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EDUARDO MARCEL FERNANDES NASCIMENTO

THE EFFECT OF CAFFEINE CHEWING GUM ON NEUROMUSCULAR AND CARDIORESPIRATORY RESPONSES AFTER SEVERE INTENSITY EXERCISE IN TRAINED CYCLISTS

Florianópolis
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Projeto de Tese submetida ao Programa de Pós-Graduação em Educação Física da Universidade Federal de Santa Catarina como requisito parcial para a obtenção do título de Doutor em Educação Física.
Orientador: Prof. Dr. Ricardo Dantas de Lucas
Coorientador: Prof. Dr. Luiz Guilherme Antonacci Guglielmo

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Eduardo Marcel Fernandes Nascimento

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Até breve...

RESUMO

Acredita-se que o efeito ergogênico da cafeína atenue a fadiga neuromuscular e interfira positivamente no desempenho em exercícios de alta-intensidade. Assim, a presente Tese investigou (por meio de três estudos) o efeito da goma de mascar com cafeína (GUM_{CAF}) nas respostas neuromusculares e cardiorrespiratórias após exercícios de intensidade severa em ciclistas treinados. No primeiro estudo, vinte ciclistas masculinos realizaram dois sprints de 7 s em cicloergômetro com freio eletromagnético em modo isolinear (ISO_{LIN}) e seis sprints em modo isocinético (ISO_{VEL}) com cadências entre 90 e 180 rpm. Uma função linear foi utilizada para modelar os sprints e extrapolar cadência máxima (C_{MAX}) e torque (T_{MAX}), e uma função quadrática foi usada para extrapolar o ápice definido como cadência ótima para potência máxima (OPT_{CAD}) e potência máxima (P_{MAX}). No segundo e terceiro estudo quinze ciclistas masculinos realizaram quatro visitas, cada uma composta por duas sessões de ciclismo de intensidade severa (Δ_1 e Δ_2) com duração de 6 minutos, separadas por 5 minutos de recuperação. GUM_{CAF} e goma placebo (GUM_{PLA}) foram administrados em um procedimento duplo-cego randomizado, sendo duas visitas para cada condição. No segundo estudo, o desempenho/fadiga muscular foi avaliado de forma isométrica pela força de contração voluntária máxima (IMVC) e dinâmica por sprints de ciclismo, antes e ao final dos exercícios (Δ_2). No terceiro estudo, a fase fundamental e o componente lento da cinética da extração muscular de oxigênio (HHb+Mb) e do consumo sistêmico de oxigênio ($\dot{V}O_2$) foram avaliados em decorrência do modelo de exercício prévio. Os resultados do primeiro estudo sugerem que as medidas da relação potência-cadência não foram diferentes entre os modos ISO_{LIN} e ISO_{VEL}. No segundo estudo, GUM_{CAF} não foi eficaz para atenuar o decréscimo da força muscular desencadeado pelos dois exercícios de ciclismo de intensidade severa, independentemente do método de avaliação (isto é, isométrico vs. dinâmico). Além disso, a variação percentual do Pré GUM para Pós-Exercício não mostrou diferença para medidas dinâmicas e isométricas, e entre GUM_{CAF} e GUM_{PLA}. No terceiro estudo, acerca da cinética da HHb+Mb, o exercício prévio acelerou a fase rápida (constante de tempo: GUM_{CAF} 16,0 ± 4,0 vs 13,9 ± 2,9 s; GUM_{PLA} 15,7 ± 6,1 vs 13,2 ± 2,5 s), atenuou a magnitude do componente lento e aumentou a HHb+Mb final ($p < 0,001$) no segundo exercício. De forma similar, na cinética do $\dot{V}O_2$, o exercício prévio também atenuou a magnitude do componente lento e aumentou o $\dot{V}O_2$ final ($p < 0,017$). A cafeína combinada ao exercício prévio (Δ_1 e Δ_2) não modificou em relação ao placebo qualquer parâmetro da cinética de HHb+Mb ou $\dot{V}O_2$. Concluindo, usando a relação potência-cadência, P_{MAX} e OPT_{CAD} puderam ser detectados de forma semelhante entre os dois modos de sprint (ISO_{LIN} e ISO_{VEL}).

A GUM_{CAF} não foi efetiva para atenuar o decréscimo da força muscular desencadeado pelos exercícios de ciclismo de intensidade severa, quando medido por ambos os métodos (isométrico vs. dinâmico). Finalmente, ao contrário do exercício prévio em exercícios de intensidade severa, GUM_{CAF} não é uma estratégia eficaz para acelerar a cinética de HHb+Mb ou $\dot{V}O_2$.

Palavras-chave: Consumo de oxigênio; Goma de mascar com cafeína; Funções neuromusculares; Fadiga; Potência máxima; Contração voluntária isométrica máxima.

ABSTRACT

It is believed that the ergogenic effect of caffeine attenuates neuromuscular fatigue and positively interferes with performance in high-intensity exercises. Thus, the present Thesis investigated (through three studies) the effect of caffeine chewing gum (GUM_{CAF}) on neuromuscular and cardiorespiratory responses after severe intensity exercise in trained cyclists. In the first study, twenty male trained cyclists performed 2 sprints of 7 s on an electromagnetically braked cycle ergometer in ISO_{LIN} and six sprints in ISO_{VEL} mode with cadences between 90 and 180 rpm. A linear function was used to model the sprints and extrapolate maximal cadence (C_{MAX}) and torque (T_{MAX}), and a quadratic function was used to extrapolate the apex defined as optimal cadence power (OPT_{CAD}) and peak power output (P_{MAX}). In the second and third study, fifteen trained male cyclists performed four visits, each one composed of two severe intensity cycling bouts (Δ_1 and Δ_2) with duration of 6 min, separated by 5-min of recovery. GUM_{CAF} and placebo gum (GUM_{PLA}) were administered in a randomized double-blind procedure, being two visits for each condition. In the second study muscle fatigue/performance was assessed isometrically by maximal voluntary contraction force (IMVC) and by cycling sprints, before and at the end of the exercises (Δ_2). In the third study, the fundamental phase and slow component of muscle oxygen extraction (HHb+Mb) and systemic oxygen uptake ($\dot{V}O_2$) kinetics were evaluated as a result of priming exercise model. The results of the first study suggest that the measures from the power-cadence relationship were not different between the ISO_{LIN} and ISO_{VEL} modes. In the second study, GUM_{CAF} was not effective in attenuating the decrease in muscle force triggered by the two severe intensity cycling exercises, regardless of the assessment method (i.e., isometric vs. dynamic). In addition, the percentage change from Pre GUM to Post Exercise showed no difference for dynamic and isometric measurements, and between GUM_{CAF} and GUM_{PLA}. In the third study, regarding HHb+Mb, priming exercise accelerated the fast kinetics (time constant: GUM_{CAF} 16.0 ± 4.0 vs 13.9 ± 2.9 s; GUM_{PLA} 15.7 ± 6.1 vs 13.2 ± 2 .5 s), attenuated the magnitude of the slow component and increased the final HHb+Mb ($p < 0.001$). Likewise, for $\dot{V}O_2$ kinetics, priming exercise also attenuated the magnitude of the slow component and increased the final $\dot{V}O_2$ ($p < 0.017$). Caffeine combined with priming exercise (Δ_1 and Δ_2) did not change any parameter of HHb+Mb or $\dot{V}O_2$ kinetics in relation to placebo. In conclusion, using the power-cadence relationship, P_{MAX} and OPT_{CAD} could be detected similarly between the two sprint modes (ISO_{LIN} and ISO_{VEL}). GUM_{CAF} was not effective to attenuating the muscle force decrement triggered by the severe intensity cycling exercises, when measured by both methods (isometric

vs. dynamic). Finally, unlike priming exercise in severe intensity exercise, GUM_{CAF} is not an effective strategy to accelerate HHb+Mb or $\dot{V}O_2$ kinetics.

Keywords: Oxygen uptake; caffeine chewing gum; neuromuscular functions; fatigue; maximal power output; isometric maximal voluntary contraction.

RESUMO EXPANDIDO

Introdução

Desde 2004, a Agência Mundial Antidoping considera que a cafeína não faz mais parte da lista de substâncias proibidas e, apesar de ser considerada uma droga, é socialmente aceita, sendo amplamente consumida por 90% dos adultos em todo o mundo. Os atletas, dentre os diferentes grupos de pessoas que consomem cafeína, destacam-se como os interessados no efeito ergogênico da cafeína no exercício. Estudos prévios demonstraram benefícios com a ingestão de cafeína, principalmente em relação ao desempenho de resistência, mostrando melhora em torno de 6% em diferentes modalidades de exercício. Portanto, muita atenção tem sido dada ao seu uso para melhorar o desempenho e/ou mitigar a fadiga. No campo da fisiologia do exercício, duas áreas podem ser destacadas no que diz respeito à fadiga induzida pelo exercício, sendo uma delas relacionada aos mecanismos de eficiência energética e suas relações com a cinética do consumo de oxigênio pulmonar (VO_2) e a oxigenação muscular; e outra com a função neuromuscular. Em relação à cinética do VO_2, do ponto de vista prático, a melhora na tolerância ao exercício está ligada a uma cinética mais rápida do VO_2 e menor componente lento (VO_{2SC}), o que permite menor perturbação da homeostase intracelular, pois reduz a dependência de fontes de energia anaeróbica. Vale ressaltar que de fato o exercício prévio tem sido utilizado para melhorar a tolerância ao exercício e, consequentemente, de alguma forma atenuar o aparecimento da fadiga muscular. Portanto, uma melhor compreensão dos efeitos da cafeína na cinética do VO_2 imposta por diferentes contextos de tarefas, como as vinculadas ao exercício prévio, seria importante para a compreensão dos fatores determinantes do desempenho de endurance. Além disso, aproveitando uma análise da cinética da oxigenação muscular usando espectroscopia no infravermelho próximo (NIRS), que tem sido usada para examinar a correspondência relativa do fornecimento de O_2 à utilização de O_2 nos tecidos durante carga de trabalho constante, também pode contribuir para uma visão mais holística dos aspectos fisiológicos pulmonares e musculares relacionados à fadiga muscular. Já em relação aos efeitos da cafeína e o desempenho neuromuscular, apesar dos possíveis benefícios da ingestão de cafeína no sistema neuromuscular, não está claro como essas alterações podem afetar a capacidade de gerar força máxima durante um exercício dinâmico como o ciclismo. Além disso, não há estudos na literatura que tenham investigado os efeitos da ingestão de cafeína, ao comparar medidas isométricas como contração voluntária máxima isométrica (IMVC) e medidas dinâmicas como torque máximo, cadência e potência (T_{MAX}, C_{MAX} e P_{MAX}, respectivamente) obtidas através do desempenho do sprint no ciclismo. Estas medidas têm a

vantagem de se aproximarem da especificidade da modalidade (ciclismo). Portanto, medir o desempenho do sprint ligado aos efeitos da cafeína pode ser uma excelente estratégia que permite fazer algumas inferências sobre a fadiga muscular. Por fim, a maioria dos estudos envolvendo cafeína foi realizada com ingestão em forma de cápsula e ingerida aproximadamente 60 minutos antes do exercício. Mais recentemente, diferentes abordagens de consumo de cafeína foram propostas e discutidas. Dentre estes, destaca-se o uso de enxaguantes bucais com cafeína e gomas de mascar com cafeína (GUM_{CAF}), devido à sua absorção mais rápida quando comparado ao uso de cápsulas com cafeína. Além disso, vale ressaltar que a cafeína pode levar ao aumento do fluxo sanguíneo tecidual e ao fornecimento de oxigênio ao músculo em exercício (devido ao aumento da produção de óxido nítrico), potencialmente aumentando a contratilidade muscular e atenuando a fadiga induzida pelo exercício. Nesse sentido, embora tenha sido demonstrado um potencial efeito benéfico do cafeína no sistema nervoso central (SNC) e na contração muscular esquelética, nenhum estudo relacionou os efeitos da ingestão de GUM_{CAF} no modelo de exercício prévio nas respostas neuromusculares e cardiorrespiratórias. Assim, investigamos (por meio de três estudos) o efeito da GUM_{CAF} nas respostas neuromusculares e cardiorrespiratórias após exercícios de intensidade severa em ciclistas treinados.

