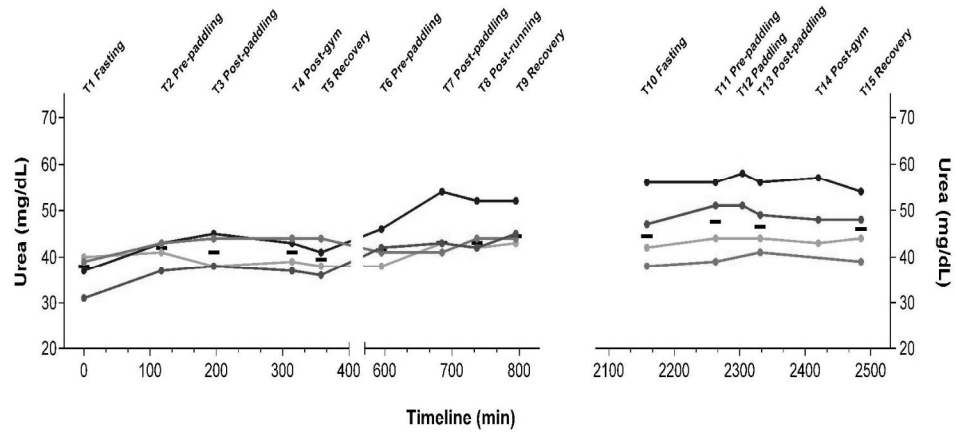
BLOOD CELLS, MUSCLE STRESS MARKERS, METABOLIC PATHWAYS SUBSTRATES, INTERMEDIATES AND PRODUCTS



