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FLÁVIO MÁRCIO MACEDO MENDES

ASSOCIAÇÃO ENTRE AMINOÁCIDOS/ACILCARNITINAS PLASMÁTICAS E O DESEMPENHO FÍSICO DE NADADORES ADOLESCENTES

Bragança Paulista
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Orientador: Prof. Dr. Leonardo Henrique Dalcheco Messias

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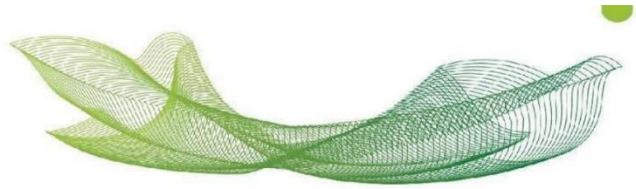

Educando para a paz

MENDES, Flávio Márcio Macedo. "Associação entre aminoácidos/acilcarnitinas plasmáticas e o desempenho físico de nadadores adolescentes**.** Tese defendida e aprovada no programa de Pós-Graduação *Stricto Sensu* em Ciências da Saúde da Universidade São Francisco em 19 de dezembro de 2022 pela Banca examinadora constituída pelos membros:

Prof. Dr. Leonardo Henrique Dalcheco Messias - Orientador e Presidente
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Universidade São Francisco

Prof. Dr. Pedro Paulo Menezes Scariot
Faculdade de Ciências Aplicadas

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RESUMO

Pesquisadores e fisiologistas do exercício estão constantemente buscando estabelecer e classificar fatores associados ao desempenho físico no esporte, sobretudo na natação, quanto as capacidades e potências aeróbia e anaeróbias, aspectos técnicos, psicológicos e abordagens nutricionais. Poucas informações estão disponíveis sobre metabólitos e biomarcadores relevantes ao desempenho físico em nadadores adolescentes. Este estudo associou as carnitinas e aminoácidos com o desempenho dos nadadores adolescentes em diferentes distâncias e à velocidade crítica (VC), verificando também a influência do sexo nos resultados. A esportômica é a ciência onde se avalia a metabolômica no esporte e para melhor entender as alterações metabólicas sofridas em jovens nadadores, desenvolvemos uma metodologia analítica em um espectrômetro de massas triplo quadrupolo da marca Waters® Xevo TQD associado ao cromatógrafo líquido da marca Shimatzu®, operado em electrospray positivo (ESI +). O método foi padronizado de forma individual e um canal de monitoramento (MRM) foi criado para cada carnitina e aminoácido. Com objetivo de analisar o maior número de AAs e carnitinas e relacionar com o desempenho dos jovens nadadores em diferentes distâncias, com a VC e mesmo sendo um projeto exploratório as informações correlacionadas podem nos elucidar sobre muitos fatores que ainda são pouco estudados. Foram selecionados 20 nadadores adolescentes do sexo masculino e dezoito do sexo feminino, todos competidores ativos de níveis regionais e nacionais. Cinco sessões experimentais foram realizadas pelos atletas. Onde no primeiro dia as amostras sanguíneas foram coletadas após jejum de 12 horas. Os ensaios de desempenho (100, 200, 400 e 800 m) foram realizados aleatoriamente nos quatro dias seguintes em uma piscina de 25 metros, e a VC foi determinada pela função matemática da distância linear versus tempo. O presente estudo verificou que a tirosina plasmática foi correlacionada com os desempenhos das nadadoras adolescentes nas distâncias de 100, 400 e 800 metros, onde avaliamos a diminuição da VC dessas atletas, ou seja, observamos um aumento no tempo para as atletas percorrem as distâncias. Outras combinações de testes e variáveis também podem ser aplicadas e analisadas por esta metodologia. A ciência ômica com as ferramentas analíticas padronizadas neste estudo contribui para a futuro do esporte.

Descritores: Metabolômica. Aminoácidos. Carnitinas. Desempenho. Metabolismo. Velocidade Crítica. Nadadores.

ABSTRACT

Researchers and exercise physiologists are constantly seeking to establish and classify factors associated with physical performance in sports, especially in swimming, regarding aerobic and anaerobic abilities and potencies, technical, psychological and nutritional approaches. Little information is available on metabolites and biomarkers relevant to physical performance in adolescent swimmers. This study associated carnitines and amino acids with the performance of adolescent swimmers at different distances and at critical velocity (CV), also verifying the influence of sex on the results. Sportsomics is the science where metabolomics is evaluated in sports and to better understand the metabolic alterations suffered in young swimmers, we developed an analytical methodology in a Waters® Xevo TQD triple quadrupole mass spectrometer associated with the Shimatzu®, liquid chromatograph, operated in positive electrospray (ESI +). The method was individually standardized and a monitoring channel (MRM) was created for each carnitine and amino acid. In order to analyze the greater number of AAs and carnitines and relate to the performance of young swimmers at different distances, with CV and even being an exploratory project, the correlated information can elucidate many factors that are still poorly studied. Twenty male and eighteen female adolescent swimmers were selected, all active competitors of regional and national levels. Five experimental sessions were performed by the athletes. Where on the first day blood samples were collected after fasting for 12 hours. The performance tests (100, 200, 400 and 800 m) were randomly performed in the following four days in a 25-meter pool, and the CV was determined by the mathematical function of the linear distance versus time. The present study verified that plasma tyrosine was correlated with the performances of adolescent swimmers at distances of 100, 400 and 800 meters, where we evaluated the decrease in CV of these athletes, that is, we observed an increase in time for athletes to travel the distances. Other combinations of tests and variables can also be applied and analyzed by this methodology. The omic science with analytical tools will certainly contribute a lot to the future of the sport.

Keywords: *Metabolomics. Amino Acids. Carnitines Performance. Metabolism. Critical Velocity. Swimmers.*

LISTA DE SIGLAS

CTA- capacidade de trabalho anaeróbico;

VC- Velocidade crítica;

ESI- electrospray;

MS- monitoramento scan

MS/MS- monitoramento scan sequencial

MRM- monitoramento de reações múltiplas;

API- ionização a pressão atmosférica;

AA- aminoácidos;

CLAE-EM/EM- cromatrografia de alta eficiência acoplada a espectrometria de massas sequencial

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SUMÁRIO

1. INTRODUÇÃO

A natação é uma técnica de locomoção na água. Como espécie terrestre, os humanos não são completamente adaptados aos ambientes aquáticos, ou pelo menos na mesma medida que outras espécies. Pode ser usada para fins de sobrevivência, lazer, recreação, saúde e esporte. É considerada um dos esportes mais completos para a saúde, pois trabalha o sistema respiratório e cardiovascular, auxiliando na redução de gordura corpórea. O desempenho na natação é multifatorial e medido pelo tempo gasto para percorrer uma determinada distância (BARBOSA et al., 2015).

Pesquisadores e fisiologistas do exercício estão constantemente buscando estabelecer e classificar fatores associados ao desempenho físico na natação, sobretudo quando das capacidades e potências aeróbia e anaeróbias, aspectos técnicos, psicológicos e abordagens nutricionais (LEMON et al., 1991). Estratégias para aprimoramento do desempenho contemplam grandes volumes de treinos semanais. Por conta dessas abordagens, a rotina de treinamentos para o atleta de natação é intensa durante seu macrociclo. Evidências sugerem que o volume de treinamento de natação está associado ao desempenho do atleta. Por esse motivo, todos os fatores atrelados ao aprimoramento físico do nadador devem ser analisados visando um melhor rendimento no esporte, sendo a ciência fundamental para esse fim (FEIJEN et al., 2020).

Outro fator relacionado a estratégias de treinamentos e desempenho físico é a idade, sobretudo a adolescência que é a fase da vida que se estende entre a infância e a idade adulta, e sua definição ainda é discutível, pois a puberdade prematura acelerou o início da adolescência em quase todas as populações, enquanto o crescimento contínuo retardou o seu final para casa dos vinte anos de acordo com (SAWYER et al., 2018). Atletas adolescentes requerem uma ingestão de macro e micro nutrientes que devem ser ajustados para atender às demandas de sua formação e desenvolvimento fisiológicos. Essa fase é caracterizada por um crescimento intenso e uma alta maturação biológica (LEE et al., 2019).

O início do desenvolvimento puberal é caracterizado pela rapidez nas mudanças físicas, no tamanho corporal, forma e composição (CHRISTIAN et al., 2018). No entanto, dismorfismos sexuais são evidenciados nas relações entre idade, crescimento e maturação sexual. Meninas normalmente iniciam um rápido crescimento linear com a primeiro sinal do desenvolvimento mamário (Estágio de mama de Tanner 2), e completam o ciclo de crescimento linear em torno dos

13 anos. Já os meninos geralmente atingem o pico da velocidade de crescimento linear mais tardiamente, em torno dos 14 anos. Essas mudanças físicas são acompanhadas em ambos os sexos por aumento das exigências energéticas (MENESES et al., 2008).

Nadadores adolescentes que participam de competições podem ser expostos a elevadas cargas de treinamento físico, exigindo assim, ajustes mais individualizados até mesmo em sua dieta para alcançar melhor desempenho e desenvolvimento de crescimento (SHAW et al., 2014). Além do dismorfismo sexual na composição corporal, a velocidade de crescimento e desenvolvimento puberal também podem fazer com que adolescentes do sexo masculino consumam mais alimentos do que o feminino (SAWYER et al., 2018).

Ferramentas para prescrever e monitorar treinamentos físicos a nadadores são imprescindíveis para alcançar maior desempenho. Uma importante ferramenta é a Velocidade Crítica, definida aqui como a velocidade máxima teórica que pode ser mantida sem exaustão por um longo período de tempo. Pode ser avaliado pela regressão linear determinada entre a distância e o tempo necessário para percorre-la. Com base nessa aplicação, um parâmetro associado ao metabolismo aeróbio com similar denominação ao protocolo (i.e VC) e outro anaeróbio descrito como capacidade de trabalho anaeróbio (CTA) são determinados (**FIGURA 1**). No entanto, poucas evidências científicas foram fornecidas sobre a validade fisiológica da CTA. A VC, por outro lado, permite é amplamente utilizada na natação, sobretudo por ser correlacionada a limiares fisiológicos (DEKERLE et al., 2002; TOUBEKIS et al., 2006). Nesse sentido, esse parâmetro tem sido utilizado como parâmetro para monitorar e avaliar o treinamento de nadadores, sobretudo por sua essência não invasiva e de fácil aplicação (NEIVA et al., 2011; TOUBEKIS et al., 2011).

Avanços tecnológicos em análises laboratoriais têm promovido novos horizontes dentro da ciência esportiva. Grupos de pesquisa vêm demonstrando e caracterizando cascatas intracelulares ativadas em detrimento de esforços agudos e, sobretudo, do treinamento físico, sendo essas respondíveis de forma específica ao estímulo fornecido (BARTLETT et al., 2014; BARR, 2014). Não obstante, a identificação de genes associados ao desempenho físico tem fornecido subsídio para técnicos e preparadores físicos priorizarem o treinamento de forma individualizada e refinada (WIDMANN et al., 2019).

Nesse contexto, aparte da análise genética e vias de sinalização intracelulares, a identificação de metabólitos séricos por meio da análise metabolômica tem ganhado notório espaço no âmbito

científico (DUFT et al., 2017; VALÉRIO et al., 2017). Especificamente na Ciência do Esporte, a aproximação entre essa análise e o contexto esportivo já possui considerável reconhecimento, sendo, inclusive, adotado o termo *Sportomics* (HEANEY et al., 2019). Estudos demonstram que o metaboloma sérico permite discriminar indivíduos com distintos níveis de treinamento (MORRIS et al., 2013). Não obstante, pesquisadores sugerem que o conjunto de metabólitos no líquido extracelular pode ser modulado via treinamento físico (BERTON et al., 2017), sendo esse um importante foco investigativo científico e indispensável ferramenta a ser considerada no âmbito prático.

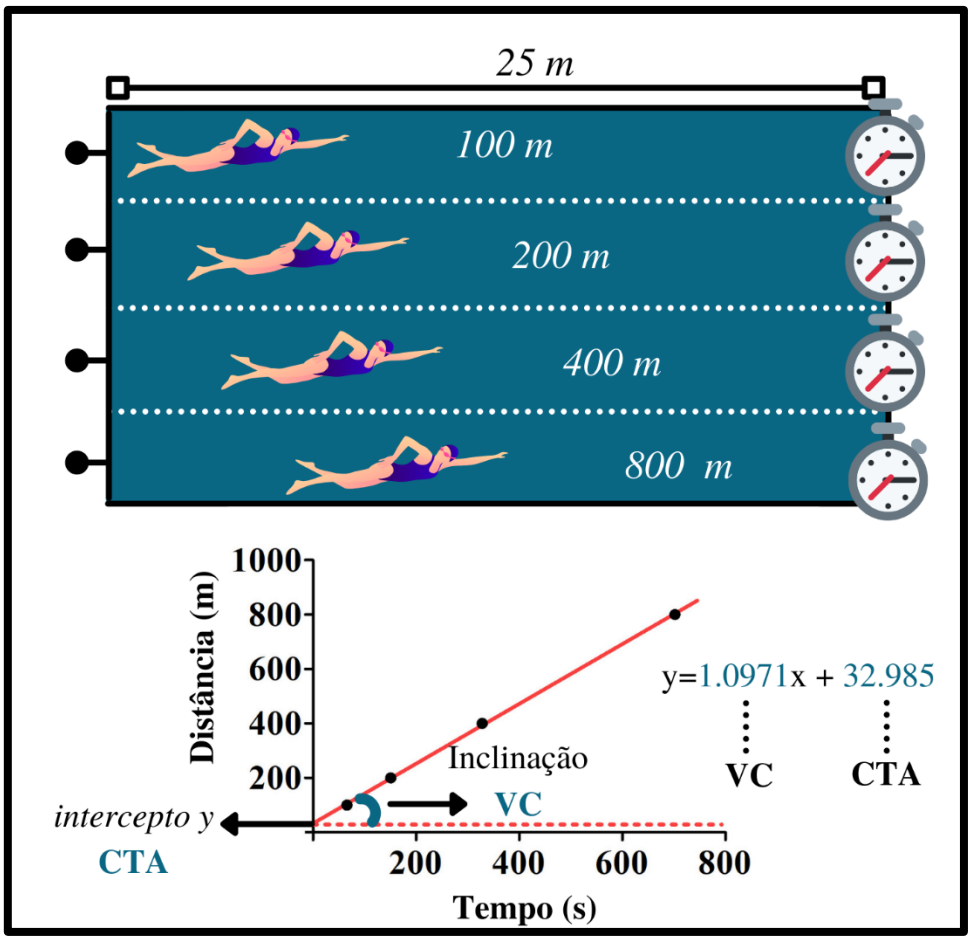

FIGURA 1. Protocolo de velocidade crítica. O painel superior exemplifica sua aplicação considerando as distâncias de 100, 200, 400 e 800 metros, sendo registrado o tempo para sua execução. O painel inferior destaca a regressão linear entre distância e o tempo. Nesse modelo matemático, a Velocidade Crítica (VC) é determinada pela inclinação da reta e a capacidade de trabalho anaeróbio (CTA) pelo intercepto no eixo y. Fonte: figura adaptada de Kraemer et al. (2022).