Objetivos

O primeiro estudo teve como objetivo comparar os parâmetros derivados do desempenho utilizando os modos de ciclismo isolinear (ISO_{LIN}) e isovelocidade (ISO_{VEL}). O propósito foi validar o modo de ciclismo ISO_{LIN} para verificar a confiabilidade e validade de um único sprint realizado sem fator de torque na estimativa dos parâmetros derivados das relações torque – cadência e potência – cadência. Consequentemente, o ISO_{LIN} foi utilizado no segundo estudo para mensurar a fadiga muscular de forma dinâmica. O segundo estudo investigou o efeito da GUM_{CAF} nas medidas de fadiga muscular (isométrica vs dinâmica) após sessões de ciclismo de intensidade severa. O terceiro estudo teve como objetivo investigar o impacto da combinação GUM_{CAF} com o exercício prévio na cinética VO_2 e a extração muscular de oxigênio (HHb + Mb) durante sessões de ciclismo realizadas no domínio de intensidade severa do exercício.

Metodologia

No primeiro estudo, vinte ciclistas masculinos realizaram dois sprints de 7 s em cicloergômetro com freio eletromagnético em modo ISO_{LIN} e seis sprints em modo ISO_{VEL} com cadências entre 90 e 180 rpm. Uma função linear modelou os sprints dentro de cada modo para extrapolar a

cadência máxima (C_{MAX}) e o torque (T_{MAX}), e uma função quadrática foi usada para extrapolar o ápice definido como potência de cadência ideal (OPT_{CAD}) e potência de pico (P_{MAX}). No segundo e terceiro estudo quinze ciclistas masculinos realizaram quatro visitas, cada uma composta por duas sessões de ciclismo de intensidade severa (Δ_1 e Δ_2) com duração de 6 minutos, separadas por 5 minutos de recuperação. GUM_{CAF} e goma placebo (GUM_{PLA}) foram administrados em um procedimento duplo-cego randomizado, sendo duas visitas para cada condição. No segundo estudo, o desempenho/fadiga muscular foi avaliado de forma isométrica pela força de IMVC e dinâmica por sprints de ciclismo, antes e ao final dos exercícios (Δ_2). No terceiro estudo, a fase fundamental e o componente lento da cinética da HHb+Mb e do VO_2 foram avaliados em decorrência do modelo de exercício prévio.

Resultados e Discussão

Os resultados do primeiro estudo sugerem que as medidas da relação potência-cadência não foram diferentes entre os modos ISO_{LIN} e ISO_{VEL}. No segundo estudo, a GUM_{CAF} não foi eficaz para atenuar o decréscimo da força muscular desencadeado pelos dois exercícios de ciclismo de intensidade severa, independentemente do método de avaliação (isto é, isométrico vs. dinâmico). Além disso, a variação percentual do Pré GUM para o Pós-Exercício não mostrou diferença para medidas dinâmicas e isométricas, e entre GUM_{CAF} e GUM_{PLA}. No terceiro estudo, acerca da cinética da HHb+Mb, o exercício prévio acelerou a fase rápida (constante de tempo: GUM_{CAF} 16,0 ± 4,0 vs 13,9 ± 2,9 s; GUM_{PLA} 15,7 ± 6,1 vs 13,2 ± 2,5 s) e atenuou a magnitude do componente lento e aumentou a HHb+Mb final ($p < 0,001$) no segundo exercício. De forma similar, na cinética do VO_2 o exercício prévio também atenuou a magnitude do componente lento e aumentou o VO_2 final ($p < 0,017$). A cafeína combinada ao exercício prévio (Δ_1 e Δ_2) não modificou em relação ao placebo qualquer parâmetro da cinética de HHb+Mb ou VO_2.

Considerações Finais

Concluindo, usando a relação potência-cadência, P_{MAX} e OPT_{CAD} puderam ser detectados de forma semelhante entre os dois modos de sprint (ISO_{LIN} e ISO_{VEL}). A GUM_{CAF} não foi efetiva para atenuar o decréscimo da força muscular desencadeado pelos exercícios de ciclismo de intensidade severa, quando medido por ambos os métodos (isométrico vs. dinâmico). Finalmente, ao contrário do exercício prévio em exercícios de intensidade severa, GUM_{CAF} não é uma estratégia eficaz para acelerar a cinética de HHb+Mb ou $\dot{V}O_2$.

Palavras-chave: Consumo de oxigênio; Goma de mascar com cafeína; Funções neuromusculares; Fadiga; Potência máxima; Contração voluntária isométrica máxima.

LIST OF FIGURES

LIST OF TABLES

LIST OF EQUATIONS

LIST OF ABBREVIATIONS AND SYMBOLS

CAF	Caffeine
C_{MAX}	Maximal cadence
C_{OPT}	Optimal cadence power
EM	Movement economy
EMG	Electromyography
GUM_{CAF}	Caffeine chewing gum
GUM_{PLA}	Placebo chewing gum
HbO_2/Mb	Oxyhemoglobin and/or myoglobin
HHb/HMb	Deoxyhemoglobin and/or myoglobin
HR	Heart rate
HR_{MAX}	HR maximal values
ISO_{LIN}	Isolinear sprint cycling modes
IMVC	Isometric maximal voluntary contraction
ISO_{VEL}	Isovelocity sprint cycling modes
[La]	Blood lactate concentration
MO_2	Muscle Oxygenation
NIRS	Near-infrared spectroscopy
P_{MAX}	Maximal power
PFM	Pedal force measurement
PLA	Placebo
PPO	Peak power output
SNC	Central Nervous System
τ	$\dot{V}O_2$ time constant
T_{MAX}	Maximal torque
V_{MAX}	Maximal velocity
VT_1	First ventilatory threshold
VT_2	Second ventilatory threshold
$\dot{V}O_2$	Pulmonary oxygen uptake
$\dot{V}O_{2MAX}$	Maximal oxygen uptake
$\dot{V}O_{2SC}$	Slow component of VO_2
$\dot{V}O_{2SC}$ trajectory	Slow component trajectory
$\dot{V}O_{2END}$	The average VO_2 value over the last 15 s

TABLE OF CONTENTS

1. CHAPTER ONE

1.1 INTRODUCTION

Since 2004, the World Anti-Doping Agency has considered that the caffeine (CAF) is no longer part of the list of prohibited substances, and despite being considered a drug, it is socially accepted, being widely consumed by 90% of adults around the world (BURKE, 2008). Athletes, among the different groups of people who consume CAF, stand out as those interested in the ergogenic effect of CAF on exercise (GRGIC et al., 2020). Previous evidence corroborates this statement, since the results of urine tests of athletes between 2004 and 2008, carried out as part of anti-doping control, found values around 73.8% of urine samples with CAF amounts above the limit of detection (DEL COSO et al., 2011). In fact, previous studies have demonstrated benefits with CAF ingestion, mainly in relation to endurance performance, showing improvement of around 6% in different exercise modalities (BURKE, 2008; GRGIC et al., 2020). Therefore, much attention has been paid to its use for improving performance and/or mitigate fatigue (ALTIMARI et al., 2006; BAZZUCCHI et al., 2011; COUTO et al. 2022; RYAN et al., 2013; KALMAR & CAFARELLI, 1999; SANTOS et al., 2020).

In this line, international associations confirm that CAF presents strong evidence for improving performance. For instance, the Australian Institute of Sport (AIS) which classifies CAF within the group A in the ABCD Classification System, as it is considered with solid scientific evidence for their use in sport (AIS Position Statement: Supplements and Sports Foods, 2022); as well as the International Olympic Committee consensus statement is that the CAF is a stimulant that possesses well-established benefits for athletic performance across endurance-based situations, and short-term, supramaximal and/or repeated sprint tasks (MAUGHAN et al., 2018).

Regarding exercise-induced fatigue, this is a multifactorial phenomenon and although significant advances in science and technology have been made mainly in recent decades, it is still not possible to determine precisely why an individual becomes fatigued under various conditions (ENOKA & DUCHATEAU, 2008). Specifically, the muscle fatigue is denoted as a decline in the maximum strength or power capacity of the muscle, which is a transient decrease in the ability to perform physical actions (ENOKA & DUCHATEAU, 2008). In the exercise physiology field, two areas can be highlighted in regard of exercise-induced fatigue, one being related to the energy-efficiency mechanisms and its relationships with the pulmonary oxygen consumption (VO_2) (CANNON et al., 2011) and muscle oxygenation (MO_2) kinetics (do

NASCIMENTO SALVADOR et a., 2018); and another with the neuromuscular function (KRUGER et al., 2019; TEMESI et al., 2017).

Concerning VO_2 kinetics, from a practical point of view, the improvement in exercise tolerance is linked to faster VO_2 kinetics and lesser slow component (VO_{2SC}), which allows less disturbance of intracellular homeostasis, as it reduces dependence on anaerobic energy sources (MATURANA et al., 2018). It is worth mentioning that in fact prior exercise has been used to improve exercise tolerance and, consequently, somehow mitigate the onset of muscle fatigue (Grassi et al. 1996; Burnley et al. 2002). Therefore, a better understanding of the effects of CAF on the VO_2 kinetics imposed by different task contexts, such as linked to prior exercise would be important for understanding the determining factors of endurance performance. This because the effects of CAF ingestion on VO_2 kinetics, the results of previous research are controversial. On the one hand, research suggest that the ergogenic effect of CAF at high-intensity endurance exercise may be partly mediated by an attenuation on the VO_{2SC} (SANTALLA et al., 2001), which is an excess of oxygen consumption (VO_2) for the intensity predicted through a linear relationship between VO_2 and workload below the first ventilatory threshold (JONES et al., 2008; GURD et al., 2006; SAHLIN et al., 2005). On the other hand, researchers have reported no changes in VO_2 kinetics responses following CAF administration (BELL et al., 1999; POWERS et al., 1986; SIMMONDS et al., 2010). However, despite the evident benefits of prior exercise on the behavior of the VO_{2SC}, no study has investigated the effects of previous exercise combined with CAF ingestion. Additionally, harnessing an analysis of MO_2 kinetics using near-infrared spectroscopy (NIRS), which has been used to examine the relative correspondence of O_2 supply to tissue O_2 utilization during constant workload (COLOSIO et al., 2021; GRASSI & QUARESMA, 2016), can also contribute to a more holistic view of the pulmonary and muscular physiological aspects related to muscle fatigue. It is noteworthy that some studies suggest that CAF is related to increased nitric oxide production, which leads to an increased tissue blood flow and oxygen supply to the exercising muscle (RUÍZ-MORENO et al. 2020; UMEMURA et al. 2006). Previous studies also suggest that CAF acts as a powerful vasoconstrictor of inactive regions during dynamic leg exercise (cycle ergometry) at moderate intensity (Daniels et al. 1998). However, no data exist related to high-intensity whole-body exercise. Additionally, fatigue of the respiratory muscles can also compromise O_2 transport to active muscles. Furthermore, the mechanisms by which CAF increases VE and prevents arterial O_2 desaturation during high-intensity whole-body exercise are not fully understood (Lima Silva et al. 2021). Therefore, these effects of CAF on the physiological aspects at pulmonary and

muscular levels together have been understudied and there is no deeper scientific knowledge on this topic, mainly when involving whole-body severe intensity exercise.