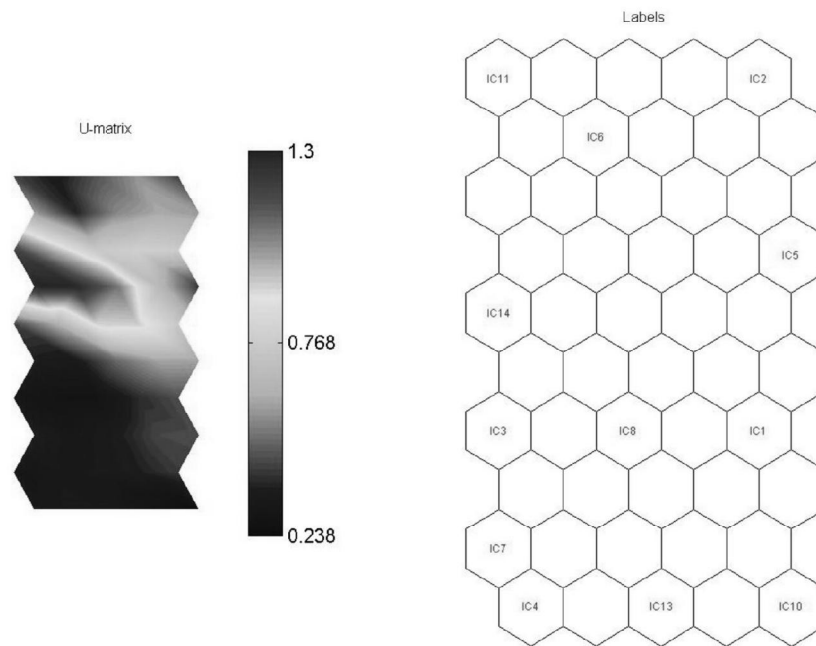


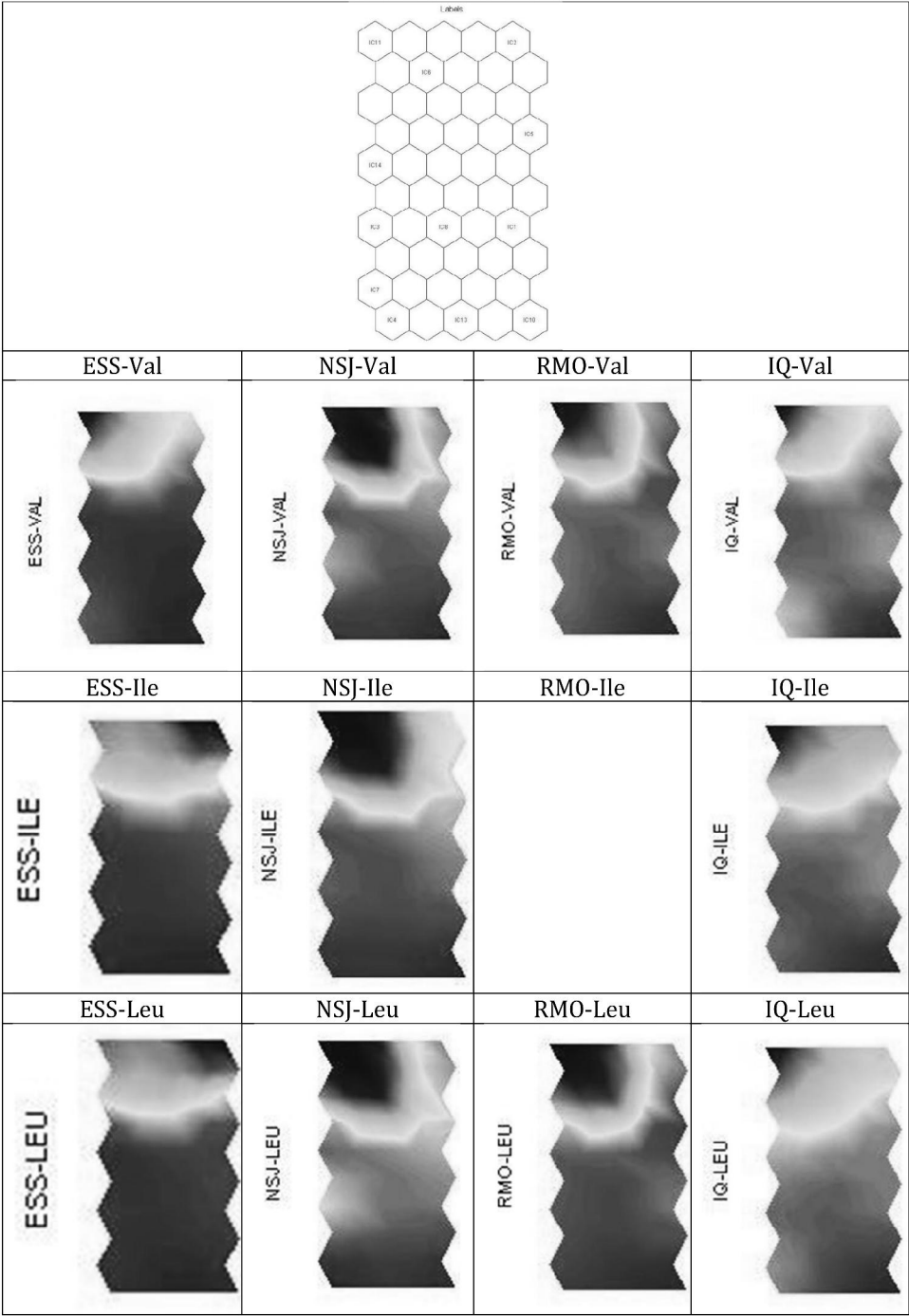


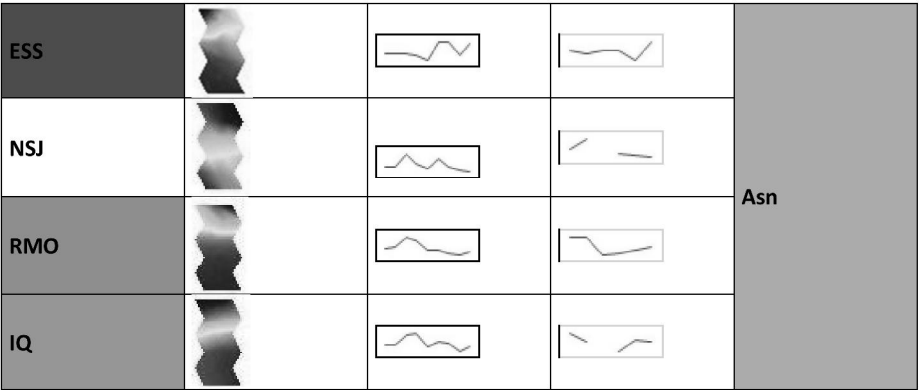
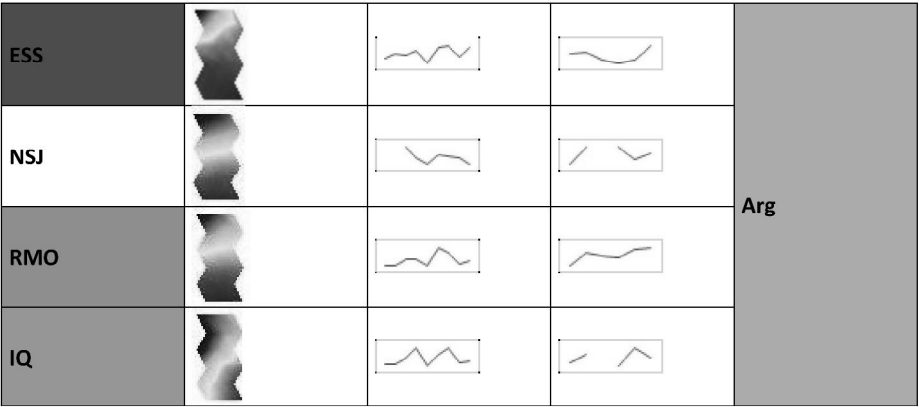