Várias ômicas estão sendo aplicadas no segmento esportivo, metabolômica, genômica, transcriptômica e proteômica, e uma ferramenta analítica que pode contribuir muito para o sucesso das ômicas, é a Técnica de cromatografia líquida acoplada a espectrometria de massas (CLAE-EM/EM) (**FIGURA 2**).

A cromatografia líquida é o método cromatográfico utilizado com mais frequência, onde misturas de compostos são empurrados com auxílio de bombas. A fase de arraste (fase móvel), é constituída por líquidos como água, acetonitrila, metanol, ácidos ou bases normalmente misturados com a função de ionizar e transportar os analitos por uma coluna recheada com micropartículas compactadas, por exemplo sílica, chamada de fase estacionária (coluna), responsável pela separação das misturas (HARRIS et al., 2005). A espectrometria de massas possui um detector com alta sensibilidade e seletividade e também uma boa resposta a um grande número de classes de compostos. Análises de compostos orgânicos já tem sido bastante utilizada pelas indústrias farmacêuticas para controle de qualidade, produto de degradação, bioequivalência e por laboratórios e centros de pesquisa utilizada para exames antidoping, toxicológicos, química forense e para análises de compostos em diversas matrizes (SKOOG et al., 2001). Associação entre as duas técnicas se deu pelo desenvolvimento da ionização a pressão atmosférica como a ESI e API que consiste na ionização em solução, separação de cargas e transferência de gotas carregadas para fase gasosa, o que possibilitou tal acoplamento. Uma grande vantagem dessa técnica é a simplicidade da preparação das amostras.

Em se tratando de nadadores adolescentes, existe pouca relação com a perspectiva *Sportomics*. Enquanto Couto et al. (2017) verificaram que metabólitos associados a marcadores de estresse oxidativo diminuíram após uma sessão de treinamento na natação, Cai et al. (2022) identificaram potenciais metabólitos séricos para discriminação do nível atlético de nadadores chineses.

Considerando o intenso desenvolvimento fisiológico e matural durante o período da adolescência, a ingestão adequada de macro e micro nutrientes deve ser ajustada para atender as necessidades do atleta jovem (CHRISTIAN et al., 2018; LEE et al., 2019). Considerando que nadadores adolescentes podem ser expostos a grandes volumes de treinamento físico, ajustes em sua dieta são necessários para obter melhor desempenho físico e maturação fisiológica (SHAW et

al., 2014). Embora a aproximação entre análises ômicas e aspectos nutricionais seja de extrema valia no âmbito científico, nenhum estudo com essa essência foi publicado até o momento.

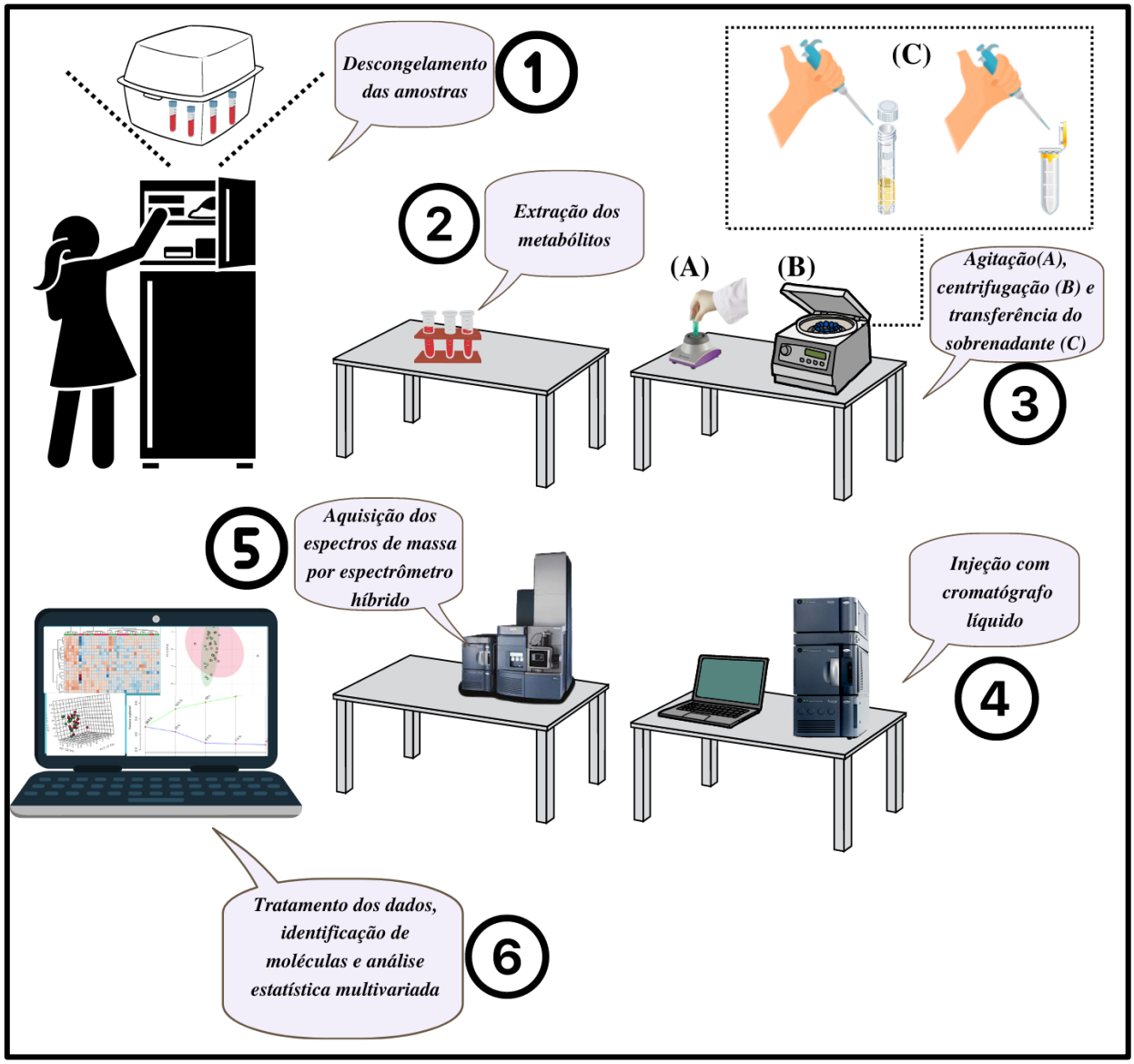

FIGURA 2. Etapas associadas a análise metabolômica considerando o plasma como matriz orgânica.

Dentro do contexto esportivo, a relevância das carnitinas e aminoácidos (AA) no desempenho físico ganharam atenção ao longo dos anos (KREIDER et al., 1993). Carnitina é um derivado de aminoácidos amplamente conhecido por seu envolvimento no transporte de ácidos graxos de cadeia longa na matriz mitocondrial. Seu nível plasmático é mantido principalmente por produtos alimentícios de origem animal e, em menor grau, por biossíntese endógena no fígado e rim. O

músculo humano contém altas quantidades de carnitinas e depende da absorção deste composto da corrente sanguínea, devido à incapacidade muscular de sintetizar esse composto. Oxidação de ácido graxo mitocondrial representa uma importante fonte de energia para metabolismo muscular particularmente durante o exercício físico (**FIGURA 3**). O teor de carnitina no corpo é de ~300 mg/kg e é distribuído principalmente no coração e nos tecidos musculares esqueléticos, enquanto apenas 0,5% são encontrados no plasma (FLANAGAN et al., 2010).

Embora suas funções em metabolismos intermediários tenham sido bem documentadas, principalmente no que se refere à oxidação de ácidos graxos musculares e à homeostase glicemia, não há evidências científicas que sustentem que a suplementação de carnitina melhore o desempenho físico (GNONI et al., 2020). Por outro lado, os AA foram amplamente discutidos no cenário ergogênico. Frente a ingestão proteica (**FIGURA 4**), as funções dos AA na síntese proteica (**FIGURA 5**), aumento no volume muscular e relacionada à saúde são bem estabelecidas, embora uma meta-análise recente tenha observado efeitos mínimos sobre os resultados de desempenho físico de indivíduos saudáveis. O aumento da quantidade relativa de proteína na dieta dos atletas tem sido sugerido para otimizar processos anabólicos e melhoram as respostas fisiológicas ao treinamento e ao desempenho (LEMON et al., 1991).

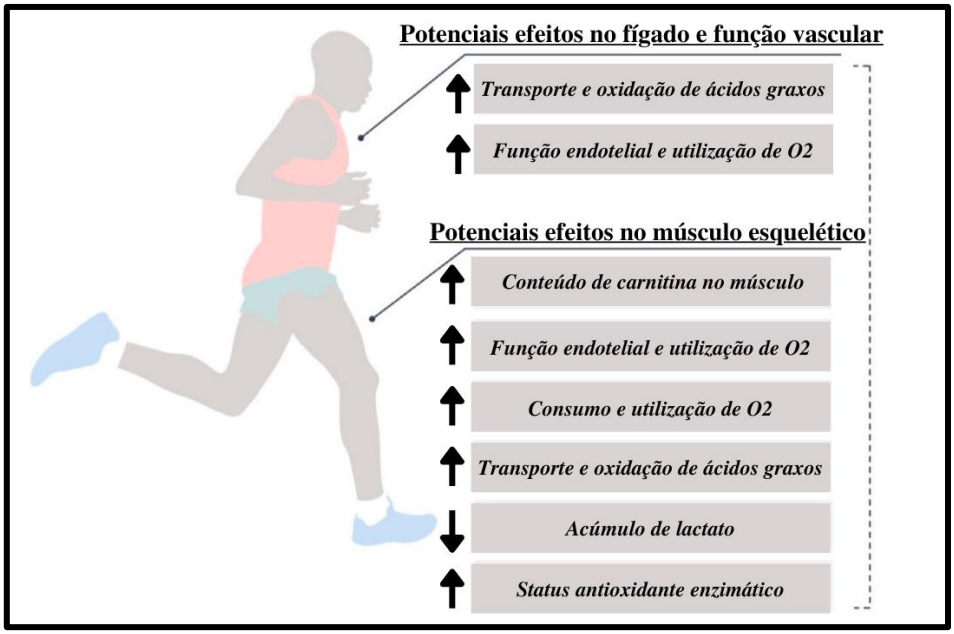

FIGURA 3. Potenciais efeitos da suplementação oral de carnitina no fígado, sistema vascular e músculo estriado esquelético. Fonte: figura adaptada de Ayuso et al. (2021).

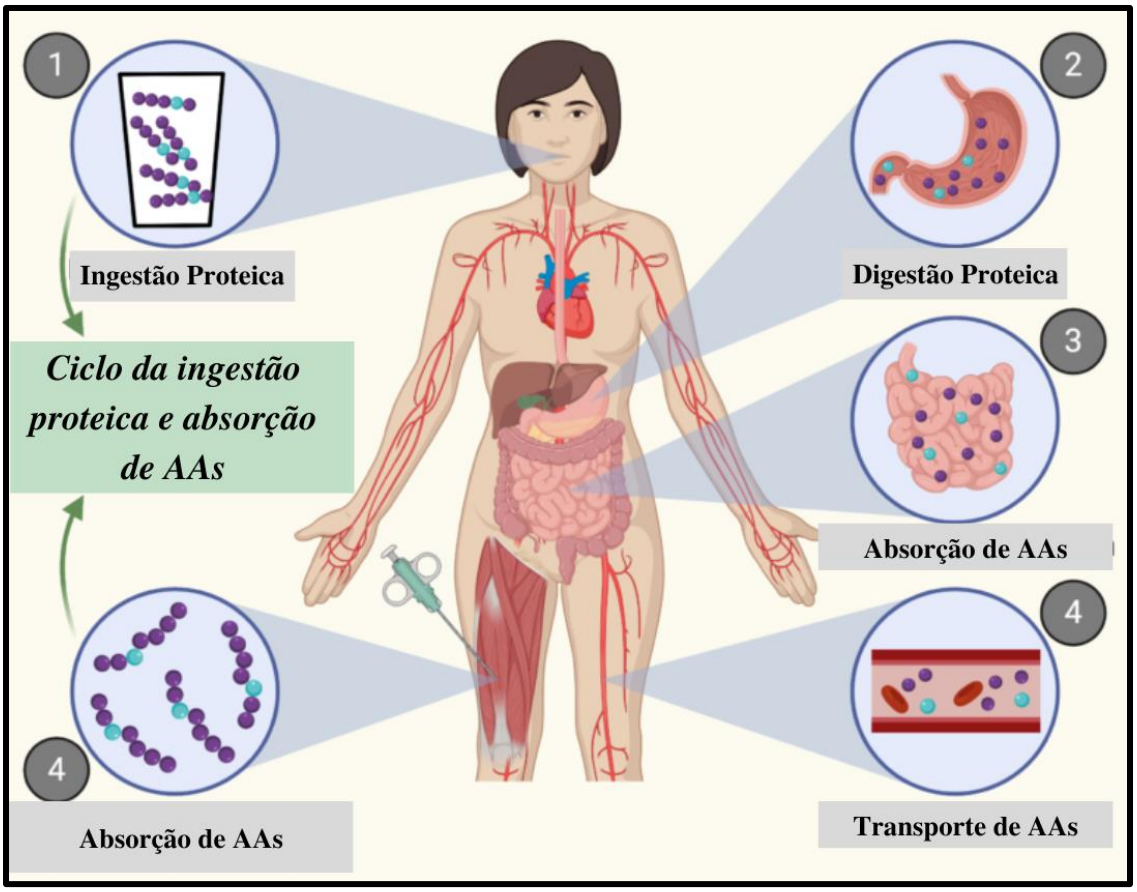

FIGURA 4. Ciclo que envolve a ingesta proteica e absorção de aminoácidos (AA) pelo organismo. Fonte: figura adaptada de Trommelen et al. (2020).

Recentemente, os AA tornaram-se um suplemento nutricional popular comercializado para atletas. Em atletas de força, sua suplementação foi proposta para aumentar a disponibilidade de AA essenciais, melhorar processos anabólicos promovendo desenvolvimento tecidual, e acelerar a taxa de recuperação durante o treinamento. Em atletas de resistência, a suplementação de AA tem sido proposta para melhorar as respostas fisiológicas e psicológicas durante o exercício de resistência (NUNES et al., 2022).