Regarding the effects of CAF and neuromuscular performance, studies point to benefits of CAF ingestion on the neuromuscular system (KALMAR and CAFARELLI, 1999; 2004). For example, Kalmar and Cafarelli (1999) observed an increase in the maximal isometric force generated by the quadriceps muscles after an ingestion of 6 $mg.kg^{-1}$ of CAF. In this study, there was also an increase in maximal voluntary activation. On the other hand, Plaskett and Cafarelli (2001) reported a positive effect of CAF on the time to exhaustion obtained during an isometric quadriceps activity with no difference in the voluntary activation of the muscle between the placebo (PLA) and CAF conditions. Additionally, previous studies suggest that CAF improves cycling endurance performance during closed-loop and open-loop exercise, without influence on post-exercise central and peripheral fatigue (COUTO et al., 2021). Therefore, the effects of CAF on the attenuation of muscle fatigue are not yet very well understood. Despite the possible benefits of CAF ingestion on the neuromuscular system, it is unclear how these changes may affect the ability to generate maximal force during a dynamic exercise such as cycling. Furthermore, there are no studies in the literature that investigated the effects of CAF ingestion, when comparing isometric measurements such as isometric maximal voluntary contraction (IMVC) and dynamic measurements such as maximal torque, cadence and power (T_{MAX}, C_{MAX} and P_{MAX}, respectively) obtained through the cycling sprint performance. These measures have the advantage of approaching the specificity of the modality (cycling). Therefore, measuring sprint performance linked to the effects of CAF, can be an excellent strategy that allows making some inferences about muscle fatigue.

Finally, most studies involving CAF have been carried out with ingestion in capsule form and taken approximately 60 minutes before exercise. More recently, different CAF consumption approaches have been proposed and discussed (WICKHAM and SPRIET, 2018). Among these, the use of CAF mouthwashes and CAF chewing gum (GUM_{CAF}) stands out (WICKHAM and SPRIET, 2018), due to their faster absorption when compared to the use of CAF capsules (KAMINORI et al., 2002). Additionally, CAF might lead to increased tissue blood flow and oxygen supply to the exercising muscle (due to increased nitric oxide production) (RUIZ-MORENO et al. 2020; UMEMURA et al., 2006), potentially enhancing muscle contractility and reducing exercise-induced fatigue (LIMA-SILVA et al., 2021). In this sense, although a potential beneficial effect of CAF on the central nervous system (CNS) and on skeletal muscle contraction has been demonstrated, no study has related the effects of GUM_{CAF} ingestion on the prior exercise model on neuromuscular and cardiorespiratory

responses. Therefore, considering the aforementioned assumptions, the following questions will be investigated: 1. Is there an additional effect of GUM_{CAF} intake over prior exercise on neuromuscular and cardiorespiratory responses, during a cycling task performed at severe domain intensity? 2. Is there a possible interchangeability between measures of muscle fatigue carried out in isometric and dynamic modes? In this way, this Thesis was divided into three studies, each of which sought to answer the questions involved in this overview.

1.2 OBJECTIVES

1.2.1 Main objective

To investigate the effect of GUM_{CAF} ingestion on neuromuscular and cardiorespiratory responses during and after severe intensity exercise in trained cyclists

1.2.2 Specific objectives

1.2.2.1 (Study 1)

Verify the reliability and validity of a single sprint in estimating the parameters derived from Torque–Cadence and Power–Cadence relationships.

1.2.2.2 (Study 2)

Investigate the effects of GUM_{CAF} on muscle fatigue measures (isometric vs dynamic) after severe intensity cycling bouts.

1.2.2.3 (Study 3)

Investigate the combined effects of GUM_{CAF} and priming exercise on VO_2 and muscle NIRS-derived kinetics, during cycling at severe-intensity domain.

1.3 HYPOTHESIS

The hypothesis of the present study is that there will be an additional effect of GUM_{CAF} ingestion over priming exercise on neuromuscular and cardiorespiratory responses, since CAF might lead to an increased tissue blood flow and oxygen supply to the exercising muscle (due to increased nitric oxide production) potentially enhancing muscle contractility and reducing exercise-induced fatigue during severe intensity cycling bouts.

1.3.1 Hypothesis study 1

The hypothesis is that a single sprint assessment using as low as possible braking resistance for the Excalibur Sport cycle ergometer could provide valid and reliable estimates of T_{MAX}, C_{MAX} and P_{MAX} and optimal cadence (OPT_{CAD}).

1.3.2 Hypothesis study 2

The hypothesis is that there would be an effect of CAF on muscle fatigue measures (isometric vs dynamic) after severe intensity cycling bouts. Additionally, to compare and correlate dynamic measures, being the maximal torque, velocity, and power output (T_{MAX}, V_{MAX}, and P_{MAX}, respectively) with isometric (i.e., IMVC) measures of fatigue, after a sequence of two bouts of severe intensity cycling. The hypothesis is that a similar magnitude of changes in these properties occurs in response to fatigue after severe intensity cycling bouts.

1.3.3 Hypothesis study 3

The hypothesis posits that the effects of GUM_{CAF} could affects priming exercise, resulting in faster VO_2 and/or mVO_2 kinetics, particularly during the second bout. It is suggested that prior intense exercise may induce significant intracellular metabolic changes, including increased blood flow and reduced metabolic inertia, and since CAF can also lead to an increased tissue blood flow and oxygen supply for the exercising muscle (due to increased production of nitric oxide), these changes combined could amplify the influence of CAF on the kinetics of VO_2 and mVO_2.

2. LITERATURE REVIEW

2.1 Official statements about caffeine use in sports

It is belived that contribution of nutrition to successful performance in elite athletes be small but potentially valuable, and dietary supplements can make a minor contribution to this nutrition programme. However, supplement's use is widespread at all levels of sport (MAUGHAN et al., 2018). Obviously the maim use of supplements is to potentiate rewards and avoid potential risks. Therefore, scientific evidence should be the basis of a sports nutrition planning when involving supplements. Furthermore, some key points must be taken into consideration, such as whether the supplement is safe, effective and whether it is allowed to be

used in competitive sport for enhancing performance (AIS POSITION STATEMENT: SUPPLEMENTS AND SPORTS FOODS, 2022).

Regarding CAF, organizations such as the ‘International Sports Nutrition Association’ (ISSN) classify CAF within category I with "strong scientific evidence supporting efficacy and apparent safety" (KERKSICK et al., 2018). In addition, the International Olympic Committee consensus statement is that the CAF it is a stimulant that possesses well-established benefits for athletic performance across endurance-based situations, and short-term, supramaximal and/or repeated sprint tasks (MAUGHAN et al., 2018).

Finally, the ABCD classification system, created by Australian Institute of Sport (AIS POSITION STATEMENT: SUPPLEMENTS AND SPORTS FOODS, 2022), provides a simple education tool for ranking sports foods and supplement ingredients according to the scientific evidence that they can safely and practically contribute to an athlete’s performance goals. Group "A" includes supplements with "sound scientific evidence for use in specific situations in sport using evidence-based protocols." Group "B" includes those with "emerging scientific support, worthy of further research". In group "C" are supplements for which "scientific evidence does not support benefits for athletes or research has not been conducted to guide an informed opinion". And, within group "D" are classified those "banned or at high risk of contamination with substances that could result in a positive doping control" (AIS POSITION STATEMENT: SUPPLEMENTS AND SPORTS FOODS, 2022). The AIS classifies caffeine (CAF) within the group A and they can be considered as with solid scientific evidence for their use in sport.

2.2 Caffeine (CAF) and its effects in different sports

CAF is a chemical compound, classified as an alkaloid belonging to the xanthine group, being chemically designated as 1,3,7-trimethylxanthine. It is a popular ergogenic resource, with athletes of all levels being the biggest consumers with the idea of improving performance (PICKERING & GRGIC, 2019; DEL COSCO et al., 2011). Its ergogenic effects on performance have a long history of use, considering the first studies were published more than 100 years ago (RIVERS & WEBBER, 1907). Among the different sports, triathlon, cycling and rowing appear as those with the highest values of CAF concentration found in urine (DEL COSO et al., 2011). In short, studies suggest that the magnitude of the CAF effect is generally greater for aerobic exercise compared to anaerobic exercise.

Among the physiological mechanisms proposed for CAF improving performance, it is the sparing of muscle glycogen through inhibition of phosphodiesterase (GRAHAM e SPRIET,

1991; CRUZ et al., 2015); the antagonistic action of CAF on adenosine A1 and A2 receptors in the CNS (DALY et al., 1994); the release of calcium from the sarcoplasmic reticulum (KLEIN et al., 1990; VON RUDEN e NEHER, 1993). Furthermore, studies suggest a reduction in perception of exertion and a decrease in the sensation of pain associated with exercise (FARHADI; HADI; SABEGH, 2011; CAPUTO et al., 2012; GUERRA; BERNARDO; GUTIÉRREZ, 2000; ALTIMARI et al., 2006; DOHERTY; SMITH, 2004;2005).

In an "umbrella" meta-analysis recently published by Grgic et al., (2020), they addresses the different results found in the literature regarding the effects of CAF on exercise performance; provides evidence regarding the quality, power and limitations of the results published by studies; and suggests existing gaps in the literature that may be fundamental for future research. According to Grgic et al., (2020) CAF can be considered an ergogenic resource for different components of exercise performance, including general endurance, muscular endurance, muscular strength/power, jumping performance and speed. Furthermore, among the main results, the authors observed that the magnitude of CAF effect on studies aimed at endurance performance are greater than those involving anaerobic performance.

In fact, the implicit concept of the ergogenic benefits of CAF related to endurance performance is historically supported by scientific evidence (TARNOPOLSKY, 1994), however, on the other hand, studies also prove the increase in performance in anaerobic exercises (DAVIS & GREEN, 2009; GRGIC & MIKULIC, 2017). For example, the CAF has been reported to have an ergogenic effect even in very short duration events such as strength tests, generating effect size values of 0.17 (GRGIC et al., 2018). However, as highlighted by Pickering & Grgic, (2019) there are many practical aspects regarding the use of CAF within sport and exercise that still remain poorly understood (with sex, time of day, genotype, habitual use, and training status, and there is a need for a greater understanding of the effects of CAF). In addition, it is noteworthy that some factors could influence the caffeine's effectiveness as an ergogenic aid, such as dose, training degree, ingestion time, time of day caffeine supply, habitual CAF consumption, proposed exercise type, genetic polymorphisms in the CYP1A2 and ADORA2A genes (Martins et al., 2020), which too represent promising avenues to better understanding and provide new insights into the use of CAF during exercise.

2.3 Caffeine and cycling sprint performance

Several studies have analyzed the effects of CAF ingestion on sprint performance. Regardless of the form which CAF is administered, sprints lasting 30s (i.e. Wingate Test) have been preferably used with the application of a torque factor that varies from 0.75 – 0.90 $N \cdot m \cdot kg^{-}$

[1] (BECK et al., 2006; GLAISTER et al., 2019; GREER et al., 2006). The results are conflicting because while some studies point to no effect of CAF on sprint performance (BECK et al., 2006; GLAISTER et al., 2012; GREER et al., 2006), others have reported a positive effect (ANSELME et al., 1992; GLAISTER et al., 2015; 2019) and that possibly, for example, the torque factor (range of values) and sprint duration (individuals produce a lower PPO during 30 s sprints than 10 s sprints), may be a limitation of studies that questioned its real effect (GLAISTER et al., 2019). Furthermore, evidence shows that the torque factor can also influence, being generally reported between 1.0 – 1.25 ($N \cdot m \cdot kg^{-1}$) to obtain the highest peak power output (GLAISTER et al., 2015; WINTER et al., 1996).