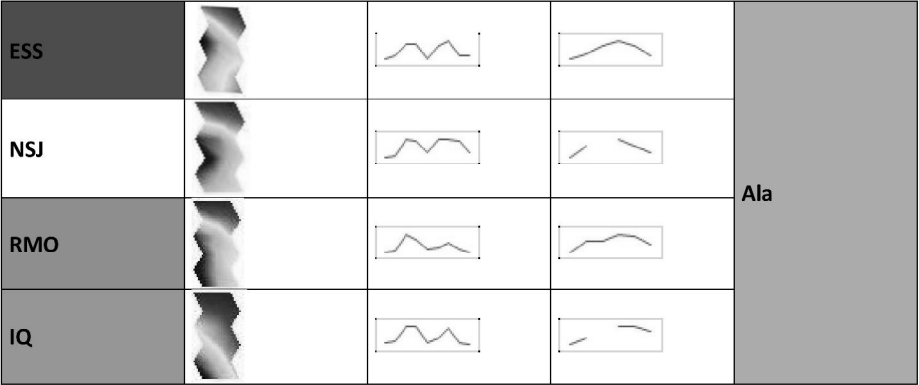


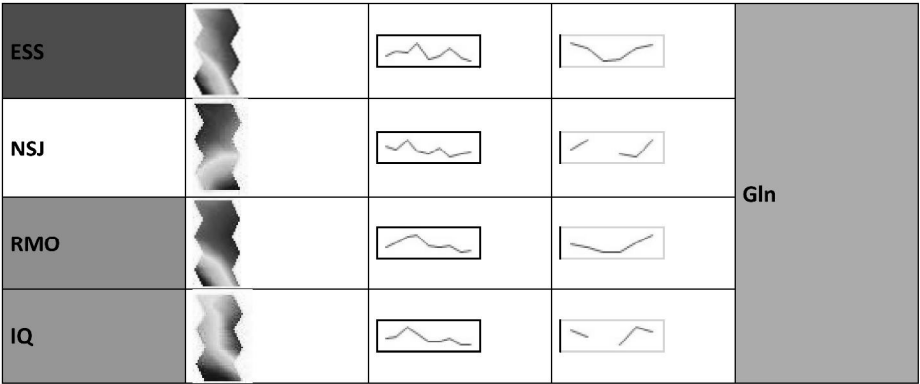
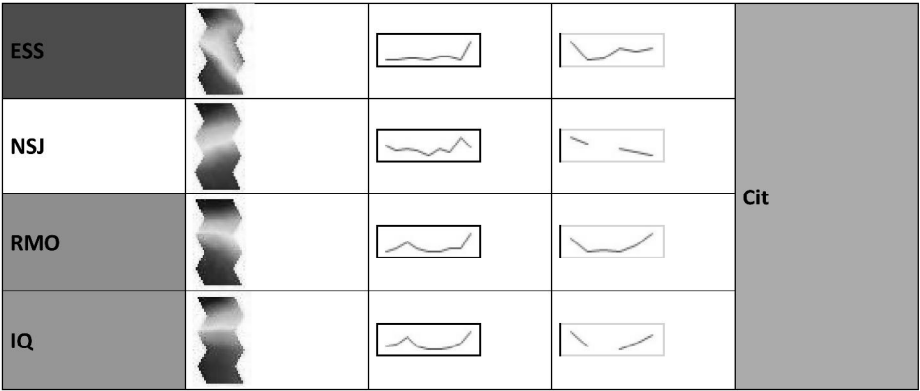
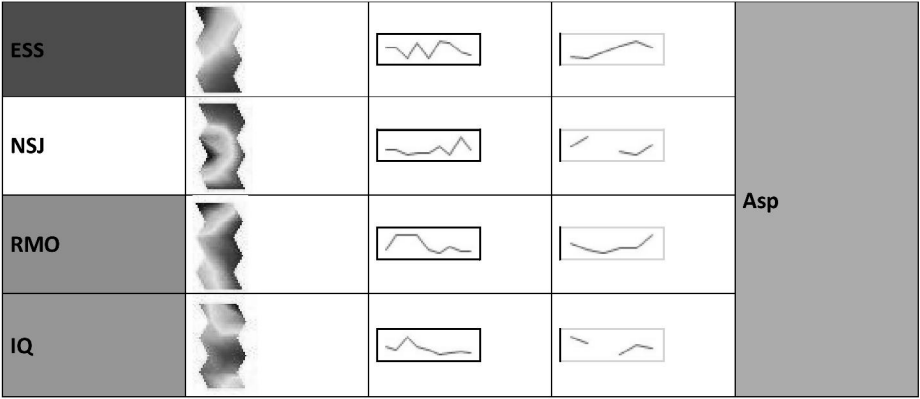