Embora evidências apontem para associações entre carnitinas/AA com o desempenho físico, ferramentas analíticas que reportem a intensidade desses metabólitos e permitam essas inferências estão em trâmite de desenvolvimento. Nesse cenário, inserir a perspectiva *Sportomics* emerge como uma importante possibilidade para aplicação de tecnologias globais. Para tanto, torna-se necessário padronizar técnicas laboratoriais como a cromatografia liquida associada a espectrometria de massas. Um detector triplo quadrupolo pode ser utilizado para análises de carnitinas e AA em

matrizes biológicas, a exemplo do plasma. O início do desenvolvimento do método é através da confirmação de massa teórica utilizando a função de MS, executando uma varredura em uma faixa de massas que compreenda a massa nominal. A segunda etapa do desenvolvimento compreende a obtenção do espectro de MS/MS. Com a massa do "íon Precursor" selecionada inicia a fragmentação da molécula e uma nova varredura de massas a fim de selecionar os fragmentos mais intensos com objetivo de selecionarmos o íon precursor e em seguida seu fragmento é obter a função MRM, obtendo assim uma análise seletiva e individualizada (HARRIS et al., 2005).

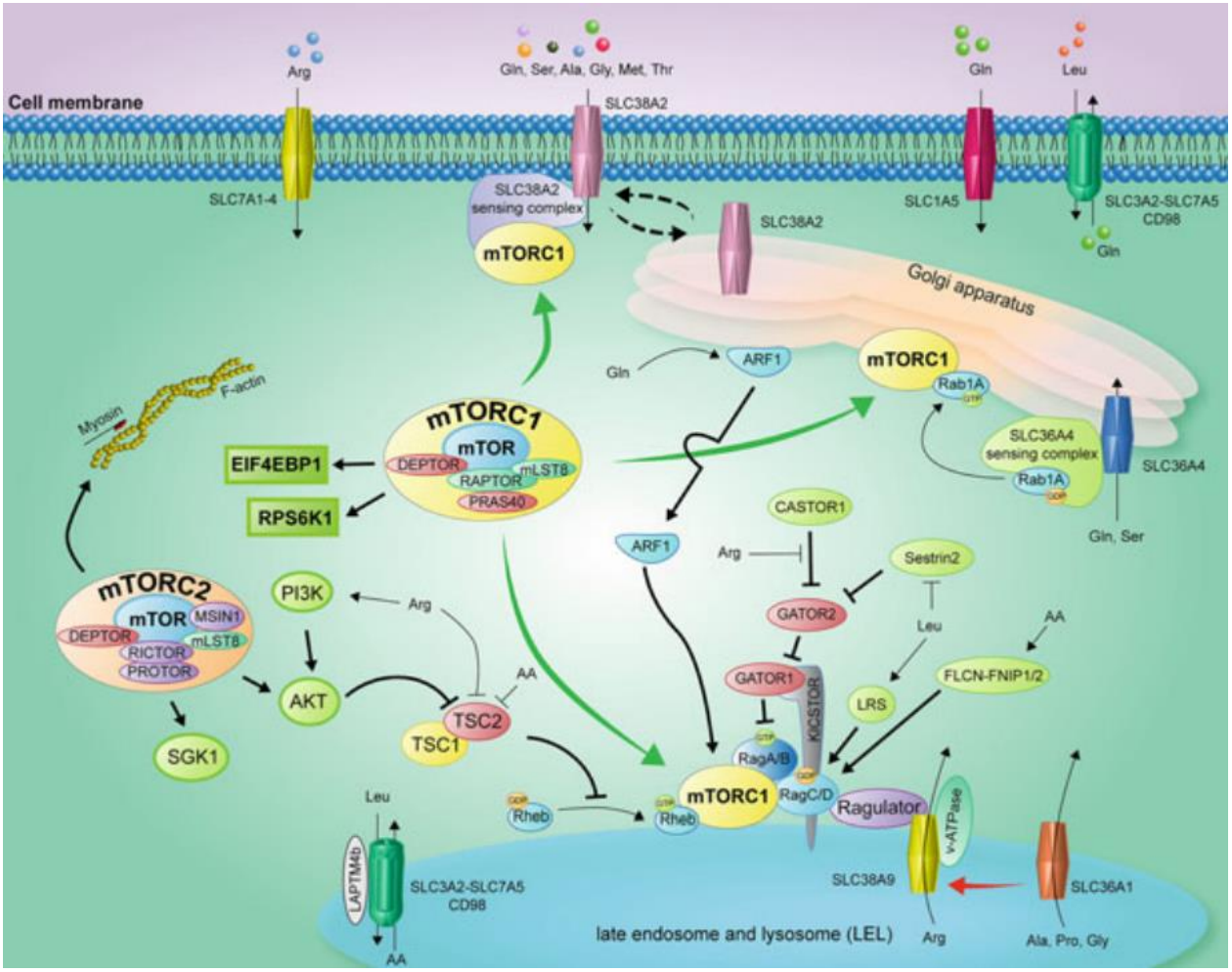

FIGURA 5. Atuação de alguns aminoácidos na síntese proteica, aqui sinalizada pelo *mammalian target of rapamycin.* Fonte: figura adaptada de Wu et al. (2019)

Essa perspectiva permitirá verificar a relação entre carnitinas/AAs plasmáticos de forma e o desempenho de nadadores adolescentes em diferentes distâncias bem como a VC, podendo acrescentar a ciência esportiva por abrir novas portas para estudos randomizados (**FIGURA 6**).

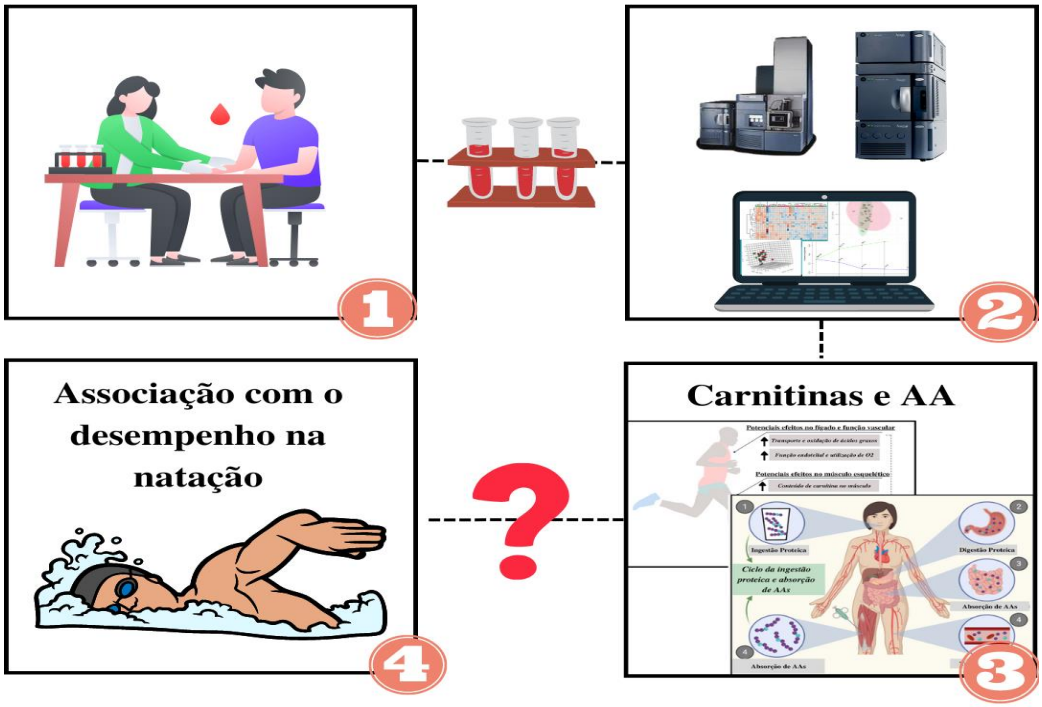

FIGURA 6. Justificativa do presente estudo. Com base na coleta sanguínea e separação do plasma (1), a cromatografia líquida acoplada à espectrometria de massas (2) permite identificar carnitinas e aminoácidos (AA) presentes nessa matriz, possibilitando a investigação sobre a associação dessas moléculas com o desempenho físico de nadadores adolescentes.

2. OBJETIVOS

2. 1. Objetivo geral

Verificar, com base na cromatografia líquida acoplada à espectrometria de massas, quais carnitinas e AA são associados com o desempenho físico de nadadores adolescentes em diferentes distâncias, bem como a VC.

2. 2. Objetivos Específicos

- Desenvolver uma técnica para análise, via cromatografia líquida acoplada à espectrometria de massas, das carnitinas e AA plasmáticos;

- Aplicar o protocolo de velocidade crítica com base nas distâncias de 100, 200, 400 e 800 metros, utilizando a VC como parâmetro a ser associado com as carnitinas e AA plasmáticos;

- Analisar se o sexo impacta nas comparações e associações sugeridas no presente estudo.

3. CAPÍTULO

MENDES, F.M.M.; SANCHES, P.H.G.; SILVA, Á.A.; REIS, I.G.M.; CARVALHO, P.D.O.; PORCARI, A.M.; MESSIAS, L.H.D. Plasma Amino Acids and Acylcarnitines Are Associated with the Female but Not Male Adolescent Swimmer's Performance: An Integration between Mass Spectrometry and Complex Network Approaches. **Biology.,** v. 11, n. 12, p. 1734, 2022.

O artigo original denominado "Plasma amino acids and acylcarnitines are associated with the female but not male adolescent swimmer's performance: an integration between mass spectrometry and complex network approaches" foi publicado no periódico Biology, o qual possui fator de impacto em 5168 e figura no quartil 1 (Biologia). O estudo apresenta os principais resultados obtidos nesta tese. Verificamos que a tirosina plasmática foi correlacionada com os desempenhos das nadadoras adolescentes nas distâncias de 100, 400 e 800 metros, onde avaliamos a diminuição da VC dessas atletas, ou seja, observamos um aumento no tempo para as atletas percorrem as distâncias.

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Plasma Amino Acids and Acylcarnitines Are Associated with the Female but Not Male Adolescent Swimmer's Performance: An Integration between Mass Spectrometry and Complex Network Approaches

Flávio Marcio Macedo Mendes [1,2], Pedro Henrique Godoy Sanches [2], Álex Ap. Rosini Silva [2], Ivan Gustavo Masselli dos Reis [1], Patrícia de Oliveira Carvalho [2], Andréia M. Porcari [2] and Leonardo Henrique Dalcheco Messias [1,*]

[1] Research Group on Technology Applied to Exercise Physiology—GTAFE, Health Sciences Postgraduate Program, São Francisco University, Bragança Paulista 12916-900, SP, Brazil

[2] MS[4]Life Laboratory of Mass Spectrometry, Health Sciences Postgraduate Program, São Francisco University, Bragança Paulista 12916-900, SP, Brazil

* Correspondence: leonardo.messias@usf.edu.br

Simple Summary: Adolescent swimmers perform a lot of physical training with the aim of improving their performance in the sport. To achieve this goal, their diet must be adequate. In this scenario, studies have suggested that amino acids and acylcarnitines can improve the physical performance of athletes. However, we still do not have information as to whether the same occurs for adolescent swimmers. Thus, we compared amino acids and acylcarnitines present in the blood of adolescent swimmers of both sexes. We also correlated these substances with the performance of athletes. Our results showed that among amino acids and acylcarnitines, only tyrosine was lower in female adolescent swimmers when compared to male athletes. Additionally, significant associations between the female swimmers with tyrosine were observed. Future studies are necessary to understand this relationship, offering new possibilities for nutrition applied to the performance of adolescent swimmers.

Abstract: The main aim of this study was to compare the performance over different distances, the critical velocity (CV), and plasma acylcarnitines/amino acids of male and female adolescent swimmers. Moreover, we applied the complex network approach to identify which molecules are associated with athletes' performances. On the first day under a controlled environment, blood samples were collected after 12 h of overnight fasting. Performance trials (100, 200, 400, and 800-m) were randomly performed in the subsequent four days in a swimming pool, and CV was determined by linear distance versus time mathematical function. Metabolomic analyses were carried out on a triple quadrupole mass spectrometer performing electrospray ionization in the positive ionization mode. No difference was observed between the performance of male and female swimmers. Except for 200-m distance (p = 0.08), plasma tyrosine was positively and significantly associated with the female times during the trials (100-m, p = 0.04; 400-m, p = 0.04; 800-m, p = 0.02), and inversely associated with the CV (p = 0.02). The complex network approach showed that glycine (0.406), glutamine (0.400), arginine (0.335), free carnitine (0.355), tryptophan (0.289), and histidine (0.271) were the most influential nodes to reach tyrosine. These results revealed a thread that must be explored in further randomized/controlled designs, improving the knowledge surrounding nutrition and the performance of adolescent swimmers.

Keywords: metabolomics; athletic performance; metabolism; critical velocity; swimming

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1. Introduction

Intense biological maturation and growth are characteristics of adolescence [1]. The physiological development during this period requires adequate macronutrient and micronutrient intake [2,3], which must be adjusted to meet the teenage athlete's training and competition demands [4]. In an exercise-nutrition context, the science surrounding nutritional recommendations for the adolescent swimmer is present. While carbohydrates of high glycemic index of 1–1.2 g per kilogram of body mass per hour should be consumedafter training, the protein supply during the same period must reach 1.2 g per kilogram of body mass per hour; regarding the daily fat intake, 2 g per kilogram of lean body mass are recommended [5]. However, competitive teenage swimmers can be exposed to mas-sive physical training volumes [6], requiring adjustments in their diet to achieve better performance and growth development [7].

Beyond exercise demands, sexual dimorphisms for body composition, peak growth velocity, and pubertal development can cause male adolescents to consume more food than females [8]. In swimming, sex differences in body composition, energy, and kinematic variables of adolescent athletes have been described [9–12], but the specific nutritional patterns between male and female teenage swimmers and their influence on performance remain to be addressed. Studies within this population are limited to muscle protein metabolism [13], amino acid intake [14], and seasonal variations in training loads on selected amino acids [15]. Additionally, Hsueh [16] concluded that branched-chain amino acids (BCAA), arginine, and citrulline, improved adolescent swimming performances in ahigh-intensity interval protocol. Despite these advances, further cross-sectional studies are required to shed light on adolescent swimmers' sex-specific nutritional patterns and their influence on performance.

In this context, acylcarnitines and amino acids have gained attention over recent years [17,18]. Carnitine content in the body is ~300 $mg.kg^{-1}$ and is mostly distributed inthe heart and skeletal muscle tissues, while only 0.5% is found in plasma [19]. Althoughits functions in intermediate metabolisms have been well documented, mainly regardingmuscle fatty acid oxidation and glucose homeostasis [20], there is no scientific evidence to support that carnitine supplementation improves physical performance [17]. On the other hand, amino acids have been largely discussed in the ergogenic scenario [21–24]. Their role in muscle mass and health-related function is well established [25], although a recent meta-analysis observed minimal effects on the physical performance outcomes of healthy subjects [26].