Lopes-Silva et al., (2018) verified the effect of CAF ingestion on performance in repeated sprints (10 x 6 s of sprints with intervals of 30 s of passive recovery) in different periods of the day (i.e. morning and afternoon). Thirteen physically active male individuals were evaluated in a randomized, double-blind manner, using CAF ingestion in capsules with concentrations of 5 $mg \cdot kg^{-1}$ or PLA, ingested 60 minutes before the tests. The results demonstrated that sprint performances were influenced by diurnal variation, with lower values found in the morning compared to the afternoon. Furthermore, CAF ingestion did not attenuate the reduction in performance in the morning period or improve performance in the afternoon period.

Glaister et al., (2012) evaluated the effect of CAF ingestion on cycling sprint performance and whether there was an effect of the dose-response relationship. Seventeen well-trained males performed seven maximal sprints of 10 s in duration. All screenings involved the ingestion of gelatin capsules containing CAF or PLA, and ingested 1 h before each sprint, and to examine the dose-response, doses of CAF in concentrations of 2, 4, 6, 8, and 10 $mg \cdot kg^{-1}$ were administered. The results demonstrated suggest that CAF ingestion did not improve sprint performance regardless of the dosage.

In another study, Glaister et al., (2019) investigated the influence of the torque factor and sprint duration on the effects of CAF supplementation on cycling sprint performance. Thirteen physically active individuals performed nine visits, the first consisting of a series of 6 s sprints with increasing torque factor to determine the optimal cadence and power relationship ($T_{OPTIMAL}$) with their respective torque factor. The other eight visits involved all combinations of torque factor (0.8 $N \cdot m \cdot kg^{-1}$ versus $T_{OPTIMAL}$), sprint duration (10 s versus 30 s) and CAF ingestion in gelatin capsules at concentrations of 5 $mg \cdot kg^{-1}$ versus PLA, administered 45 minutes prior to the exercise. The results of this study demonstrated that when the torque factor

and sprint duration (10 s) were optimized, a higher peak power production was found, suggesting the beneficial effect of CAF ingestion on sprint performance.

In addition, Lara et al., (2020) investigated the effects of CAF intake on Wingate anaerobic test performance during 3 phases of the menstrual cycle. Their results showed that the ergogenic effect of CAF on Wingate anaerobic test peak cycling power was of a similar magnitude in the follicular, preovulatory, and mid-luteal phases.

2.4 Caffeine and muscle strength performance

It is believed that the main mechanisms by which CAF increases muscular strength, muscular endurance and power performance are potentially related to its ability to increase the conduction velocity of muscle fibers and the recruitment of motor units (BLACK et al., 2015).

The results of previous studies confirm that CAF could increase the muscular strength with a magnitude of effect size varying between 0.16 - 0.20, confirming its ergogenic effect related to this kind of exercise performance (GRGIC et al., 2018). In a study conducted by Tallis et al., (2013), the authors suggest that CAF ingestion at physiological concentrations of ~ 70 μmol/L can favor the release of calcium from the sarcoplasmic reticulum and provide an increase in muscle strength. On the other hand, previous studies indicate that high acute doses of CAF (9 and 11 mg/kg of body mass) did not improve muscle strength nor muscle endurance in athletes habituated users of CAF (WILK et al., 2019).

In relation to peak and average muscle power, the ergogenic effect of CAF has been reported with a variation in its effect size from 0.18 to 0.27 (GRGIC, 2018). For studies focused on speed performance during running, cycling and rowing, the magnitude of the effect size has been demonstrated to be around 0.41 (CHRISTENSEN et al. 2017). Finally, Raya-González et al., (2020) meta-analysed the studies that explored the effects of CAF supplementation on movement velocity in resistance exercise. Their results showed that acute CAF ingestion is highly ergogenic for movement velocity in resistance exercise with the pooled effect size ranged from 0.41 to 0.82.

In addition, Grgic and Del Coso, (2021) conducted a meta-analysis and the results demonstrated that CAF ingestion has a significant ergogenic effect on the muscular endurance and muscular strength in women.

2.5 Response time in absorption and different forms of caffeine ingestion

The time of CAF ingestion before the exercise has been used with an average of 60 minutes in most of studies (GRGIC et al., 2020). However, the results of the studies are

contradictory, and its real time of effect seems uncertain, especially when involving different doses (TALANIAN & SPRIET, 2016) and even genotypes (PICKERING & GRGIC, 2019).

Taking tablets or capsules along with water or drinking coffee are traditionally used in sports and research environments (WICKHAM & SPRIET, 2018). In fact, the ingestion of CAF in capsule form has commonly been found in meta-analysis study (GRGIC et al., 2020). However, alternative forms can also be found, such as CAF gel, bars, tablets, CAF mouthwash and GUM_{CAF} are among examples. Supposedly it could provide faster absorption of CAF via oral mucosa and intestines (WICKHAM & SPRIET, 2018). Also, recent studies have shown that mouthwash can activate neuronal sensors in the oral cavity that make direct connections to the brain and can affect performance (WICKHAM & SPRIET, 2018). Furthermore, there are currently manufacturers advocating the idea of using oral and nasal aerosols, which could signal neuronal sensors in the nasal cavity primarily and provide a direct connection for absorption in the lungs, but scientific evidence has not yet been tested. However, as mentioned by Stecker et al., (2019) more studies are needed on greater variety of exercise modalities, to obtain more generalized results. Furthermore, the GUM_{CAF} administration must be better studied, as its efficacy could be different of capsules due to differences in absorption rate and bioavailability for a given time protocol.

2.6 Caffeine Chewing Gum (GUM_{CAF})

Although studies have demonstrated evidence regarding the effectiveness of CAF administered in capsules to increase wakefulness time, induce an alert state and positively alter mood (KAMIMORI et al. 2000; KAPLAN et al. 1997; PENETAR et al. 1993), the delay time of 20 to 30 minutes for CAF to reach significant quantities in the blood circulation and consequently at the CNS, is an important factor to consider, due to the absorption via gastrointestinal system,

Alternative methods have been proposed with the aim of accelerate the time of CAF absorption, and studies involving military objectives were the pioneers in the use of GUM_{CAF}. The purpose was to optimize the restoration of alert time and improve performance, assuming that GUM_{CAF} accelerates the rate of blood absorption by activating sensors in the oral mucosa, in addition to absorption via the intestine (KAMIMORI et al., 2002). The extensive vascularization of the oral cavity and the absorption on this site would be one of the advantages for the use of chewing gum (KAMIMORI et al., 2002; SHARGEL & YU, 1999). Thus, avoiding the first step of metabolism which could supposedly occur via the intestine and, consequently, in the liver could perhaps be another preponderant factor in favor of GUM_{CAF} ingestion.

In a classic study by KAMIMORI et al. (2002) investigating the absorption rate in different amounts (50, 100 or 200 mg) in the capsule and GUM_{CAF} condition, it is possible to verify the difference in the blood plasma concentration of CAF over the time. The results demonstrated that although the maximum CAF concentrations were not different between the capsule and chewing gum conditions, a shorter time to reach the maximum values was found in the chewing gum condition (44 – 80 minutes) compared to the capsule (84 – 120 minutes). Specifically in relation to the quantity, the authors suggest ingestion with 200 mg of GUM_{CAF} as the condition with the fastest rate of absorption, reaching its maximum value around 5 - 15 minutes and a lesser extent later up to 25 minutes, unlike the condition in a capsule that presented maximum values around 25 to 45 minutes. The same group of authors in another study investigated and proved the efficacy of the effect of GUM_{CAF} in a dose dependent manner, concluded that plasma levels of CAF were maintained and increased in relation to the three different dosages of 50, 100 and 200 mg (SYED et al., 2005).

These findings support evidence of GUM_{CAF} regarding the speed of absorption rate and the period of time that its effect can be maintained, this being a preponderant factor in the most different sports, such as cycling. Another point to highlight would be the redirection of blood flow to the muscles under effort and the consequent decrease in splanchnic blood flow, thus suggesting an advantage of GUM_{CAF} compared to capsules that depend exclusively on the visceral route, with the intestine and liver as the main organs (WICKHAM & SPRIET, 2018).

Finally, recently was conducted a systematic review with meta-analysis to determine the effect of GUM_{CAF} on exercise performance outcomes (BARRETOS et al., 2022). Their results showed that GUM_{CAF} supplementation may be an effective ergogenic strategy for trained athletes involved in both endurance and strength/power exercise, using a recommended dose of ≥3 mg/kg BM consumed within 15 min before the target exercise.

2.7 Caffeine chewing gum on aerobic and sprint performance in cycling

The ergogenic effect of GUM_{CAF} in cycling has been subject of different studies (RYAN et al., 2012; RYAN et al., 2013; LANE et al. 2014; OBERLIN-BROWN et al., 2016; PATON et al., 2015). Ryan et al. (2012) verified the effect of low doses of GUM_{CAF} for three different time points (-35, -5 before and 15 minutes after starting the task) during a submaximal cycling exercise. In this study, a total of 200 mg was used, divided into two pieces of 100 mg each, and the intensity of the exercise corresponded to 80% of VO_{2MAX} until the exhaustion. The time to exhaustion were not impacted in the CAF when compared to the PLA and control situations.

On the other hand, Ryan et al., (2013), evaluating trained cyclists, administered 300 mg of GUM_{CAF} 120, 60 and 5 minutes prior to a cycling exercise performed at 75% VO_{2MAX} for 15 minutes followed by a time-trial based on accumulation of total work (i.e. 7 kJ/kg of body mass). The results demonstrated the CAF improved the time-trial performance only when administered 5 minutes before the exercise.

Lane et al. (2014) verified the effect of GUM_{CAF} ingestion containing 3 mg/kg of body mass in 24 well-trained athletes (12 men and 12 women) during a time-trial simulating the London 2012 Olympic Games circuit, which lasted ~50 to 60 minutes (men 43.83 km and women 29.35 km). Individuals performed a time-trial in four visits on different days. Subjects chewed 2 mg/kg of chewing gum for 10 minutes and 40 minutes before starting time-trial and another 1 mg/kg 10 minutes before starting time-trial, and the conditions were: beetroot juice; CAF and placebo like beetroot juice; beetroot juice and CAF as placebo; and control condition with CAF placebo and beetroot placebo. The authors concluded that GUM_{CAF} ingestion induced an increase in performance in time-trial by 3% to 4%, both in men and women, and ingestion with beetroot juice did not generate an additional effect in the experimental conditions of the study.

On the other hand, a study by Oberlin-Brown et al. (2016) verified the effect of GUM_{CAF} on eleven well-trained male cyclists comparing the conditions: PLA; carbohydrate; CAF; and CAF with carbohydrate in a 90-minute exercise at 63% of VO_{2MAX} followed by a 20 km time-trial. The CAF ingestions consisted of 50 mg prior to the start of time-trial and at the distance of 5, 8 and 15 km totaling 200 mg of CAF. The performance (time and power output) was not improved by using of chewing gum for either CAF or carbohydrate. The authors suggest that the results may be related to the protocol which CAF was administered, that is, only 50 mg prior to the start of exercise, and this may have been a preponderant factor to an insufficient supply of CAF to adenosine receptors at the beginning of time-trial, thus generating a delay in the ergogenic effect.

In another study, published by Paton et al., (2015) which involved ten female and ten male trained cyclists, the authors evaluated the effect of GUM_{CAF} on a simulated cycling race with three laps of 10 km each; and sprints of ~15 s duration in the last 0.2 km of each lap. Each lap included variation of altitude with the aim of simulating a real race route; and the GUM_{CAF} contented 200-300 mg for women and men, respectively, corresponding to relative doses around 3-4 $mg \cdot kg^{-1}$. Significant differences were not found in the initial 20 km of the time-trial, but the CAF increased the average power by 3.8 ± 2.3%, increasing the speed of the last 10 km by 1.9%; and also improved sprint performance by around 4.0 ± 3.6% during the final sprint.