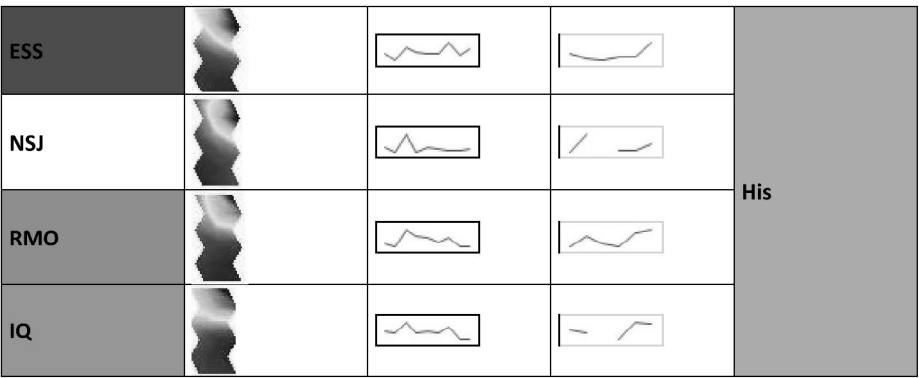
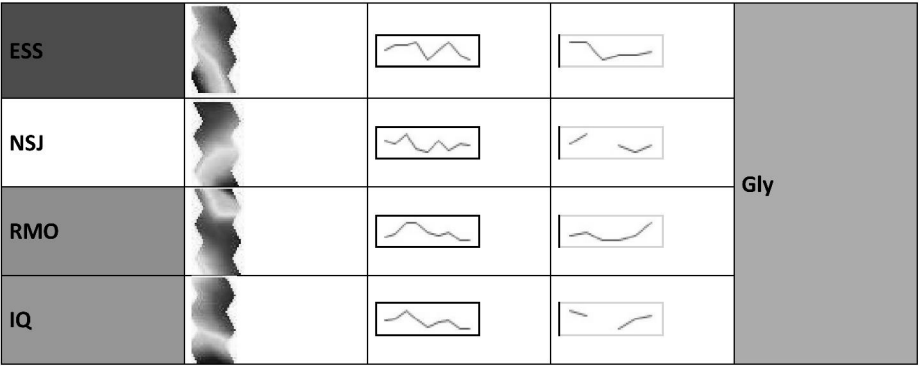
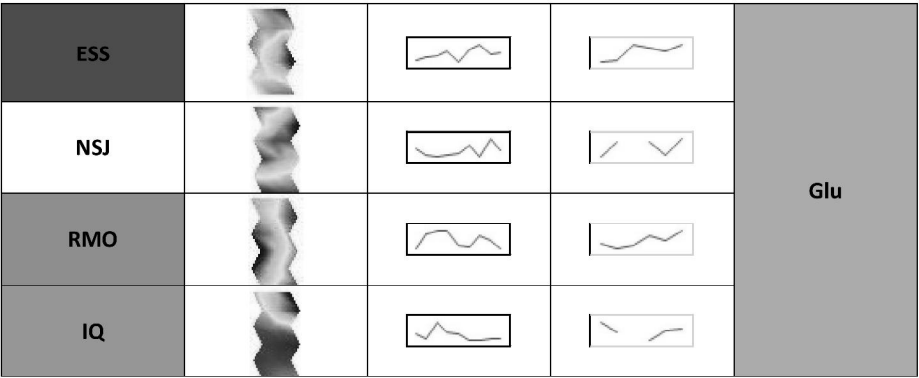
CLUSTERIZAÇÃO