To properly analyze the impact of physical performance on the acylcarnitines and amino acids plasma abundance, blinded, randomized, and controlled studies are necessary. However, without initial and cross-sectional reports, the wide range of possibilities can overshadow and delay scientific advances. The sportomics approach can advance the matter, since it has elucidated modulations generated by physical efforts in athletes [27–30]. Within a small biological matrix (e.g., blood, urine, tissue), thousands of molecules can be accurately identified and used to understand acute and chronic exercise effects [31–33]. Despite its application in cross-combat [34], soccer [35], and canoeing [36], the identification of metabolites by this method and its relationship with swimming performance is still atan embryonic stage.

Therefore, the main aim of this study was to compare swimming performances over four distances (100, 200, 400, and 800-m) and the critical velocity (CV) [37–40], using plasma acylcarnitines/amino acids from male and female adolescent athletes. Given the distinct pubertal development between male and female adolescents and their particular nutritional patterns, we hypothesized that significative differences in plasma acylcarnitines/amino acids would be observed between the athletes, which in turn could possibly be related to physical performance.

2. Materials and Methods

2.1 Subjects

Twenty male and eighteen female adolescent swimmers from regional levels were evaluated. The athletes had a daily training volume of 120 min a week. All swimmers had competed in regional and national competitions over the last two years. The Fédération Internationale de Natation points (i.e., FINA points) were calculated based on the period 01.09.2021–31.08.2022 and were obtained for males at 100-m=214±29, 200-m=201±21, 400-m=205±29, and 800-m=202±27, and for females at 100-m=222±16, 200-m=212±14, 400-m=220±15, and 800-m=214±16. Evaluations were conducted during the general preparatory period according to their training periodization. Informed consent was obtained from athletes and a parent and/or legal guardian. The experiments were approved by the Research Ethics Committee of the São Francisco University (24892219.3.0000.5514) and were conducted in agreement with the ethical recommendations of the Declaration of Helsinki.

2.2 Experimental Design

Swimmers were instructed to keep the same individual hydration/food habits through-out the experiment. No athlete reported the use of nutritional or ergogenic supplements.The swimmers completed five experimental sessions without performing any training sessions during the period. On the first day, under a controlled environment (laboratory facility), blood samples were collected after 12 h overnight fasting, and anthropometric mea-surements were performed in sequence. The performance trials were randomly performed in the subsequent four days in a swimming pool (25-m), 24 h apart. Efforts were initiatedat the same hour (2:00 p.m.), and the exact order to dive off the block was maintained (Figure 1).

2.3 Metabolomic Analysis

The swimmers' plasma was collected in a tube containing EDTA and stored in a freezer at 80 °C until analysis. The sample preparation followed the study of Sarafian [41]. All samples were thawed at room temperature and randomized before extraction to avoid analytical bias. A pooled sample was formed before sample extraction from equal parts of each sample (25 µL) and then aliquoted into different quality control (QC) samples, extracted with the swimmers' samples. Plasma samples (150 µL) were randomized and cold isopropanol (200 µL) was added, followed by vortexing (30 seg) and centrifugation (12,000 rpm, 4 °C, 10 min). Then, the supernatant (200 µL) was collected and dried in N2.Blank samples were prepared using ultra-pure water instead of plasma. QC samples weredistributed every 10 injections for instrumental monitoring, resulting in 10 QC samples for system suitability and 5 QC samples for intra-batch monitoring.

2.4 Tandem MS Analysis

Data acquisition was carried out on a Waters® Xevo TQD triple quadrupole mass spectrometer (Waters Corporation, Milford, CT, USA) equipped with a Shimadzu® SCL-10A controller, a Shimadzu® LC-20AD pump controller, and a Shimadzu® SIL-20A automatic sampler injector (Shimadzu Corporation, Kyoto, Japan). The procedures were adapted from Cicalini [42]. The analysis was performed using Flow Injection Analysis (FIA), with no chromatographic separation, using 10 µL as the injection volume. A flow gradient ranging from 0.01–0.50 mL/min was applied from 0.1 to 0.51 min and then kept for 3 min, after which the flow rate was decreased to 0.1 mL/min, resulting in 4 min of run time. The mobile phase was composed of water:acetonitrile:formic acid (80:20:0.1, *v/v/v*). The electrospray ionization source was operated in the positive ionization mode (ESI+) withthe following parameters: desolvation gas flow of 850 L/h; source temperature of 150 °C;desolvation temperature of 500 °C; voltages of capillary and cone set at 3.0 kV and 60.0 V,respectively. The instrument was operated using the multiple reaction monitoring (MRM)mode, and the precursor > fragment transitions were optimized for each of the analyzed compounds (amino acids: n = 20; acylcarnitines: n = 12), as described in SupplementaryTable S1. Peak

areas of the transitions were recorded and integrated using Target Lynx software (Waters Corporation, Milford, CT, USA).

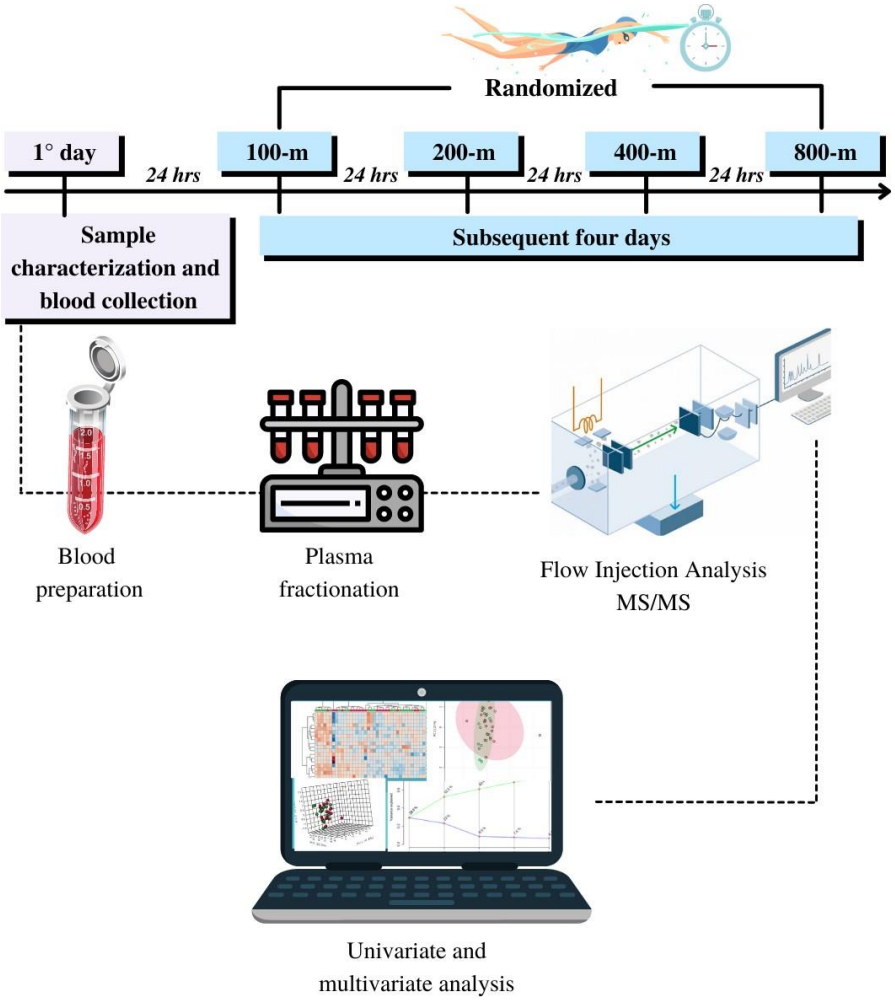

Figure 1. Experimental design of the study; MS—Mass Spectrometry.

2.5 *Performance Trials and Critical Velocity Assessment*

The performance trials comprised distances of 100, 200, 400, and 800-m (4, 8, 16, and 32 turns, respectively). The warm-up was standardized in all tests and based on 10 min of stretching outside the pool followed by 5 min of swimming at low intensity. This warm-up is the same that the athletes perform before their training. Swimmers were instructed to provide their best performances during each effort. The time was recorded by the same researcher using a stopwatch and registered when the athlete touched the swimming pool edge. Commands such as "ready" and "go" were used and were standardized for all athletes. All tests were performed under a competition situation; one swimmer per lane. The CV was determined based on the four time trials. The linear equation D = CV t + AWC was applied for the assessment of the critical velocity (CV); where D is the distance covered (D), t refers to the time to cover the distance, CV relates to the slope of regression, and AWC relates to y-intercept. The CV is defined as the velocity that can be maintained without exhaustion [43–45], regularly associated with aerobic indexes [46–49]. The AWC represents a finite amount of work performed until exhaustion [47]. However, its physiological significance requires further investigation. Therefore, only CV and the 100, 200, 400, and800-m times were considered for association with acylcarnitines and amino acid levels.

The coefficient of determination (R^2) was used to indicate the reliability of the linear adjustment.

2.6 Univariate and Multivariate Analysis

Statistical analyses were conducted using the STATISTICA 7.0 package (Statsoft, Tusla, OK, USA), MetaboAnalyst 5.0, and Python 3.9.3 environments. GraphPad Prism 5.0 software (GraphPad Software, San Diego, CA, USA) and the Canva environment were used to produce the figures. Pearson product-moment for the correlation analyses was performed in STATISTICA. Effect sizes (ES) were calculated at the following inferences: small if 0 |d| 0.5; medium if 0.5 < |d| 0.8; and large if |d| > 0.8).

The MetaboAnalyst environment [50] was used for most of the univariate and multivariate analyses. Data transformation (log transformation base 10) and scaling (Pareto) were adopted for the normalization of the data. We used the independent t-test followed by the false discovery rate (FDR) approach to compare the plasmatic levels of acylcarnitines/amino acids between male and female adolescent swimmers. Principal component analysis (PCA) and Partial Least-Squares Discriminant Analysis (PLS-DA) discriminated the male and female swimmers according to plasma acylcarnitines/amino acids. A random forest model was developed to predict the data into male or female swimmers based on these metabolite panels, and the out-of-bag score (OOB) was adopted to measure its prediction error.

A complex network topology analysis was created based on the significant ($p < 0.05$) correlations among the variables [51]. The variables (i.e., acylcarnitines/amino acids) were the nodes, and associations between these were represented by linking edges. As shown below in the results section, Tyrosine was the only molecule associated with the female adolescents' swimmer performances. In this sense, a weighted and targeted complex network was created using Tyrosine as the target. In this analysis, both significant positive and inverse correlations were equally treated and received positive weights regardless of the correlation direction.

The edge weights were calculated as the product of the edge degree of proximity to the Tyrosine node (this can vary from 0.01 to 1; higher means closer) and the correlationcoefficient between the nodes connected by the edge (this can vary from 0.01 to 1; higheris better). Therefore, edges received a weight equivalent to their respective correlation coefficient when they were directly linked to the node of interest (Tyrosine). Second- degree connections with the node of interest were equivalent to 0.5 (half) of the correlation coefficients, while third, fourth, and fifth-degree connections were equivalent to 0.250, 0.125,and 0.0625, respectively. Thus, the edge weights were used as the connection strength inthe calculation of the target eigenvector scores. Centrality eigenvector values were obtained utilizing the NetworkX 2.5 library [52] inside Python. The eigenvector centrality for node iis the ith element of the vector x defined by the equation $Ax = \lambda x$, where A is the adjacencymatrix of the graph G with eigenvalue λ. There is a unique solution x, for which all entriesare positive if λ is the largest eigenvalue of the adjacency matrix A [53]. The targeted eigenvector computed the centrality of a node based on the centrality of its neighbors and the weights of its edge connections.

3. Results

Male and female swimmers presented similar age (male = 15 ± 2 yr; female = 14 ± 2 yr; $p = 0.348$), body mass (male = 60.6 ± 11.4 kg; female = 54.9 ± 9.0 kg; $p = 0.101$), and height (male = 166 ± 16 cm; female = 160 ± 7 cm; $p = 0.656$). No difference was observed between swimmer performances over the predicted trials (100 m—male = 71.3 ± 10.4 s; female = 75.7 ± 5.4 s—ES = 0.53; 200 m—male = 166.3 ± 18.7 s; female = 174.1 ± 11.2 s—ES = 0.50; 400 m—male = 352.9 ± 53.9 s; female = 355.5 ± 25.1 s—ES = 0.06; 800 m—male = 743.9 ± 103.4 s; female = 752.4 ± 58.7 s—ES = 0.10) and the CV (male = 1.01 ± 0.10 m/min; female = 1.00 ± 0.14 m/min) (Figure 2). High regression coefficients were obtained for both groups (male—R^2 = 0.99 ± 0.00; female—R^2 = 0.99 ± 0.00).

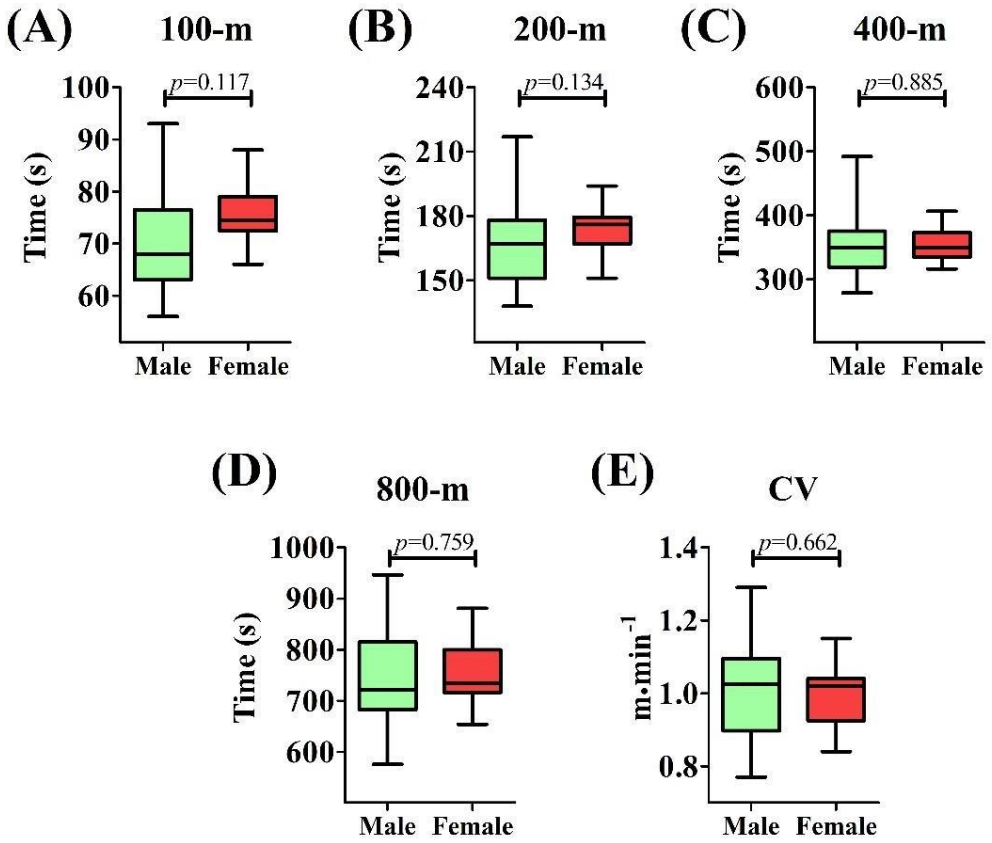

Figure 2. Comparison of performances over distinct distances and the critical velocity (CV) between male and female adolescent swimmers; (**A**) Comparison of performances on the 100-m; (**B**) Compari- son of performances on the 200-m; (**C**) Comparison of performances on the 400-m; (**D**) Comparison of performances on the 800-m; (**E**) Comparison of CV; $p \leq 0.05$.