In relation to the average power output of the final 10 km, both men and women improved by around 4.3 ± 3.4 and 3.2 ± 3.0%, respectively, as well as the race time by 1.9 ± 5, 0 and 6.2 ± 5.2%, respectively. The authors concluded that GUM_{CAF} improved mean and sprint performance in the final lap of a 30 km time trial in cyclists, without substantial differences in the ergogenic effect of CAF between genders and suggest that these findings are due to of greater CNS activation caused by inhibition of adenosine receptors at the brain level. It is worth mentioning that, studies have suggested an ergogenic effects of GUM_{CAF} when administered between 200 and 300 mg, both in male and female cyclists; this being provided before or during an endurance exercise (WICKHAM & SPRIET, 2018).

Paton et al. (2010) investigated the ergogenic effect of GUM_{CAF} on fatigue and hormonal responses during repeated sprints in nine competitive male cyclists. The sprint protocol consisted of 4 sets of five sprints of 30 s each with 30 s of active recovery at 100 W. The GUM_{CAF} or placebo gum were administered during the 10min recovery period after the second set. The results demonstrated a reduction in decline of cadence and power output in sets 3 and 4 in the CAF versus PLA condition. Therefore, the authors suggest that GUM_{CAF} ingestion attenuated fatigue during repeated, high-intensity sprint exercise in competitive cyclists.

2.8 Slow component of oxygen consumption and muscle fatigue

The mechanisms that control the rate of adjustment of oxidative phosphorylation to an instantaneous increase in energy demand have been debated over the last three decades (GRASSI, ROSSITER, & ZOLADZ, 2015). If, on the one hand, it is consensual that exercising at the moderate intensity domain the behavior of VO_2 is exponential in nature, on the other hand, there are potential mechanisms that control the adjustment of VO_2 (GRASSI et al 1996.). One is based on the supply of oxygen (O_2) to active tissues, which plays a critical role as a rate limiting factor (MURIAS & PATERSON, 2015; MURIAS, SPENCER, & PATERSON, 2014) and the other is based on control that resides in the supply of intracellular substrate and enzyme activation (GRASSI, 2001; POOLE, et al., 2008; ROSSITER, 2011). From a practical point of view, the improvement in exercise tolerance in different task contexts is linked to faster VO_2 kinetics, which allows less disturbance of intracellular homeostasis, as it reduces dependence on anaerobic energy sources (MATTIONI MATURANA et al., 2017). Therefore, a better understanding of the VO_2 kinetics imposed by different task contexts, such as in prior exercise, which is common in different types of exercise, would be important for understanding the factors determining performance.

Since prior exercise has been widely used for exploring the mechanistic bases of $\dot{V}O_2$ kinetics and possible limitations to human exercise tolerance (BARSTOW et al., 1990; BURNLEY et al., 2000, 2002; do NASCIMENTO SALVADOR et al., 2018), the precise cause of the altered VO_2 kinetics after priming exercise still needs to be further clarified. However, some well-established aspects suggest an increase in muscle O_2 supply and/or a partial reduction in muscle oxidative metabolic inertia and/or changes in motor unit recruitment profiles (JONES et al., 2008; BURNLEY et al., 2002; GURD et al., 2006; KRUSTRUP et al., 2001; SAHLIN et al., 2005). A clearly supported advantage of prior exercise at a determined intensity, is that there is an enhanced oxidative contribution to energy turnover during the transition to exercise, which allows for faster overall VO_2 kinetics following prior exercise and less disruption of intracellular homeostasis, with consequent reduction the rate at which muscular fatigue develops, reducing the magnitude of muscular 'O_2 deficit' (BURNLEY AND JONES, 2007).

Do Nascimento Salvador et al. (2018) investigated whether prior exercise would attenuate muscle fatigue accompanied by VO_{2CL} behavior during a subsequent cycling exercise at very heavy intensity, in a group of physically active individuals. The results demonstrated a decrease in VO_{2CL} after a previous exercise of very heavy intensity that was accompanied by greater metabolic acidosis and cardiovascular responses. However, considering that the VO_{2CL} attenuation was not accompanied by muscle force production behavior, the authors suggest that these findings do not convincingly support the hypothesis of a causal relationship between the time course of muscle fatigue and VO_{2CL} during high-intensity exercise.

Of note, according to Grassi, Rossiter, & Zoladz (2015) during high-intensity exercise in humans, the muscle inefficiency and fatigue show reciprocal association, and during constant power and incremental exercise protocols the two phenomena seem to be strictly intertwined. However, these authors question whether the dynamics of intramuscular fatigue *per se* are associated with progressive muscle inefficiency since that the VO_{2SC} of the metabolic processes are derived largely from within the muscles generating the external locomotive power. In fact, some data support the notion that muscle fatigue accompanies the reduced efficiency during exercise above the critical power (CANNON et al., 2011; FROYD et al., 2013). However, as suggested by Grassi, Rossiter, & Zoladz (2015) further work is required to establish whether fatigue and muscle inefficiency progress with the same time course. This is because the association between skeletal muscle fatigue and decreased efficiency seems obvious, but the evidence for common denominators/common mechanisms be between the two phenomena yet not very well understood.

2.9 Muscle oxygenation and caffeine

Near-Infrared Spectroscopy (NIRS) is a non-invasive equipment that allow to measure an arbitrary rate of oxygen extraction from a certain tissue, such as the skeletal muscles, and which reflects the imbalance between supply and use within the muscle (COLOSIO et al., 2021).

Although the use of NIRS applied to skeletal muscle has often been viewed with some skepticism by many exercise physiologists, mainly due to the various limitations involved, technological advances have attempted to alleviate these concerns (GRASSI & QUARESMA, 2016). However, inevitably previous studies have demonstrated that NIRS is a valuable, reliable tool for evaluating muscles in humans, presenting precision and reproducibility regarding muscle oxidative metabolism (COLOSIO et al., 2021; GRASSI & QUARESMA, 2016). Furthermore, the equipment is non-invasive and has a reasonably long time of high resolution, suggesting that it is an extremely important method not only for athletes and physically active individuals, but also has fundamental importance in the investigation of individuals with some pathological situation that impair the oxidative capacity of muscles.

The assessment of muscle oxygenation during an exercise such as cycling, along with the behavior of VO_2 at the lung level, allows a comprehensive view of metabolic demand and their contributions to different exercise intensity. Indeed, a previous study has suggested the validity and possibility of implementing measurements of peripheral metabolism via NIRS to evaluate the relative contribution of metabolic instability (measured with NIRS and haematochemical markers) and muscle activation (measured with EMG) to the VO_{2sc} in different intensity domains. (COLOSIO et al., 2021).

Furthermore, CAF is related to an increased nitric oxide production, which consequently lead to augmented tissue blood flow and oxygen supply for the exercising muscles (Ruíz Moreno et al. 2020), and potentially enhance muscle contractility and reduce exercise induced fatigue (LIMA-SILVA et al. 2021). Ruíz-Moreno et al., (2020) investigated the effects of CAF on muscle oxygen saturation during exercise of increasing intensity. Their results suggest that CAF induced changes in muscle oxygen saturation during submaximal workloads, suggesting that this mechanism might also contribute to CAF's ergogenic effect.

Therefore, investigating these concurrent mechanisms when using gum can shed light on CAF's impact on active muscles, blood flow redistribution, and oxygenation maintenance, as no previous research has explored the link between GUM_{CAF} intake and $\dot{V}O_2$ and HHb+Mb kinetics.

2.10 Neuromuscular function and caffeine

The classical method for assessing the neuromuscular function is performed by the measurement of force/torque from isometric maximal voluntary contractions (IMVC). Commonly, these measures are performed together with transcranial magnetic stimulation (ABOODARDA et al., 2018) or percutaneous electrical stimulation at nerve locations (MIRA et al., 2018). Although this approach is widely used, there are several concerns for using that technique, especially in the "real life" sports context. Considering that muscle fatigue is a transient event (ALLEN et al., 2008), a single measurement performed in only selected muscle groups may not reflect the total muscle fatigue developed in certain sports contexts. However, it is important to recognize that is the only way to measure the neural and muscular underpinnings of the performance decline. The intensity and duration of exercise, the engaged muscle mass, the mode and speed of muscle actions are factors that strongly influence the magnitude of decline in maximal voluntary force and the relative contribution of changes in contractile function (BEHRENS et al., 2023), along with environmental conditions (temperature, altitude) (PLACE & MILLET, 2019). All these aspects should be taken into account when measuring the muscle performance decline (i.e. fatigue). In addition, the specificity of exercising mode (i.e. sports) characterized mainly by dynamic contractions could be another limitation in detecting fatigue during the IMVC. Finally, the measurement timing is a preponderant factor in the assessment of muscle performance/fatigue (PLACE & MILLET, 2019). The duration between the completion of exercise and testing of muscle performance/fatigue, commonly takes 1 to 4 min, and this delayed assessment is problematic since recovery starts immediately after exercise has stopped (PLACE & MILLET, 2019). Thus, this time delay can affect the proper detection of both neural and contractile properties. In this way, alternative methods that involve a more realistic approach are necessary, for a better understanding of fatigue induced by exercise.

Sun et al., (2022) conducted a systematic review and meta-analysis to summarize current experimental findings on the effects of CAF on physiological indexes before and after neuromuscular fatigue. Their main findings suggest that CAF intake had a relatively large effect on the voluntary activation (VA), potentiated twitch, M-wave, which can be used as characteristic indexes of CAF's impact on neuromuscular fatigue. However, this conclusion tends to indicate the effects of caffeine on neuromuscular fatigue during endurance running or jumping or muscle bending and stretching. Thus, according to the authors future research needs to explore other physiological indicators and their indicative effects in order to determine effective and accurate characteristic indicators of CAF on neuromuscular fatigue.

In addition, Couto et al., (2022) investigated whether endurance performance and neuromuscular fatigue would be affected by CAF (capsules, 5 mg/kg) ingestion during closed- and open-loop exercises. Their results showed that CAF improved endurance performance in both modes of exercise without influence on post-exercise central and peripheral fatigue. Black et al. (2015) have demonstrated that CAF and PLA capsules triggered an improvement in MVC (5.7 and 2.5%, respectively) 60 min after ingestion. Furthermore, the IMVC of the knee extensors was reduced to a similar extent for both conditions, after 30 min cycling at 60% of $\dot{V}O_{2MAX}$ followed by a time trial of 10 min. Although post-exercise neuromuscular fatigue measurements performed by Black et al. (2015) were assessed 20 min after exercise cessation, compromising greater inferences and comparisons with other studies, once when central and peripheral fatigue might have been largely recovered.

2.11 Placebo ingestion and cognitive aspects and exercise performance

The ingestion of PLA has been considered as an inert substance or a procedure of a given treatment or intervention that improves the outcome (BENEDETTI, 2008; PAINELLI et al., 2020). When it comes specifically to the expectation of ergogenic effects experienced after ingesting CAF, this is an extremely relevant subject that has aroused great interest in sports science. For example, Shabir et al. (2018) reported potential effects of CAF expectancy in 76% of a total of 17 intervention studies on sports, exercise, and cognitive performance. Given this, the authors suggest that if an individual believes that they have consumed CAF and believes that CAF is ergogenic, they are likely to experience a performance benefit even if CAF was not consumed.

In the study of Smith (2009) investigating the effects of supplementation with 40 mg of GUM_{CAF}, placebo chewing gum (PLA) and control, demonstrated among other results that the PLA condition was associated with a more positive mood. The author suggests that cognitive benefits associated with chewing reflect changes induced by the act of chewing *per se* (e.g. increased muscle tension or heart rate). In addition, it is believed that regular chewing has been associated with a reduction in stress (FRC RESEARCH CORPORATION, 2006) which may be related to stimulation of the vagus nerve (SHIBA et al., 2002).