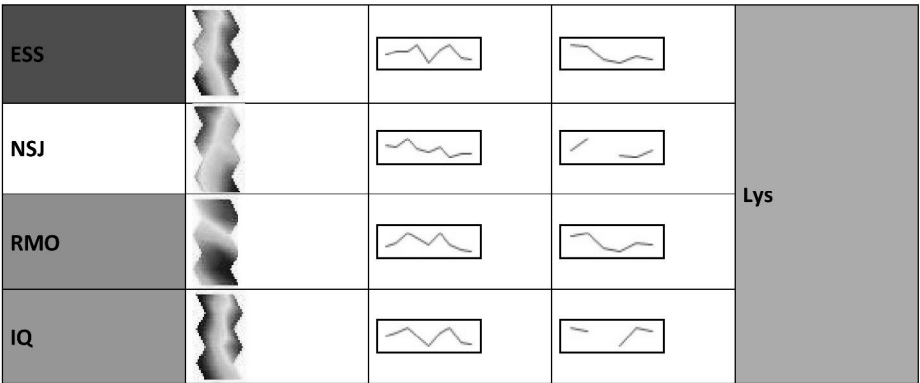
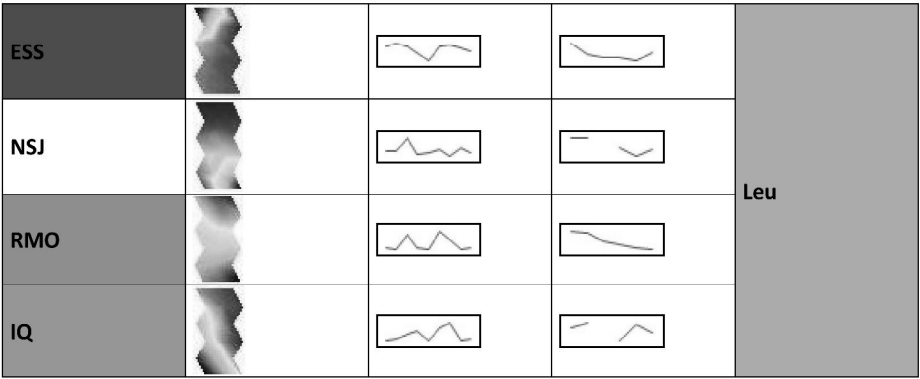
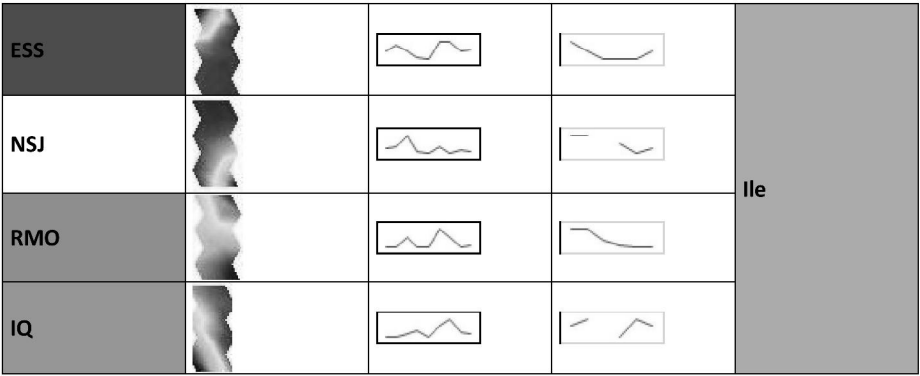









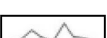

































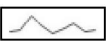
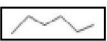





























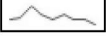


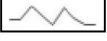

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