Only tyrosine was significantly higher (1.56±0.22 normalized intensity) in males when compared to females (1.30±0.26 normalized intensity) among the amino acids (Figure 3A and Supplementary Figure S1), and this difference was not able to discriminate the groups according to PCA or PLS-DA analyses (Figure 3B). Additionally, the OOB score was 0.289, with an error of 0.278 for females and 0.300 for males (Figure 3C).

A similar profile regarding the plasma acylcarnitines was observed between groups (Figure 4A and Supplementary Figure S2). Moreover, these molecules were also not able to discriminate between male and female adolescent swimmers by PCA or PLS-DA analysis (Figure 4B). The classification error was 0.500 for females and 0.350 for males, and the OOB was obtained at 0.421 (Figure 4C).

Although no group differentiation was achieved, the significant difference observedfor plasmatic tyrosine levels also revealed positive and significant correlations with the performance of 100, 400, and 800-m by female swimmers. Additionally, this amino acid was inversely and significantly associated with CV for the same group. By inspectingthe correlation of tyrosine with other detected metabolites, glycine (0.406), glutamine (0.400), arginine (0.335), free carnitine (0.355), tryptophan (0.289), and histidine (0.271) were identified by the targeted network as being the most influential nodes to reach tyrosine (Figure 5). For the complete correlation matrix between the adolescent swimmer performances and the amino acids/acylcarnitines, see Supplementary Tables S2 and S3.

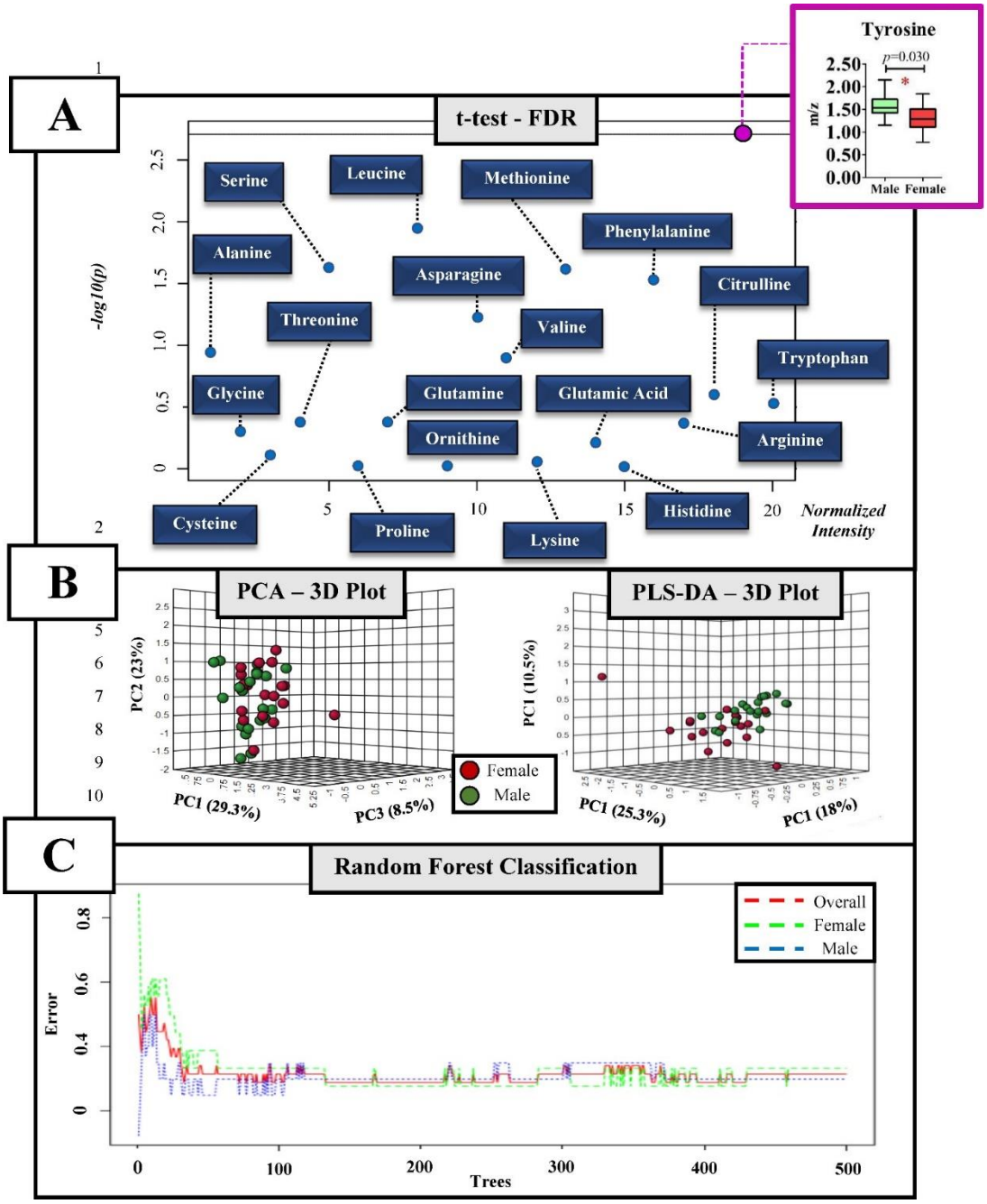

Figure 3. Univariate and multivariate analyses on the plasma amino acid of adolescent swimmers. (**A**) Independent *t*-test and False Discovery Rate (FDR) of plasma amino acids between male and fe- male adolescent swimmers; (**B**) discriminatory approach by the Principal Component Analysis (PCA) and the Partial Least Squares-Discriminant Analysis (PLS-DA); (**C**) Random Forest classification;* Significant difference; $p \leq 0.05$.

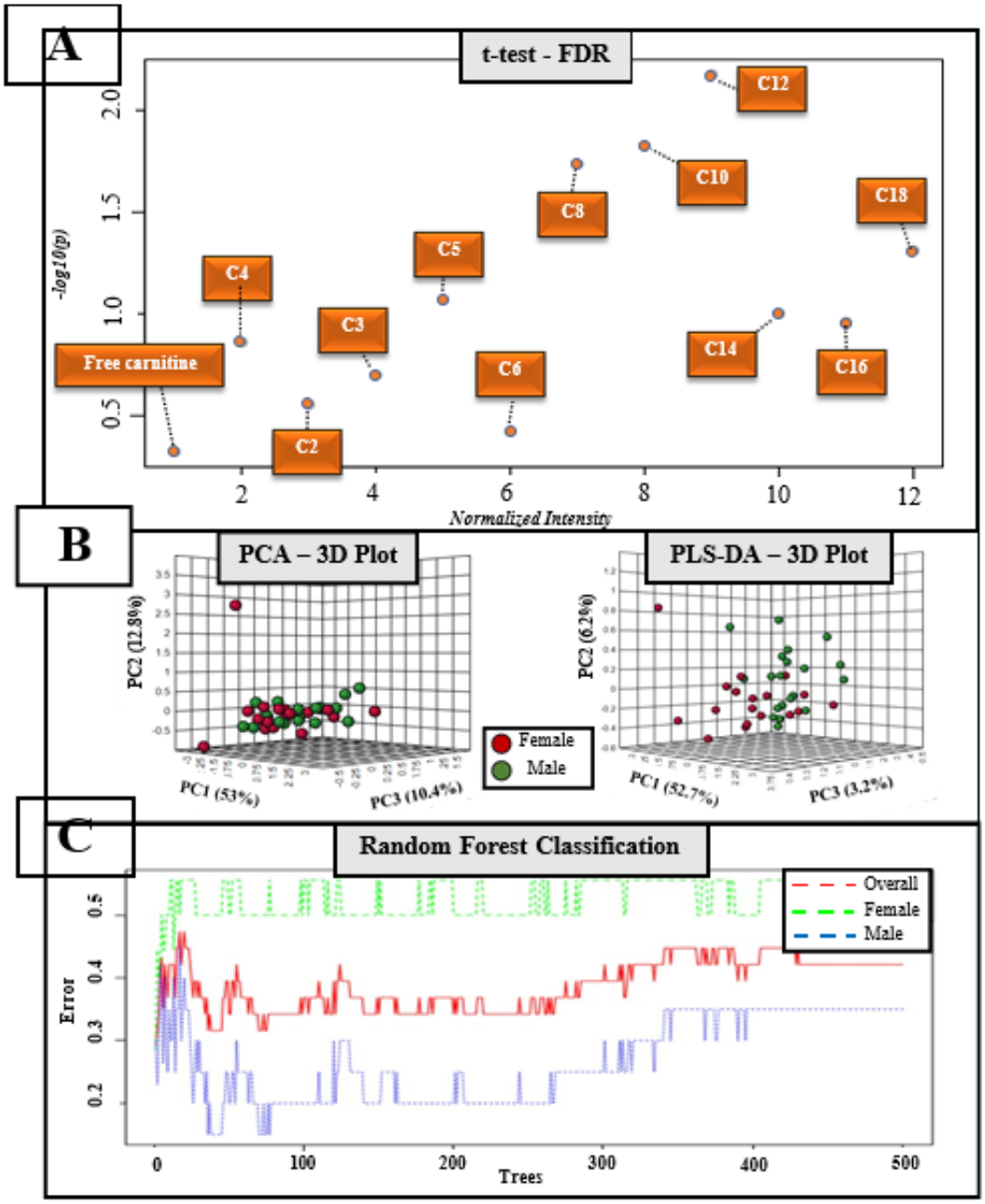

Figure 4. Univariate and multivariate analyses on the acylcarnitines of adolescent swimmers. (**A**) In- dependent t-test and False Discovery Rate (FDR) of plasma acylcarnitines between male and female adolescent swimmers; (**B**) discriminatory approach by the Principal Component Analysis (PCA) andthe Partial Least Squares-Discriminant Analysis (PLS-DA); (**C**) Random Forest classification.

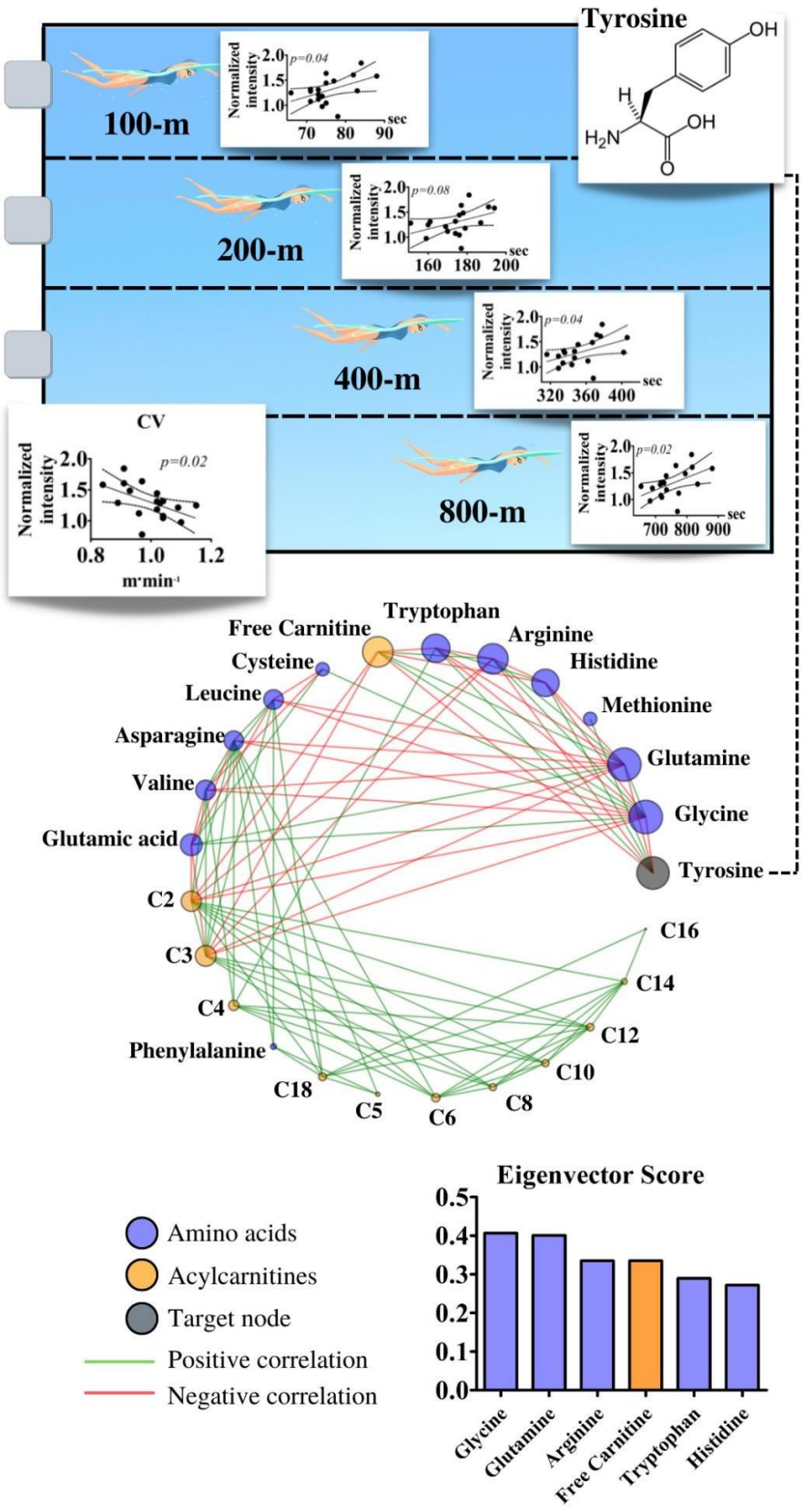

Figure 5. Upper panel presents the correlations between the performance trials for 100, 200, 400, and 800 m in addition to the critical velocity (CV) and plasma tyrosine of female adolescent swimmers. The lower panel presents the complex network based on the amino acids and acylcarnitines considering tyrosine as the target node.

4. Discussion

This is the first study to describe associations between female adolescent swimmerperformances and plasma tyrosine. Furthermore, the eigenvector centrality showed thatglycine, glutamine, arginine, free carnitine, tryptophan, and histidine may influence this molecule. Together, these results revealed a thread that must be explored in further randomized and controlled designs, improving the knowledge surrounding nutrition and the performance of adolescent swimmers.