In fact, some studies point out for changing in brain responses related to the expectancy for the effect of an active substance, in this case the ingestion of CAF, when using the PLA condition (PIRES et al., 2018; WAGER et al., 2014; LIU et al., 2017). Therefore, experimental designs that assess brain responses induced by the belief of ingesting CAF are preferable when involving the PLA condition (PIRES et al., 2018; WAGER et al., 2004, 2011; LIU, 2017).

According to previous studies (KIRSCH & WEIXEL, 1988; SAUNDERS et al., 2016) the use of double-blind experimental designs is questioned and a possible source of bias, since when individuals are informed that there is a 50%-50% chance of ingesting a substance, the performance results in motor tests seem to suffer a possible influence (BEEDIE et al., 2006; FOAD et al., 2008).

According to Panelli et al. (2020), the scenario involving placebo has been little explored. It is believed that previous experience and habituation are aspects that deserve to be highlighted, as they can provide learning in relation to the treatment, and that consequently enable an increase in participants' expectations about the ergogenic effects of CAF (PAINELLI et al., 2020; MONTGOMERY & KIRSCH, 1997). Furthermore, it is suggested that an enhancement of individuals' responses to CAF ingestion may occur due to prior knowledge of the treatment or the intention to use an ergogenic resource (HURST et al., 2017; GEERS et al., 2005). The personal characteristics is another point that would affect the effects of PLA, such as optimism, pessimism, anxiety and fear, which can increase the perception of positive effects or generate non-effects of PLA through an increased negative effect on the perception of the treatment (PAINELLI et al., 2020).

Therefore, in short, the ingestion of PLA perceived as CAF seems to induce improved performance according to the results of some studies (FOAD et al., 2008; PIRES et al., 2018), challenging the real effects of ingesting this ergogenic.

2.12 Torque/speed and power/speed relationship in cycling

Cadence is considered the number of revolutions per minute (rpm) during a typical pedaling movement. Crank torque is the product of the perpendicular force applied to the pedal versus the length of the crank arm (lever). The power output expressed in watts (W) is derived multiplying the torque value in Newtons per meter (N·m) by the angular velocity (i.e. cadence) expressed in radians per second (rad/s). This dynamic propulsion torque has been used as a powerful tool in the studies looking at the mechanical efficiency in cycling (COYLE, et al., 1988; 1991). Besides that, the measurement of force/torque applied in the pedals during the cycling activity could provide important information regarding the performance and fatigue.

In this way, as mentioned above when a force is applied to the pedal a perpendicular force is transmitted to the pedal, generating a component called “effective force”, which is responsible for the power production, when combined with the cadence. The quality of pedaling technique has often been analyzed by the behavior of “effective force”.

The first studies to analyze the forces applied to the pedal during pedaling were conducted in the middle of the last century. The results demonstrated that at an angle of 90°, that is, the pedal in a horizontal position was the point of greatest application of force. Later in the early 1980s, studies were conducted to find ways to improve mechanical efficiency in applying force to the pedals and discovered that the use of clips and overloads would be efficient methods for improve the efficiency (DAVIS & HULL, 1981).

The application of force during pedaling provided a tangential force and it would be the most related to the efficient force applied. On the other hand, the centrifugal force, which is a perpendicular force to the tangential, does not contribute to mechanical efficiency (Figure 1). Using the ratio between the tangential force and all other forces applied to the pedal, it is possible to calculate the mechanical efficiency.

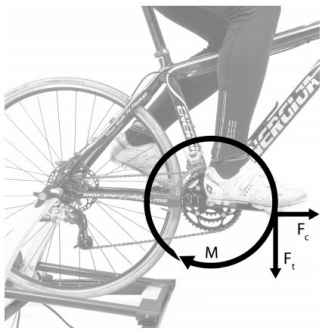

Figure 1. Overview of the forces applied to the pedal during the "downstroke" phase at 90°, where M= propulsive torque; Fc = centrifugal force and Ft = tangential force (adapted from FONDA & SARABON, 2010).

Measuring the maximum capacity of generate force in a short period of time is essential for determining the performance during certain competitive events or even for measure the fatigue. Consequently, when the relationship between force and speed is obtained, it is possible to calculate the maximum power output (SARGENT et al., 1981; BARON et al., 1999). However, the relationship of force (or torque) and speed denotes an exponential one, that is, the greater the speed of a concentric contraction, the smaller the applied force, that is, the greater forces are applied at lower speeds. This paradigm between force and speed has theoretical support from Huxley's theory of cross-bridges (1957), which states that maximum force production can be found at the ideal speed of myofibril coupling, and is achieved in isometric contractions, i.e. velocity equal to zero.

2.13 The cycling sprint

The PPO and the capacity for anaerobic energy production can be measured by work and mechanical power on the cycle ergometer (VANDELWALLE et al., 1985). As aforementioned, in cycling, PPO is a very important index for predicting performance (DOREL et al., 2005), which can be obtained by short and maximum efforts (sprints) between 5 and 10 s (GLAISTER 2019; KORDI et al., 2019; KRUGER et al., 2019).

From the studies using isokinetic cycle ergometers, the maximum capacity to generate force began to be widely studied in cycling. The two components for calculating external power output are the effective force and pedal-level speed. In this way, when a sprint is performed and the moment of inertia of the flywheel is known, if the applied frictional force and the speed and acceleration of the flywheel can be accurately measured, the force and power can be monitored (DOREL, 2018). Thus, depending on the sampling rate, torque-speed and torque-power relationships during a single sprint (Figure 2) can be tracked and this methodology continues to be very useful when using inertial-loaded friction (DOREL et al. 2003) or just cycle ergometers with inertial load (MARTIN et al. 1997; DOREL, 2018). Numerous investigations have been conducted focusing on the relationship between pedaling rate and torque-power; and a linear relationship is commonly reported between pedaling torque-rate and quadratic pedaling power-rate (GARDNER et al., 2007). Although it is worth highlighting that due to different types of ergometers, the comparisons between studies are difficult. However, with the advancement of the new technology commercially available, it has become possible to measure the force directly at the crank or pedal by using strain gauges, and the estimation of the resistive torque (braking or magnetic) is no longer necessary.

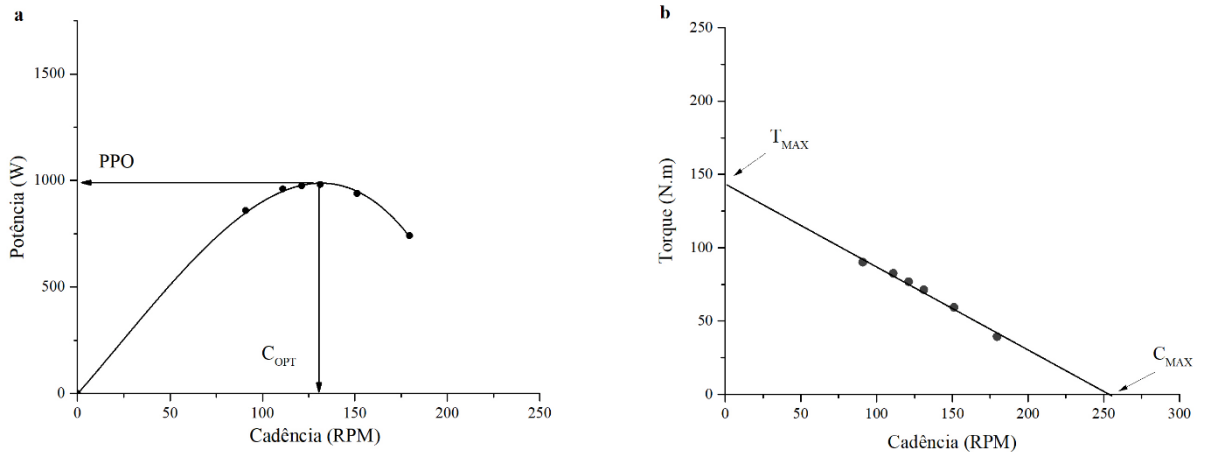

Figure 2. a The power-cadence relationship in sprint cycling. The apex of the parabolic relationship represents the peak power (PPO) and the cadence at PPO represents the optimal cadence (C_{OPT}); **b** Torque-cadence relationship from a cycling sprint test. Linear relationships were extrapolated to axis intersections to calculate maximum torque (T_{MAX}) and cadence (C_{MAX}).

The results of previous studies have suggested a torque/power relationship commonly obtained through a sprint lasting between 7 and 10s (GLAISTER 2019; KORDI et al., 2019; KRUGER et al., 2019), using a torque factor between 1.00 – 1.25 ($N \cdot m \cdot kg^{-1}$), since higher W_{PICO} values have been found in this range (BUSKO, 2005; WINTER, et al., 1996). Consequently, an important variable that has been widely investigated is the relationship between power output (PPO) and cadence, which obtains the intensity referring to the optimal cadence for achieving peak PPO, which is called OPT_{CAD}. In this way, studies have compared protocols with increments of the torque factor, aiming to determine the OPT_{CAD} (GLAISTER et al., 2019) and compare the magnitude of its values with traditional inertial methods, such as the classic Wingate protocol. Glaister et al. (2019) reported that OPT_{CAD} can vary between individuals because it is directly related to the level of physical conditioning and the type of muscle fiber. Therefore, it is believed that optimal velocity could be found with values of approximately 120 rpm, however a variation of 100 to 135 rpm may occur in endurance and sprint/power athletes, respectively.

Since previous studies demonstrated similar results of pedaling rate and torque-power relationships when applied in laboratory and field environments (GARDNER et al., 2007), different models were proposed. Among the main cycling sprint models used in the laboratory are isokinetic (SARGEANT et al., 1981), force-velocity (VANDEWALLE et al., 1985) and inertial load (MARTIN et al., 2007); and similar relationships were found, but on the other hand

also questioned (KORDI et al., 2019). Kordi et al. (2019) investigating and comparing the magnitude and reliability of PPO, maximum torque (T_{MAX}), maximum cadence (C_{MAX}) and optimal cadence (C_{OPT}) measured with isovelocity (isokinetic method) and isoinertial sprint methods (torque factor and free cadence), found that the functional measures were highly reliable, but suggest that these models should not be used interchangeably. This was pointed out by the authors due to the results of higher PPO and T_{MAX} using the isoinertial method; and higher C_{OPT} and C_{MAX} using the isovelocity method. Therefore, there is a question about which sprint model should be used.

Obviously, an analysis of all the factors inherent to the construction of the hypothetical model to obtain the different variables such as T_{MAX}, PPO, C_{MAX} and C_{OPT} must be carried out with caution. If, on the one hand, models that use a torque factor (isoinertial load) supposedly allow greater force production and a free cadence, closer to the cyclist's reality; on the other hand, isokinetic models have the main advantage of collecting more data and precision in obtaining points at a determined and specific cadence, with higher C_{OPT} and C_{MAX} values being able to be found (KORDI et al., 2019). Therefore, as only one previous study compared two models (isoinertial vs isovelocity) on the same sample and ergometer, and although it showed high levels of reliability between sessions, the conclusion was that these models are not interchangeable (KORDI et al., 2019), and therefore, the need to investigate new models becomes evident.

Perhaps as a suggestion, in the isoinertial model, the torque factor used makes it possible to achieve higher T_{MAX} than the isovelocity model, and perhaps the use of a linear isoinertial model without torque factor (ISO_{LIN}) and free cadence, could not produce higher values like the isoinertial load model, but close to the isovelocity method and allow the exchange of functional measurements between the models. These methods are relatively easy to conduct, allow valid PPO measurements (DRISS & VANDEWALLE, 2013; MARTIN et al., 1997) and their use is essential for coaches, athletes and those practicing the sport to monitor speed cycling performance.

3. CHAPTER TWO

3.1 STUDY ONE: Reliability and validity of cycling sprint performance at isolinear mode without torque factor: A preliminary study in well-trained male cyclists.

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Original research

Title

Reliability and validity of cycling sprint performance at isolinear mode without torque factor: A preliminary study in well-trained male cyclists.

Running Head: Cycling sprint performance at isolinear mode

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Abstract

Purpose: This study aimed to compare the performance-derived parameters utilizing isolinear (ISO_{LIN}) and isovelocity (ISO_{VEL}) sprint cycling modes. Method(s): For that, 20 male trained cyclists performed 2 sprints of 7 s on an electromagnetically braked cycle ergometer in ISO_{LIN} and six sprints in ISO_{VEL} mode with cadences between 90 and 180 rpm, each separated by 3-min. A linear function modeled the sprints within each mode to extrapolate maximal cadence (C_{MAX}) and torque (T_{MAX}), and a quadratic function was used to extrapolate the apex defined as optimal cadence power (OPT_{CAD}) and peak power output (P_{MAX}). Fifteen subjects performed another 4 sprints at ISO_{LIN} mode on different days to verify the reliability. Results: The measures from the power-cadence relationship were not different between the ISO_{LIN} and ISO_{VEL} modes. Although significant differences were detected in the T-C relationship, T_{MAX} was greater at ISO_{LIN} than ISO_{VEL} ($p = 0.006$). On the other hand, C_{MAX} was higher at ISO_{VEL} than ISO_{LIN} ($p < 0.001$). The correlation between parameters was large to very large ($r = 0.51$ to 0.89). However, high limits of agreement were verified. The ISO_{LIN} presented consistency during the trials, and the random errors were acceptable (CV = 5.3% to 11.5%). Conclusion(s): Using the power-cadence relationship, P_{MAX} and OPT_{CAD} could be detected similarly between the two sprint modes (ISO_{LIN} and ISO_{VEL}). Thus, the findings demonstrated that a single ISO_{LIN} sprint test could be a suitable tool for quantifying the time course of muscle fatigue during and after cycling exercises in well-trained male cyclists.

Keywords: Maximal power, force-velocity test, isokinetic cycling, cadence

3.1.1 Introduction

In cycling exercises, the maximal power output can be assessed by work and mechanical power throughout sprint tests on a cycle ergometer (Driss & Vandewalle, 2013; Vandewalle et al., 1985). Several studies have reported a hyperbolic power-pedaling rate relationship and the 'quasi' linear torque/force-pedaling rate relationship for maximal cycling (Gardner et al., 2007). While the former derivates the maximal power (P_{MAX}) and its optimal cadence (OPT_{CAD}), the latter provides estimates of maximal torque (T_{MAX}) and cadence (C_{MAX}). It is well known that these parameters during cycling are essential to predict sprint performance (Dorel et al., 2005) and to detect changes induced by muscle fatigue (Sargeant, 1994; Kruger et al., 2019)

Previous studies have demonstrated similar results in pedaling rate-torque or -power relationships when applied in laboratory and field settings (Gardner et al., 2007). Among the leading sources for obtaining the torque-cadence (T-C) and power-cadence (P-C) relationship from sprint cycling in the laboratory is the functioning mode of the ergometer, such as the isokinetic/isovelocity (Sargent et al., 1981) or the isoinertial (Vandewalle et al., 1985; Martin et al., 2007). Although estimated parameters seem similar (Wackwitz et al., 2021), several factors could affect this relationship (Kordi et al., 2019). While isokinetic protocols have the advantage of more data in obtaining maximal torque values at specific cadences, they require a set of tests in various cadences (Kordi et al., 2019). On the other hand, one practical advantage of the isoinertial mode would be that T–C and P–C relationships could be obtained from a single test. This would be instrumental in quantifying the time course of muscle fatigue, particularly during continuous cycling. Previous studies have been based on 2 to 3 fixed cadences for measuring peak isokinetic torque (and fatigue) over time during cycling exercise protocols (Cannon et al., 2011; do Nascimento Salvador et al., 2018). Apart from requiring several laboratory visits for each participant, such an experimental approach does not permit the T–C spectrum analysis, characterized by the dynamic force production capabilities at low (T_{MAX}) and high (C_{MAX}) cycling cadences. These parameters could provide a better estimation of the effects of fatigue on muscle performance than the instantaneous torque/power measured at a given cycling cadence. Therefore, a single reliable and valid test to estimate the parameters from T–C and P–C relationships would be helpful in quantifying muscle fatigue during and after cycling exercises.

The assessment of changes in T–C and P–C relationships may be equally critical to quantify the impact of a fatiguing task, particularly during dynamic whole-body exercises, because of task specificity. In fact, Kruger et al. (2019) demonstrated that a single 7 s all-out acceleration test was very reliable and valid for identifying distinct fatigue responses following

different durations of dynamic exercise. Their findings indicate that when compared to the isometric assessment of fatigue, dynamic and isometric assessments are not interchangeable following dynamic exercise with a large muscle mass. Such results could be specific to the recumbent cycle ergometer utilized due to a more stabilized body position for maximal force production. Furthermore, it was demonstrated that the braking loads could affect the goodness of fit in T–C and P–C relationship curves, which is particularly important when single sprint evaluations are required (Kruger et al., 2020). This study has also shown that reducing the sprint load for tests occurring at a fatigued state improved the fit of the data. Therefore, the ergometer characteristics (gear ratio, flywheel inertia, and radius) and, hence the braking load are important aspects to consider when a single sprint evaluation is required to assess the effect of fatigue and recovery. One of the most used cycle ergometers is the Excalibur Sport from LODE, with over 3000 occurrences on Google Scholar. Since Excalibur Sport has a high flywheel inertia and the reduction in sprint load provides a better fit of data in a fatigued state, all-out sprint tests without a torque factor could be "the more generalized" load for a single sprint evaluation. Thus, this investigation aimed to verify the reliability and validity of a single sprint performed without torque factor in estimating the parameters derived from T–C and P–C relationships. The hypothesis is that a single sprint evaluation using as low as possible braking resistance for the Excalibur Sport cycle ergometer could provide valid and reliable estimates of P_{MAX}, T_{MAX}, C_{MAX}, and OPT_{CAD}.

3.1.2 Methods

Subjects

This study used an intentional non-probabilistic sample of twenty male cyclists (mean ± standard deviation [SD]; age: 26.5 ± 6.0 years; height: 177.2 ± 5.4 cm; body mass: 70.6 ± 4.7 kg; maximal oxygen uptake 57.3 ± 6.4 mL·kg-1·min-1) that volunteered to participate in the study. All participants had at least two years of training and competition experience. Just prior to the period of investigation, cyclists were training at least of 8 h per week of cycling sessions. According to De Pauw et al. (2013), the participants were classified as "well-trained cyclists (performance level 3)". The Institutional Ethics Committee approved the study protocol and complied with the Declaration of Helsinki. The participants were instructed to avoid participating in any strenuous exercise the 48-72 hours before the testing day and refrain from consuming CAF and alcohol for 12 h before each test session.

Procedures

Participants performed five experimental sessions in the laboratory under controlled laboratory conditions (temperature 22 ± 1 °C and relative humidity 50 ± 2%), and all testing was conducted at the same time of day (± 1 h). The sprint efforts were performed on an electronically braked cycle ergometer (Excalibur Sport PFM, Lode BV, Groningen, Netherlands) equipped with a pedal force measurement system that allowed the cadence (rpm), power (W), and right/left crank torques (N·m) to be collected, with a crank length of 175 mm. The seat and handlebar heights were individually adjusted for all the tests. All participants completed a maximal incremental cycling test and passively rested for 15 min before the performance of cycling sprints (ISO_{LIN} and ISO_{VEL}).

Sprints

After 3 min warming-up at 50 W, each subject performed two ISO_{LIN} sprints followed by six ISO_{VEL} sprints, resting 3 min between each sprint. All the sprints were performed with a duration of 7 s keeping the seated position, preceded by a 5 s countdown to start the effort, in which they were instructed to pedal at their preferred cadence. The ISO_{VEL} sprints were performed in the following order: 90, 110, 120, 130, 150, and 180 rpm. The interval among sprints comprised 2.5 min of passive rest plus 30 s pedaling at 50 W at the preferred cadence. Then, the “flying start sprint” was performed to avoid the typical inertia force delay of static start sprint. ISO_{LIN} sprints started at the preferred cadence because the purpose was to assess a suitable tool for quantifying the time course of muscle fatigue during and after cycling exercises. Figure 1 details the design of study where it was required six repetitions in ISO_{VEL} and only twice in ISO_{LIN}. During the sprints, the cyclists remained seated. They did not assume a standing position during the acceleration phase, which is known to allow greater power production (Reiser et al. 2002), and used standard pedals fitted with toe clips and straps.

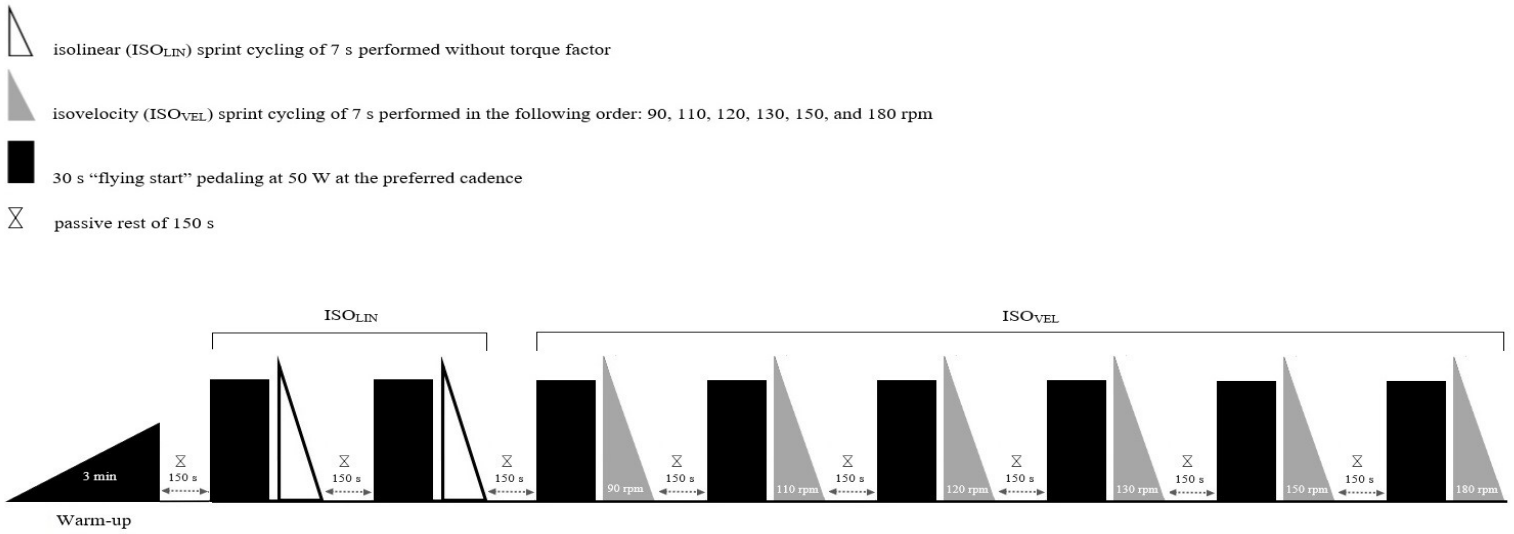

Figure 1. Study Design. After 3 min warming-up at 50 W (at preferred cadence), each subject performed two ISO_{LIN} sprints followed by six ISO_{VEL} sprints, resting 3 min among sprints. All the sprints were performed with a 7-s duration with the subjects assuming a sitting position, preceded by a 5 s countdown to start the effort. The ISO_{VEL} sprints were performed in the following order: 90, 110, 120, 130, 150, and 180 rpm. The interval among sprints comprised 2.5 min of passive rest plus 30 s pedaling at 50 W before the "flying start sprint" (i.e. avoiding the inertia force delay when the sprint is started from the static start).