Tyrosine (C9H11NO3) is a precursor to the catecholamines dopamine and norepinephrine. Its supplementation increases the circulating concentrations of these hormones in both the periphery and central nervous system [54]. When hormonal function is maintained intact, but dopamine and/or norepinephrine are temporarily depleted, tyrosine supplementation may enhance cognition [55]. Given the role of these hormones in motivation and thermoregulation [56–61], some research focus on the possible ergogenic activity of tyrosine on central fatigue and heat has been provided [62–68]. Furthermore, the role of tyrosine in body temperature is not limited to dopamine and norepinephrine synthesis. This amino acid is also a precursor of iodinated thyroid hormones [69], which exert crucial functions on many biological processes, including thermogenesis [70]. However, studies have drawn opposite conclusions regarding the tyrosine effect of physical exercise performed in heat [62,66–68]. Moreover, Sutton [71] showed that even with significantly increased plasma tyrosine levels by supplementation (150 g per kilogram of body mass consumed 30 min before performance tasks) the endurance, muscle strength, and anaerobic performance of moderately-highly active males were not improved.

Our results add to the omics-swimming area by demonstrating that the higher the tyrosine plasmatic levels of female adolescent swimmers, the worse the performance for 100 m, 200 m, 400 m, and the CV. Others have adopted the omics approach for swimmers, but with distinct purposes. By evaluating asthmatic and non-asthmatic adolescent swimmers using the breathomics approach, Couto [72] verified that metabolites associated with oxidative stress markers decreased after a swimming session, regardless of the group. Recently, Cai [73] identified serum metabolites (high-density lipoprotein, unsaturated fatty acid, lactic, methanol, isoleucine, 3-hydroxybutyric acid, acetoacetate, glutamine, glycine, and α-glucose) that discriminate between sub-elite and elite Chinese swimmers.

Based on the relationship between plasma tyrosine and female swimming performance, we conducted the targeted complex network approach to reveal connections between this amino acid and the remaining identified molecules. Plasmatic glycine, glutamine, arginine, and free carnitine were inversely correlated with tyrosine. Regarding this, a discussion exists regarding the latter three molecules on physical performance [17,74–78], including for swimmers [79–81]. While glutamine may promote glycogen synthesis and serve as fuel for lymphocytes and macrophages [73,82,83], carnitine is essential in beta-oxidation and can also modulate coenzyme-A metabolism [84,85]. Arginine is relevant for protein synthesis and can also be used to produce energy [86,87]. Histidine and tryptophan, on the other hand, were positively associated with tyrosine. Histidine may increase intracellular carnosine, offering an improved buffering capacity during exercise [88]. Being a precursor for serotonin synthesis, tryptophan supplementation has been deemed ergogenic for improving pain tolerance during exercise, but opposite results have also been drawn [89,90].

Arguments, coupled with the association of these molecules with tyrosine, suggest that some of these metabolites play an essential role in physical performance. However, this preliminary study could not detect the correlation between the plasmatic levels of glycine, glutamine, arginine, free carnitine, tryptophan, histidine, and the swimmer's performance. On the other hand, since some of these metabolites participate in a similar biochemical process, it is possible to speculate on their indirect impact on female swimming performance. In this sense, the Pentose Phosphate pathway [91] leads to aromatic amino acids biosynthesis (tyrosine and tryptophan ($r = 0.66$)) and histidine hepatic catabolism [92,93]. In the liver, histidine takes its place in nitrogen metabolism, which catalyzes L-histidine in L-glutamate and NH3 [91], and is transported to the Urea Cycle via glutamine, to be finally converted to arginine, and participating in both nitrogen excretion and protein turnover [92].

Another point worth discussing is the difference in plasma tyrosine between male and female adolescent swimmers. Moller [94] verified that plasma tyrosine in healthy women was significantly reduced in the luteal phase when compared to the follicular. Recently, He [95] showed that plasma levels of alanine, glutamine, threonine, and tyrosine in eumenorrheic women significantly varied throughout the menstrual cycle, and significantly dropped to the lowest level close to Day 21. Although little data is available regarding amino acid fluctuations across the menstrual cycle, based on these studies, it is possible to suggest that the significant difference in plasma tyrosine of adolescent female swimmers can be associated with the menstrual cycle. However, given the age of athletes and the possible discomfort in providing this information for the research group, the phase or even the menarche attainment was not collected. Therefore, this explanation is merely speculative and requires further confirmation.

Limitations and Future Perspectives

This study employed a descriptive and cross-sectional approach. Further studies are required to confirm that, when tyrosine levels are decreased, the physical performance of female adolescent swimmers improves. Moreover, blood samples were collected after 12 h overnight fasting before and on a distinct day of the performance trials. Future studies can advance this by collecting blood samples before and after swimming efforts to properly verify plasma tyrosine levels and their influence on a swimmer's performance. Despite the important and new findings provided by the targeted network, it is not possible to assume a cause-and-effect relationship. Attention should also be paid to athlete levels. The tested swimmers were from regional to national levels; thus, our insights cannot be transposed to high-class swimmers. Another limitation was associated with the menstrual cycle and menarche attainment of female adolescent swimmers, as tyrosine levels were reported to change according to the menstrual cycle. Lastly, the critical velocity protocolis a non-invasive procedure; therefore, oxygen consumption and other classic parameters such as blood lactate were not collected. Further studies are warranted to associate the amino acids and acylcarnitines with these physiological data.

5. Conclusions

This study applied a sportomics approach and verified positive and significant correlations between tyrosine and female's swimmer times for 100, 400, and 800 m, and inversely associated with CV. Moreover, the targeted complex network approach revealed that glycine, glutamine, arginine, free carnitine, tryptophan, and histidine may influence this molecule. However, these results, can only be transposed to female athletes. Future blinded, randomized, and controlled studies are necessary to advance on the new findings provided by this report, offering new avenues of investigation into the nutrition applied to the performance of swimmers.

Supplementary Materials: The following supporting information can be downloaded at: https: //www.mdpi.com/article/10.3390/biology11121734/s1, Figure S1: Comparison of plasma aminoacids between male and female adolescent swimmers by the false discovery rate. Values are expressed as normalized intensity; Figure S2: Comparison of plasma acylcarnitines between male and female adolescent swimmers by the false discovery rate. Values are expressed as normalized intensity; Table S1: Optimization of multiple reaction monitoring transitions were for each compound analyzed; Table S2: Correlations among amino acids and the swimmers' performances over distances and the critical velocity (CV); Table S3: Correlations among acylcarnitines and the swimmers' performances over distances and the critical velocity (CV).

Author Contributions: Conceptualization, F.M.M.M. and L.H.D.M.; Data curation, Á.A.R.S., I.G.M.d.R.and L.H.D.M.; Formal analysis, F.M.M.M., I.G.M.d.R. and L.H.D.M.; Funding acquisition, P.d.O.C., A.M.P. and L.H.D.M.; Investigation, F.M.M.M. and L.H.D.M.; Methodology, F.M.M.M., P.H.G.S.,Á.A.R.S., P.d.O.C., A.M.P. and L.H.D.M.; Project dministration, F.M.M.M. and L.H.D.M.; Resources, P.d.O.C., A.M.P. and L.H.D.M.;

Software, P.H.G.S., Á.A.R.S., I.G.M.d.R. and L.H.D.M.; Supervision, L.H.D.M.; Validation, P.d.O.C., A.M.P. and L.H.D.M.; Visualization, P.d.O.C., A.M.P. and L.H.D.M.; Writing—original draft, F.M.M.M. and L.H.D.M.; Writing—review and editing, P.H.G.S., Á.A.R.S., P.d.O.C., A.M.P. and L.H.D.M. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki, and approved by the Institutional Review Board (or Ethics Committee) of University ofSão Francisco (24892219.3.0000.551/25 November 2019).

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

Data Availability Statement: Not applicable.

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References

1. Sawyer, S.M.; Azzopardi, P.S.; Wickremarathne, D.; Patton, G.C. The age of adolescence. *Lancet Child Adolesc. Health* **2018**, *2*,223–228. [CrossRef]
2. Christian, P.; Smith, E.R. Adolescent Undernutrition: Global Burden, Physiology, and Nutritional Risks. *Ann. Nutr. Metab.* **2018**, *72*, 316–328. [CrossRef] [PubMed]
3. World Health Organization. *Nutrition in Adolescence: Issues and Challenges for the Health Sector: Issues in Adolescent Health andDevelopment*; WHO Press: Geneva, Switzerland, 2005.
4. Lee, S.; Lim, H. Development of an Evidence-based Nutritional Intervention Protocol for Adolescent Athletes. *J. Exerc. Nutr. Biochem.* **2019**, *23*, 29–38. [CrossRef]
5. Ferens, K.; Przeliorz-Pyszczek, A. Nutritional principles of adolescent swimmers. *Cent. Eur. J. Sport Sci. Med.* **2019**, *25*, 51–61.[CrossRef]
6. Feijen, S.; Tate, A.; Kuppens, K.; Claes, A.; Struyf, F. Swim-Training Volume and Shoulder Pain Across the Life Span of theCompetitive Swimmer: A Systematic Review. *J. Athl. Train.* **2020**, *55*, 32–41. [CrossRef]
7. Shaw, G.; Boyd, K.T.; Burke, L.M.; Koivisto, A. Nutrition for swimming. *Int. J. Sport Nutr. Exerc. Metab.* **2014**, *24*, 360–372. [CrossRef]
8. Shomaker, L.B.; Tanofsky-Kraff, M.; Savastano, D.M.; Kozlosky, M.; Columbo, K.M.; Wolkoff, L.E.; Zocca, J.M.; Brady, S.M.; Yanovski, S.Z.; Crocker, M.K.; et al. Puberty and observed energy intake: Boy, can they eat! *Am. J. Clin. Nutr.* **2010**, *92*, 123–129.[CrossRef] [PubMed]
9. Barbosa, T.M.; Morais, J.E.; Marques, M.C.; Silva, A.J.; Marinho, D.A.; Kee, Y.H. Hydrodynamic profile of young swimmers: Changes over a competitive season. *Scand J. Med. Sci. Sports* **2015**, *25*, e184–e196. [CrossRef]
10. Sammoud, S.; Nevill, A.M.; Negra, Y.; Bouguezzi, R.; Chaabene, H.; Hachana, Y. 100-m Breaststroke Swimming Performance in Youth Swimmers: The Predictive Value of Anthropometrics. *Pediatr. Exerc. Sci.* **2018**, *30*, 393–401. [CrossRef]
11. Zacca, R.; Toubekis, A.; Freitas, L.; Silva, A.F.; Azevedo, R.; Vilas-Boas, J.P.; Pyne, D.B.; Castro, F.A.S.; Fernandes, R.J. Effects of detraining in age-group swimmers performance, energetics and kinematics. *J. Sports Sci.* **2019**, *37*, 1490–1498. [CrossRef] [PubMed]
12. Kraemer, M.B.; Garbuio, A.L.P.; Kaneko, L.O.; Gobatto, C.A.; Manchado-Gobatto, F.B.; Dos Reis, I.G.M.; Messias, L.H.D. Associations among sleep, hematologic profile, and aerobic and anerobic capacity of young swimmers: A complex network approach. *Front. Physiol.* **2022**, *13*, 948422. [CrossRef]
13. Tipton, K.D.; Ferrando, A.A.; Williams, B.D.; Wolfe, R.R. Muscle protein metabolism in female swimmers after a combination ofresistance and endurance exercise. *J. Appl. Physiol.* **1996**, *81*, 2034–2038. [CrossRef]
14. Matsuda, T.; Kato, H.; Suzuki, H.; Mizugaki, A.; Ezaki, T.; Ogita, F. Within-Day Amino Acid Intakes and Nitrogen Balance inMale Collegiate Swimmers during the General Preparation Phase. *Nutrients* **2018**, *10*, 1809. [CrossRef]
15. Krause, S.; Langrock, M.; Weiss, M. Influence of seasonal variations in training loads on selected amino acids and parameters ofthe psychoimmunological network in a swimming team. *Int. J. Sports Med.* **2002**, *23*, 380–387. [CrossRef]

16. Hsueh, C.F.; Wu, H.J.; Tsai, T.S.; Wu, C.L.; Chang, C.K. The Effect of Branched-Chain Amino Acids, Citrulline, and Arginine onHigh-Intensity Interval Performance in Young Swimmers. *Nutrients* **2018**, *10*, 1979. [CrossRef]
17. Gnoni, A.; Longo, S.; Gnoni, G.V.; Giudetti, A.M. Carnitine in Human Muscle Bioenergetics: Can Carnitine Supplementation Improve Physical Exercise? *Molecules* **2020**, *25*, 182. [CrossRef]
18. Kreider, R.B.; Miriel, V.; Bertun, E. Amino acid supplementation and exercise performance. Analysis of the proposed ergogenicvalue. *Sports Med.* **1993**, *16*, 190–209. [CrossRef]
19. Flanagan, J.L.; Simmons, P.A.; Vehige, J.; Willcox, M.D.; Garrett, Q. Role of carnitine in disease. *Nutr. Metab.* **2010**, *7*, 30. [CrossRef]
20. Brass, E.P. Supplemental carnitine and exercise. *Am. J. Clin. Nutr.* **2000**, *72*, 618S–623S. [CrossRef]
21. Blomstrand, E. Amino acids and central fatigue. *Amino Acids* **2001**, *20*, 25–34. [CrossRef]
22. Lemon, P.W.; Proctor, D.N. Protein intake and athletic performance. *Sports Med.* **1991**, *12*, 313–325. [CrossRef] [PubMed]
23. Pasiakos, S.M.; Lieberman, H.R.; McLellan, T.M. Effects of protein supplements on muscle damage, soreness and recovery ofmuscle function and physical performance: A systematic review. *Sports Med.* **2014**, *44*, 655–670. [CrossRef] [PubMed]
24. Williams, M. Dietary supplements and sports performance: Amino acids. *J. Int. Soc. Sports Nutr.* **2005**, *2*, 63–67. [CrossRef] [PubMed]
25. Wu, G. Dietary protein intake and human health. *Food Funct.* **2016**, *7*, 1251–1265. [CrossRef] [PubMed]
26. Nunes, E.A.; Colenso-Semple, L.; McKellar, S.R.; Yau, T.; Ali, M.U.; Fitzpatrick-Lewis, D.; Sherifali, D.; Gaudichon, C.; Tome, D.;Atherton, P.J.; et al. Systematic review and meta-analysis of protein intake to support muscle mass and function in healthy adults.
J. Cachexia Sarcopenia Muscle **2022**, *13*, 795–810. [CrossRef]
27. Bassini, A.; Cameron, L.C. Sportomics: Building a new concept in metabolic studies and exercise science. *Biochem. Biophys. Res.Commun.* **2014**, *445*, 708–716. [CrossRef] [PubMed]
28. Bongiovanni, T.; Pintus, R.; Dessi, A.; Noto, A.; Sardo, S.; Finco, G.; Corsello, G.; Fanos, V. Sportomics: Metabolomics applied to sports. The new revolution? *Eur. Rev. Med. Pharmacol. Sci.* **2019**, *23*, 11011–11019. [CrossRef]
29. Bragazzi, N.L.; Khoramipour, K.; Chaouachi, A.; Chamari, K. Toward Sportomics: Shifting From Sport Genomics to Sport Postgenomics and Metabolomics Specialties. Promises, Challenges, and Future Perspectives. *Int. J. Sports Physiol. Perform.* **2020**, *15*, 1201–1202. [CrossRef]
30. Sellami, M.; Elrayess, M.A.; Puce, L.; Bragazzi, N.L. Molecular Big Data in Sports Sciences: State-of-Art and Future Prospects of OMICS-Based Sports Sciences. *Front. Mol. Biosci.* **2021**, *8*, 815410. [CrossRef] [PubMed]
31. Kuehnbaum, N.L.; Gillen, J.B.; Kormendi, A.; Lam, K.P.; DiBattista, A.; Gibala, M.J.; Britz-McKibbin, P. Multiplexed separations for biomarker discovery in metabolomics: Elucidating adaptive responses to exercise training. *Electrophoresis* **2015**, *36*, 2226–2236. [CrossRef]
32. Sakaguchi, C.A.; Nieman, D.C.; Signini, E.F.; Abreu, R.M.; Catai, A.M. Metabolomics-Based Studies Assessing Exercise-Induced Alterations of the Human Metabolome: A Systematic Review. *Metabolites* **2019**, *9*, 164. [CrossRef] [PubMed]
33. Schranner, D.; Kastenmuller, G.; Schonfelder, M.; Romisch-Margl, W.; Wackerhage, H. Metabolite Concentration Changes in Humans After a Bout of Exercise: A Systematic Review of Exercise Metabolomics Studies. *Sports Med. Open.* **2020**, *6*, 11. [CrossRef]
34. Goncalves, L.C.O.; Magalhaes-Neto, A.M.; Bassini, A.; Prado, E.S.; Muniz-Santos, R.; Verli, M.V.A.; Jurisica, L.; Lopes, J.S.S.; Jurisica, I.; Andrade, C.M.B.; et al. Sportomics suggests that albuminuria is a sensitive biomarker of hydration in cross combat.*Sci. Rep.* **2022**, *12*, 8150. [CrossRef]
35. Pintus, R.; Bongiovanni, T.; Corbu, S.; Francavilla, V.C.; Dess, I.A.; Noto, A.; Corsello, G.; Finco, G.; Fanos, V.; Cesare Marincola, F.Sportomics in professional soccer players: Metabolomics results during preseason. *J. Sports Med. Phys. Fitness* **2021**, *61*, 324–330. [CrossRef]
36. Coelho, W.S.; Viveiros de Castro, L.; Deane, E.; Magno-Franca, A.; Bassini, A.; Cameron, L.C. Investigating the Cellular and Metabolic Responses of World-Class Canoeists Training: A Sportomics Approach. *Nutrients* **2016**, *8*, 719. [CrossRef] [PubMed]
37. Berthoin, S.; Baquet, G.; Dupont, G.; Blondel, N.; Mucci, P. Critical velocity and anaerobic distance capacity in prepubertal children. *Can. J. Appl. Physiol.* **2003**, *28*, 561–575. [CrossRef] [PubMed]
38. Neiva, H.P.; Fernandes, R.J.; Vilas-Boas, J.P. Anaerobic critical velocity in four swimming techniques. *Int. J. Sports Med.* **2011**, *32*,
195–198. [CrossRef]
39. Toubekis, A.G.; Tokmakidis, S.P. Metabolic responses at various intensities relative to critical swimming velocity. *J. Strength Cond. Res.* **2013**, *27*, 1731–1741. [CrossRef] [PubMed]
40. Toubekis, A.G.; Tsami, A.P.; Tokmakidis, S.P. Critical velocity and lactate threshold in young swimmers. *Int. J. Sports Med.* **2006**, *27*, 117–123. [CrossRef] [PubMed]
41. Sarafian, M.H.; Gaudin, M.; Lewis, M.R.; Martin, F.P.; Holmes, E.; Nicholson, J.K.; Dumas, M.E. Objective set of criteria for optimization of sample preparation procedures for ultra-high throughput untargeted blood plasma lipid profiling by ultra performance liquid chromatography-mass spectrometry. *Anal. Chem.* **2014**, *86*, 5766–5774. [CrossRef]
42. Cicalini, I.; Valentinuzzi, S.; Pieragostino, D.; Consalvo, A.; Zucchelli, M.; Donzelli, S.; Ambrogi, D.; Brown, H.A.; Calton, L.J.;Stuppia, L.; et al. Analytical Evaluation of the Ideal Strategy for High-Throughput Flow Injection Analysis by Tandem Mass Spectrometry in Routine Newborn Screening. *Metabolites* **2021**, *11*, 473. [CrossRef]
43. Wakayoshi, K.; Ikuta, K.; Yoshida, T.; Udo, M.; Moritani, T.; Mutoh, Y.; Miyashita, M. Determination and validity of critical velocity as an index of swimming performance in the competitive swimmer. *Eur. J. Appl. Physiol. Occup. Physiol.* **1992**, *64*, 153–157.[CrossRef] [PubMed]

44. Wakayoshi, K.; Yoshida, T.; Kasai, T.; Moritani, T.; Mutoh, Y.; Miyashita, M. Validity of critical velocity as swimming fatiguethreshold in the competitive swimmer. *Ann. Physiol. Anthropol.* **1992**, *11*, 301–307. [CrossRef] [PubMed]
45. Wakayoshi, K.; Yoshida, T.; Udo, M.; Kasai, T.; Moritani, T.; Mutoh, Y.; Miyashita, M. A simple method for determining criticalspeed as swimming fatigue threshold in competitive swimming. *Int. J. Sports Med.* **1992**, *13*, 367–371. [CrossRef] [PubMed]
46. Jones, A.M.; Vanhatalo, A. The 'Critical Power' Concept: Applications to Sports Performance with a Focus on Intermittent High-Intensity Exercise. *Sports Med.* **2017**, *47*, 65–78. [CrossRef] [PubMed]
47. Jones, A.M.; Vanhatalo, A.; Burnley, M.; Morton, R.H.; Poole, D.C. Critical power: Implications for determination of V O2max andexercise tolerance. *Med. Sci. Sports Exerc.* **2010**, *42*, 1876–1890. [CrossRef] [PubMed]
48. Poole, D.C.; Burnley, M.; Vanhatalo, A.; Rossiter, H.B.; Jones, A.M. Critical Power: An Important Fatigue Threshold in ExercisePhysiology. *Med. Sci. Sports Exerc.* **2016**, *48*, 2320–2334. [CrossRef] [PubMed]
49. Vanhatalo, A.; Jones, A.M.; Burnley, M. Application of critical power in sport. *Int. J. Sports Physiol. Perform.* **2011**, *6*, 128–136.[CrossRef]
50. MetaboAnalyst. Available online: https://www.metaboanalyst.ca/ (accessed on 20 June 2022).
51. Gobatto, C.A.; Torres, R.S.; Moura, F.A.; Cunha, S.A.; Giometti, C.B.; Araujo, G.G.; Sousa, F.A.B.; Manchado-Gobatto, F.B. Corresponding Assessment Scenarios in Laboratory and on-Court Tests: Centrality Measurements by Complex Networks Analysis in Young Basketball Players. *Sci. Rep.* **2020**, *10*, 8620. [CrossRef]
52. Hagberg, A.A.; Schult, D.A.; Swart, P.J. Exploring network structure, dynamics, and function using NetworkX. In Proceedings ofthe 7th Python in Science Conference, Gäel Varoquaux. Pasadena, CA, USA, 19–24 August 2008; Vaught, T., Millman, J., Eds.; 2008; pp. 11–15.
53. Newman, M.E.J. *Networks: An Introduction*; Oxford University Press: Oxford, UK, 2010.
54. Meeusen, R.; Decroix, L. Nutritional Supplements and the Brain. *Int. J. Sport Nutr. Exerc. Metab.* **2018**, *28*, 200–211. [CrossRef][PubMed]
55. Jongkees, B.J.; Hommel, B.; Kuhn, S.; Colzato, L.S. Effect of tyrosine supplementation on clinical and healthy populations understress or cognitive demands—A review. *J. Psychiatr. Res.* **2015**, *70*, 50–57. [CrossRef] [PubMed]
56. Espana, R.A.; Schmeichel, B.E.; Berridge, C.W. Norepinephrine at the nexus of arousal, motivation and relapse. *Brain Res.* **2016**, *1641*, 207–216. [CrossRef] [PubMed]
57. Harley, C.W. Norepinephrine and dopamine as learning signals. *Neural Plast.* **2004**, *11*, 191–204. [CrossRef] [PubMed]
58. Lee, T.F.; Mora, F.; Myers, R.D. Dopamine and thermoregulation: An evaluation with special reference to dopaminergic pathways. *Neurosci. Biobehav. Rev.* **1985**, *9*, 589–598. [CrossRef] [PubMed]
59. Maki-Marttunen, V.; Andreassen, O.A.; Espeseth, T. The role of norepinephrine in the pathophysiology of schizophrenia. *Neurosci.Biobehav. Rev* **2020**, *118*, 298–314. [CrossRef] [PubMed]
60. Myers, R.D. Thermoregulation and norepinephrine. *Science* **1969**, *165*, 1030–1031. [CrossRef] [PubMed]
61. Zheng, X.; Hasegawa, H. Central dopaminergic neurotransmission plays an important role in thermoregulation and performance during endurance exercise. *Eur. J. Sport Sci.* **2016**, *16*, 818–828. [CrossRef]
62. Coull, N.A.; Watkins, S.L.; Aldous, J.W.; Warren, L.K.; Chrismas, B.C.; Dascombe, B.; Mauger, A.R.; Abt, G.; Taylor, L. Effect of tyrosine ingestion on cognitive and physical performance utilising an intermittent soccer performance test (iSPT) in a warm environment. *Eur. J. Appl. Physiol.* **2015**, *115*, 373–386. [CrossRef] [PubMed]
63. Meeusen, R.; Watson, P. Amino acids and the brain: Do they play a role in "central fatigue"? *Int. J. Sport Nutr. Exerc. Metab.* **2007**, *17*, S37–S46. [CrossRef]
64. Meeusen, R.; Watson, P.; Dvorak, J. The brain and fatigue: New opportunities for nutritional interventions? *J. Sports Sci.* **2006**, *24*, 773–782. [CrossRef]
65. Struder, H.K.; Hollmann, W.; Platen, P.; Donike, M.; Gotzmann, A.; Weber, K. Influence of paroxetine, branched-chain amino acids and tyrosine on neuroendocrine system responses and fatigue in humans. *Horm. Metab. Res.* **1998**, *30*, 188–194. [CrossRef] [PubMed]
66. Tumilty, L.; Davison, G.; Beckmann, M.; Thatcher, R. Oral tyrosine supplementation improves exercise capacity in the heat. *Eur J. Appl. Physiol.* **2011**, *111*, 2941–2950. [CrossRef] [PubMed]
67. Tumilty, L.; Davison, G.; Beckmann, M.; Thatcher, R. Failure of oral tyrosine supplementation to improve exercise performance in the heat. *Med. Sci. Sports Exerc.* **2014**, *46*, 1417–1425. [CrossRef] [PubMed]
68. Watson, P.; Enever, S.; Page, A.; Stockwell, J.; Maughan, R.J. Tyrosine supplementation does not influence the capacity to perform prolonged exercise in a warm environment. *Int. J. Sport Nutr. Exerc. Metab.* **2012**, *22*, 363–373. [CrossRef] [PubMed]
69. Sirakov, M.; Plateroti, M. Thyroid Hormone Signaling and Function: News from Classical and Emerging Models. *Cells* **2022**, *11*,
453. [CrossRef]
70. Citterio, C.E.; Targovnik, H.M.; Arvan, P. The role of thyroglobulin in thyroid hormonogenesis. *Nat. Rev. Endocrinol.* **2019**, *15*, 323–338. [CrossRef] [PubMed]
71. Sutton, E.E.; Coill, M.R.; Deuster, P.A. Ingestion of tyrosine: Effects on endurance, muscle strength, and anaerobic performance. *Int. J. Sport Nutr. Exerc. Metab.* **2005**, *15*, 173–185. [CrossRef] [PubMed]
72. Couto, M.; Barbosa, C.; Silva, D.; Rudnitskaya, A.; Delgado, L.; Moreira, A.; Rocha, S.M. Oxidative stress in asthmatic andnon-asthmatic adolescent swimmers-A breathomics approach. *Pediatr. Allergy Immunol.* **2017**, *28*, 452–457. [CrossRef] [PubMed]
73. Cai, M.; Wu, C.; Jing, C.; Shen, X.; He, M.; Wang, L.; Guo, Q.; Yan, Y.; Yan, X.; Yang, R. Blood Metabolomics Analysis Identifies

Differential Serum Metabolites in Elite and Sub-elite Swimmers. *Front. Physiol.* **2022**, *13*, 858869. [CrossRef] [PubMed]
74. Coqueiro, A.Y.; Rogero, M.M.; Tirapegui, J. Glutamine as an Anti-Fatigue Amino Acid in Sports Nutrition. *Nutrients* **2019**, *11*, 863. [CrossRef] [PubMed]
75. d'Unienville, N.M.A.; Blake, H.T.; Coates, A.M.; Hill, A.M.; Nelson, M.J.; Buckley, J.D. Effect of food sources of nitrate, polyphenols, L-arginine and L-citrulline on endurance exercise performance: A systematic review and meta-analysis of randomised controlled trials. *J. Int. Soc. Sports Nutr.* **2021**, *18*, 76. [CrossRef] [PubMed]
76. Gleeson, M. Dosing and efficacy of glutamine supplementation in human exercise and sport training. *J. Nutr.* **2008**, *138*, 2045S–2049S. [CrossRef] [PubMed]
77. Karlic, H.; Lohninger, A. Supplementation of L-carnitine in athletes: Does it make sense? *Nutrition* **2004**, *20*, 709–715. [CrossRef] [PubMed]
78. Walsh, N.P.; Blannin, A.K.; Robson, P.J.; Gleeson, M. Glutamine, exercise and immune function. Links and possible mechanisms. *Sports Med.* **1998**, *26*, 177–191. [CrossRef] [PubMed]
79. Esen, O.; Eser, M.C.; Abdioglu, M.; Benesova, D.; Gabrys, T.; Karayigit, R. Eight Days of L-Citrulline or L-Arginine Supplementation Did Not Improve 200-m and 100-m Swimming Time Trials. *Int. J. Environ. Res. Public Health* **2022**, *19*, 4462. [CrossRef][PubMed]
80. Kargotich, S.; Rowbottom, D.G.; Keast, D.; Goodman, C.; Dawson, B.; Morton, A.R. Plasma glutamine changes after high-intensity exercise in elite male swimmers. *Res. Sports Med.* **2005**, *13*, 7–21. [CrossRef]
81. Trappe, S.W.; Costill, D.L.; Goodpaster, B.; Vukovich, M.D.; Fink, W.J. The effects of L-carnitine supplementation on performance during interval swimming. *Int. J. Sports Med.* **1994**, *15*, 181–185. [CrossRef]
82. Hirabara, S.M.; Gorjao, R.; Levada-Pires, A.C.; Masi, L.N.; Hatanaka, E.; Cury-Boaventura, M.F.; da Silva, E.B.; Santos-Oliveira, L.C.D.; Sousa Diniz, V.L.; Serdan, T.A.D.; et al. Host cell glutamine metabolism as a potential antiviral target. *Clin. Sci.* **2021**, *135*,305–325. [CrossRef] [PubMed]
83. Cruzat, V.; Macedo Rogero, M.; Noel Keane, K.; Curi, R.; Newsholme, P. Glutamine: Metabolism and Immune Function, Supplementation and Clinical Translation. *Nutrients* **2018**, *10*, 1564. [CrossRef]
84. Adeva-Andany, M.M.; Carneiro-Freire, N.; Seco-Filgueira, M.; Fernandez-Fernandez, C.; Mourino-Bayolo, D. Mitochondrial beta-oxidation of saturated fatty acids in humans. *Mitochondrion* **2019**, *46*, 73–90. [CrossRef]
85. Bremer, J. Carnitine–metabolism and functions. *Physiol. Rev.* **1983**, *63*, 1420–1480. [CrossRef] [PubMed]
86. Morris, S.M., Jr. Arginine: Beyond protein. *Am. J. Clin. Nutr.* **2006**, *83*, 508S–512S. [CrossRef] [PubMed]
87. Stechmiller, J.K.; Childress, B.; Cowan, L. Arginine supplementation and wound healing. *Nutr. Clin. Pract.* **2005**, *20*, 52–61. [CrossRef] [PubMed]
88. Holecek, M. Histidine in Health and Disease: Metabolism, Physiological Importance, and Use as a Supplement. *Nutrients* **2020**, *12*, 848. [CrossRef]
89. Segura, R.; Ventura, J.L. Effect of L-tryptophan supplementation on exercise performance. *Int. J. Sports Med.* **1988**, *9*, 301–305.[CrossRef]
90. Stensrud, T.; Ingjer, F.; Holm, H.; Stromme, S.B. L-tryptophan supplementation does not improve running performance. *Int. J.Sports Med.* **1992**, *13*, 481–485. [CrossRef]
91. Kanehisa, M.; Goto, S. KEGG: Kyoto encyclopedia of genes and genomes. *Nucleic Acids Res.* **2000**, *28*, 27–30. [CrossRef]
92. Brosnan, M.E.; Brosnan, J.T. Histidine Metabolism and Function. *J. Nutr.* **2020**, *150*, 2570S–2575S. [CrossRef]
93. Stankovic, I.M.; Niu, S.; Hall, M.B.; Zaric, S.D. Role of aromatic amino acids in amyloid self-assembly. *Int. J. Biol. Macromol.* **2020**, *156*, 949–959. [CrossRef]
94. Moller, S.E.; Moller, B.M.; Olesen, M.; Fjalland, B. Effects of oral contraceptives on plasma neutral amino acids and cholesterol during a menstrual cycle. *Eur. J. Clin. Pharmacol.* **1996**, *50*, 179–184. [CrossRef]
95. He, W.; Dam, T.V.; Thogersen, R.; Hansen, M.; Bertram, H.C. Fluctuations in Metabolites and Bone Markers Across the Menstrual Cycle in Eumenorrheic Women and Oral Contraceptive Users. *J. Clin. Endocrinol. Metab.* **2022**, *107*, 1577–1588. [CrossRef] [PubMed]

4. CONCLUSÃO GERAL

Um método analítico foi desenvolvido utilizando um sistema cromatográfico acoplado a espectrômetro de massas modelo triplo quadrupolo operado em função MRM e electrospray positivo. Com base nas análises foi observado que a tirosina plasmática nas atletas do sexo feminino está diminuída comparada aos atletas do sexo masculino, diferença essa possivelmente devido as flutuações durante o ciclo menstrual, já relatados na literatura. No entanto, nesse estudo não foram coletadas as informações relacionadas ao ciclo menstrual das atletas, portanto, esta explicação é meramente especulativa e requer confirmação. Já entre as atletas do sexo feminino houve uma correlação positiva entre tirosina plasmática e o desempenho nas distâncias de 100, 400 e 800 metros, além de uma correlação inversa com a VC. Em síntese, os resultados do presente estudo sugerem que quanto maiores os níveis de tirosina, pior o desempenho de nadadores adolescentes. No entanto, em função da característica transversal e descritiva desta tese, estudos futuros randomizados são necessários para confirmar as associações observadas. Para tanto, a técnica aqui desenvolvida pode ser aplicada.

REFERÊNCIAS BIBLIOGRÁFICAS

MIELGO-AYUSO, J.; PIETRANTONIO, L.; VIRIBAY, A.; CALLEJA-GONZÁLEZ, J.; GONZÁLEZ-BERNAL, J.; FERNÁNDEZ-LÁZARO, D. Effect of acute and chronic oral l-carnitine supplementation on exercise performance based on the exercise intensity: A systematic review. **Nutrients**., v. 13, n. 12, p. 4359, 2021.

BAAR, KEITH. Using molecular biology to maximize concurrent training. **Sports. Med**., v. 44, n. 2, p. 17-25, 2014.

BARBOSA, T.M.; MORAIS, J.E.; MARQUES, M.C.; SILVA, A.J.; MARINHO, D.A.; KEE, Y.H. Hydrodynamic profile of young swimmers: changes over a competitive season. **Scand. J. Med. Sci. Sports.,** v. 25, p. 184-196, 2014.

BARTLETT, J. D.; CLOSE, G. L.; DRUST, B.; MORTON, J. P. The emerging role of p53 in exercise metabolism. **Sports. Med.,** v. 44, n. 3, p. 303-309, 2014.

BERTON, R.; CONCEIÇÃO, M.S.; LIBARDI, C.A.; CANEVAROLO, R.R.; GÁSPARI, A.F.; CHACON-MIKAHIL, M.P.T.; ZERI, A.C.; CAVAGLIERI, C.R Metabolic time-course response after resistance exercise: A metabolomics approach. **J. Sport. Sci.,** v. 35, n. 12, p. 1211-1218, 2017.

CAI, M.; WU, C.; JING, C.; SHEN, X.; HE, M.; WANG, L.; GUO, Q.; YAN, Y.; YAN, X.; YANG R. Blood Metabolomics Analysis Identifies Differential Serum Metabolites in Elite and Sub-elite Swimmers. **Front. Physiol.,** v. 13, p. 858869, 2022.

CHRISTIAN, P.; SMITH, E.R. Adolescent Undernutrition: Global Burden, Physiology, and Nutritional Risks. **Ann. Nutr. Metab.,** v. 72, p. 316-328, 2018.

COUTO, M.; BARBOSA, C.; SILVA, D.; RUDNITSKAYA, A.; DELGADO, L.; MOREIRA, A.; ROCHA, S.M. Oxidative stress in asthmatic and non-asthmatic adolescent swimmers-A breathomics approach. **Pediatr. Allergy. Immunol.,** v. 28, p. 452-457, 2017.

DEKERLE, J.; SIDNEY, M.; HESPEL, J,M.; PELAYO, P. Validity and reability of critical speed, critical stroke rate, and anaerobic capacity in relation to front crawl swimming performances. **Int. J. Sport. Med.,** v. 23, p. 93-98, 2002.

DUFT, R. G.; CASTRO, A.; BONFANTE, I. L.; BRUNELLI, D. T.; CHACON-MIKAHIL, M. P.; CAVAGLIERI, C. R. Metabolomics approach in the investigation of metabolic changes in obese men after 24 weeks of combined training. **J. Prot. Res.,** v. 16, n. 6, p. 2151-2159, 2017.

FEIJEN, S.; TATE, A.; KUPPENS, K.; CLAES, A.; STRUYF, F. Swim-Training Volume and Shoulder Pain Across the Life Span of the Competitive Swimmer: A Systematic Review. **J. Athl. Train.,** v. 55, p. 32-41, 2020.

FLANAGAN, J. L.; SIMMONS, P. A.; VEHIGE, J.; WILLCOX, M. D.; GARRETT, Q. Role of carnitine in disease. **Nutr. Metab.,** v. 7. p. 1-14, 2010.

GNONI, A.; LONGO, S.; GNONI, G.V.; GIUDETTE, M.A. Carnitine in Human Muscle Bioenergetics: Can Carnitine Supplementation Improve Physical Exercise? **Molecules.,** v. 25, p. 182, 2020.

HARRIS, D.C. **Análise Química Quantitativa.** 6. Ed. Rio de Janeiro: LTC Editora, Rio de Janeiro, 2005. 507 p.

KRAEMER, W.J.; NITKA, M. Choic of resistance used in an exercise: simple to complex interactions. **Strength. Cond. J.,** v. 44, n. 4, p.123-126, 2022.

KREIDER, R.B.; MIRIEL, V.; BERTUN, E. Amino acid supplementation and exercise performance. Analysis of the proposed ergogenic value. **Sports. Med.,** v. 16, p. 190-209, 1993.

LEE, T.F.; MORA, F.; MYERS, R.D. Dopamine and thermoregulation: An evaluation with special reference to dopaminergic pathways. **Neur. Bio. Rev.,** v 9, p. 589-598, 1985.

LEMON, P.W.; PROCTOR, D.N. Protein intake and athletic performance. **Sports Med.,** v. 12, p. 313-325, 1991

MENESES, C.O.; CAMPOS, D.L.; TOLEDO, T.B. Tanner stages: a study of reliability between the refered ande the obseved. **Adol. Saúde.,** v. 5, p. 54-56, 2008.

MORRIS, S.M. Arginine: beyond protein. **Am. J. Clin. Nutr.,** v. 83, p. 508-512, 2006.

NEIVA, H.P.; FERNANDES, R.J.; VILAS-BOAS, J.P. Anaerobic critical velocity in four swimming techniques. **Int. J. Sports. Med.,** v. 32, p. 195-198, 2010.

NUNES, E.A.; COLENSO-SEMPLE, L.; MCKELLAR, S.R. Systematic review and meta-analysis of protein intake to support muscle mass and function in healthy adults**. J. Cachexia. Sarcopenia. Muscle.,** v. 13, p. 795-810, 2022.

SAWYER, S.M.; AZZOPARDI, P.S.; WICKREMARATHNE, D.; PATON, G.C. The age of adolescence. **Lancet. Child. Adolesc. Health.,** v. 2, p. 223-228, 2018.

SHAW, G.; BOYD, K.T.; BURKE, L.M.; KOIVISTO, A. Nutrition for swimming. **Int. J. Sport. Nutr. Exerc. Metab.,** v. 24, p. 360-372, 2014.

SKOOG, D.A.; HOLLER, F.J.; NIEMAN, T.A. **Moecular mass spectometry. Principles of instrumental analysis.** 7. ed. Boston: Cengage Learning, 2001, 498 p.

TOUBEKIS, A.G.; TOKMAKIDIS, S.P. Metabolic responses at various intensities relative to critical swimming velocity. **J. Strength. Cond. Res.,** v. 27, p. 1731-1741, 2013.

TROMELEN, J.; HOLEERDA, A.M.; PINCKAERS, J.P.; LUC, J.C.; LOON, V. Comprehensive assessment of post-orandial protein nutrition and ageing application of intrinsically labelled protein in vivo human dubjects. **Proc. Nutr. Soc.,** v. 80, p. 221-229, 2021.

VALÉRIO, D.F.; BERTON, R.; CONCEIÇÃO, M.S.; CANEVAROLO, R.R.; CHACON-MIKAHIL, M.P.T.; CAVAGRLIERI, C.R.; MEIRELLES, G.V.; ZERI, A.C.; LIBARDI, C.A. Early metabolic response after resistance exercise with blood flow restriction in well-trained men: a metabolomics approach. **Appl. Physiol. Nutr. Metab.,** v. 43, n. 3, p. 240-246, 2017.

WIDMANN, M.; NIEß, A.M.; MUNZ, B. Physical exercise and epigenetic modifications in skeletal muscle**. Sports. Med.,** v. 49, n. 4, p. 509-523, 2019.

WU, G. **Amino acids in nutrition and health: Amino acids in systems function and health**. Suiça: Springer International Publishing, 2020.

ANEXO

UNIVERSIDADE SÃO FRANCISCO-SP

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: MAPEAMENTO METABOLÔMICO DE NADADORES PUBESCENTES, ADOLESCENTES E ADULTOS FRENTE A 12 SEMANAS DE TREINAMENTO FÍSICO CONTROLADO: INFERÊNCIAS À CAPACIDADE AERÓBIA

Pesquisador: Leonardo Henrique Dalcheco Messias

Área Temática:

Versão: 2

CAAE: 24892219.3.0000.5514

Instituição Proponente: Universidade São Francisco-SP

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 3.769.788

Considerações Finais a critério do CEP:

APÓS DISCUSSÃO EM REUNIÃO DO DIA 12/12/2019, O COLEGIADO DELIBEROU PELA APROVAÇÃO DO PROJETO DE PESQUISAS. APÓS A CONCLUSÃO DO PROJETO É OBRIGATÓRIO O ENVIO DO RELATÓRIO FINAL PARA ENCERRAMENTO DO PROJETO.

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_1458412.pdf	25/11/2019 16:33:01		Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	Versao_II_TCLE_menor_de_18.pdf	25/11/2019 16:29:58	Leonardo Henrique Dalcheco Messias	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	Versao_II_TCLE_adulto.pdf	25/11/2019 16:29:01	Leonardo Henrique Dalcheco Messias	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	Versao_II_TALE.pdf	25/11/2019 16:28:12	Leonardo Henrique Dalcheco Messias	Aceito
Declaração de Instituição e Infraestrutura	Anuencia_Nosso_Clube.pdf	24/10/2019 14:46:21	Leonardo Henrique Dalcheco Messias	Aceito
Declaração de Pesquisadores	Termo_de_Confidencialidade.pdf	24/10/2019 14:45:52	Leonardo Henrique Dalcheco Messias	Aceito
Declaração de Pesquisadores	Termo_de_Compromisso.pdf	24/10/2019 14:45:40	Leonardo Henrique Dalcheco Messias	Aceito
Projeto Detalhado / Brochura Investigador	Projeto_de_Pesquisa.pdf	24/10/2019 14:43:54	Leonardo Henrique Dalcheco Messias	Aceito
Folha de Rosto	Folha_de_Rosto.pdf	24/10/2019 14:43:13	Leonardo Henrique Dalcheco Messias	Aceito

Situação do Parecer:
Aprovado

Necessita Apreciação da CONEP:
Não

BRAGANCA PAULISTA, 13 de Dezembro de 2019

Assinado por:
CARLOS EDUARDO PULZ ARAUJO
(Coordenador(a))