Cadence-time and cadence-power relationship parameters

The data were collected from the hardware of the Pedal Force Measurement (PFM) option of the Lode Excalibur Sport ergometer. This system offers many possibilities for analysis like forces on the left and/or right crank, the extra visualizations in the field of pedal force measurement, and its report with statistics of the measured data afterward. Thus, torque and angular velocity data were recorded every two degrees of a pedal stroke, accumulating 180 information per pedal revolution.

After that, data was transferred to an Excel spreadsheet (Microsoft Corporation, Redmond, Washington, USA) and analyzed to calculate the average torque, power, and cadence per revolution. In the ISO_{VEL} mode, the average power output for each pedal revolution at pre-set cadence was used to produce the T-C and P-C relationships. From the two sprints performed in ISO_{LIN} mode, that with the best mathematical fit was used for further analysis, according to the criteria: a) highest R2 value; and b) greater number of points in the adjustment (Dorel, 2018). For the ISO_{LIN} method, the values of each sprint between the second and the fifth second were used initially to build the T-C and P-C relationships. Subsequently, a linear adjustment was applied, and the value of "one and a half SDs (1.5)" was adopted to insert or even remove some other points for constructing T-C and P-C relationships. Thus, a linear function was used to fit individual T-C relationships, and intercepts extrapolated in both axes correspond to T_{MAX} and C_{MAX}, respectively. In this way, we tried to approximate the same number of rotations (data points) for each method to produce the T-C and P-C relationships and enable these interchangeable functional measures. A quadratic function, with both non-constrained and constrained intercept, was used to fit values of cadence rate and power, the apex defined as optimal cadence power OPT_{CAD} and P_{MAX} and OPT_{CADCT} and P_{MAXCT}, respectively (Samozino et al. 2007). Figure 2 provides an overview of the T-C and P-C relationship parameters.

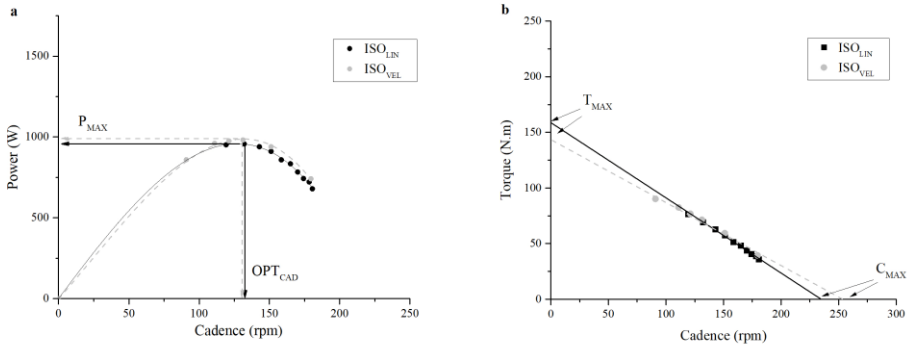

Figure 2. The Power-cadence relationship of both isolinear and isovelocity sprint cycling modes. The apex of the parabolic relationship represents maximal power output and cadence at maximal power output represents optimal cadence (**a**); Torque-cadence relationship of isolinear and isovelocity sprint cycling tests. The linear relationships have been extrapolated to the axis intercepts to calculate maximal torque and cadence (**b**).

C_{MAX}: maximal cadence; OPT_{CAD}: optimal cadence; ISO_{LIN}: isolinear sprint; ISO_{VEL}: isovelocity sprint; N·m: Newton-meters; P_{MAX}: maximal power output; rpm: rotations per minute; T_{MAX}: maximal torque; W: watts.

Reliability of isolinear-derived parameters

From the 20 subjects that performed the ISO_{LIN} and ISO_{VEL} sprints protocols, 15 subjects completed another four trials of sprints at ISO_{LIN} mode on different days, each test separated by 3 to 14 days. Procedures are the same as described above.

Statistical analysis

Descriptive data were expressed as mean ± SD, and the statistical data as mean point estimate with confidence intervals (CI) of 95%. To determine the validity of ISO_{LIN} against the ISO_{VEL} the measures P_{MAX}, P_{MAXCT}, T_{MAX}, C_{MAX}, OPT_{CAD}, and OPT_{CADCT} were compared

employing a paired t-test, the bias ± 95% of limits of agreement (LoA) of the Bland and Altman graphical analysis (Bland and Altman, 1986), and a simple linear regression with the Pearson product-moment correlation (r). A linear regression was performed to verify homoscedasticity (constant dispersion of differences across the range of averages) or heteroscedasticity (progressive increase in dispersion as the averages increase) between the ISO_{LIN} and ISO_{VEL} measures on the Bland-Altman plots (Ludbrook, 2010). For the reliability of ISO_{LIN}-derived parameters, a mixed effects model was conducted to determine the systematic errors, where the fixed effect is the trial number and the random effect is the identity of each subject. Random errors were accessed by the typical error of measurement (TEM), and the TEM normalized as percentual of the mean (i.e. coefficient of variation [CVTEM]), and the intraclass coefficient of correlation (ICC) type 3,1 (Shrout & Fleiss, 1979). The r values were interpreted as follows: <0.10, trivial; 0.10 - 0.29, small; 0.30 - 0.49, moderate; 0.50 – 0.69, large; 0.70 - 0.89, very large; and 0.90 – 1.0, almost perfect (Hopkins, 2015). ICCs of 0.14, 0.36, 0.54, 0.69 and 0.83 were defined as threshold for classifying to low, moderate, high, very high and nearly perfect, respectively (Malcata & Hopkins, 2014). The statistical significance was set at $p < 0.05$. Analyses were performed in the software GraphPad Prism version 8.1.2 (GraphPad Software, La Jolla, CA, USA) and using an Excel spreadsheet (Microsoft Corporation, Redmond, Washington, USA) for reliability analysis (Hopkins, 2015).

3.1.3 Results

Comparison of isolinear- and isovelocity-derived parameters

Regarding the goodness of fitting of T-C and P-C relationships, there were significant differences for ISOLIN and ISOVEL intra-methods when applied constrained and non-constrained intercept ($R^2 = 0.976 \pm 0.016$ vs. 0.991 ± 0.012; $p < 0.001$; and $R^2 = 0.953 \pm 0.045$ vs. 0.991 ± 0.005, $p < 0.001$ respectively), but were no significant differences inter-methods ($p = 0.074$ and $p = 0.851$, respectively).

The descriptive parameters derived from ISO_{LIN} and ISO_{VEL} are presented in Table 1. The T_{MAX} was higher for ISO_{LIN} than ISO_{VEL} mode ($p = 0.006$), while C_{MAX} and OPT_{CADCT} were higher for ISO_{VEL} than ISO_{LIN} mode ($p < 0.001$ and 0.002, respectively). No significant differences occurred between ISO_{LIN} and ISO_{VEL} for P_{MAX}, P_{MAXCT}, and OPT_{CAD} ($p = 0.144$, 0.525, and 0.838, respectively).

The relationship between ISO_{LIN}- and ISO_{VEL}-derived parameters is presented in Figure 3. Correlation coefficients were large to very large (i.e. r = 0.51 to 0.89). Figure 4 shows the agreement between the parameters derived from ISO_{LIN} and ISO_{VEL}. All comparisons presented

homoscedasticity (i.e. no linear tendency [or correlation] of the bias along the average values). Wide values of LoA occurred for P_{MAX} and P_{MAXCT} (±128 and ±124 W, respectively), moderately wide values occurred for OPT_{CAD}, OPT_{CADCT}, and C_{MAX} (±19, ±17, and ±36 rpm, respectively) and for T_{MAX} (±36 N·m).

Table 1. Comparison of isolinear- and isovelocity-derived parameters from cycling sprints.

Parameter	Isolinear	Isovelocity	p-value
P_{MAX} (W)	969 ± 145	991 ± 133	0.144
P_{MAXCT} (W)	982 ± 136	972 ± 132	0.525
OPT_{CAD} (rpm)	128 ± 10	128 ± 10	0.838
OPT_{CADCT} (rpm)	118 ± 12	125 ± 12	0.002
T_{MAX} (N·m)	159 ± 21	147 ± 19	0.006
C_{MAX} (rpm)	237 ± 24	255 ± 38	< 0.001

Values are presented as mean ± standard deviation; n = 20.

C_{MAX}: maximal cadence; OPT_{CADCT}: optimal cadence with constrained intercept; OPT_{CAD}: optimal cadence; N·m: Newton-meters; P_{MAX}: maximal power output; P_{MAXCT}: peak power output with constrained y-intercept; rpm: rotations per minute; T_{MAX}: maximal torque; W: watts

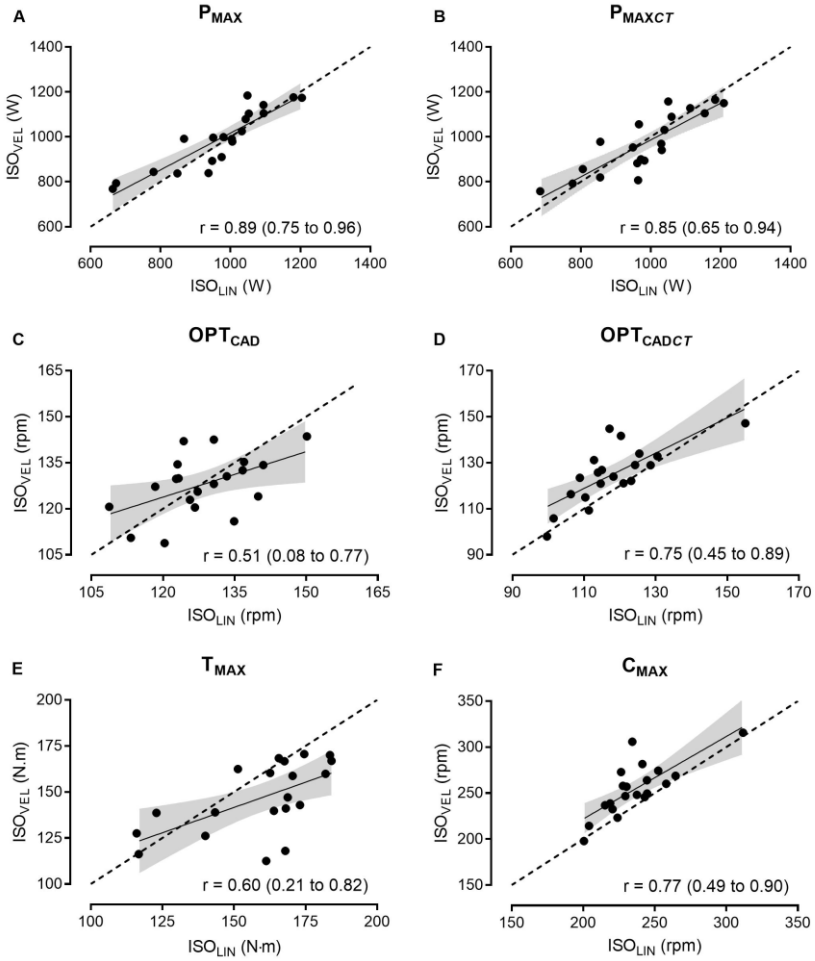

Figure 3. Relationship between isovelocity- and isolinear-derived parameters maximal power output with non-constrained (A) and constrained intercept (B), optimal cadence with non-constrained (C) and constrained intercept (D), maximal torque (E), and maximal cadence (F). For all panels n = 20, correlations are presented as mean point estimates (black continuous line) and 95% confidence intervals (grey shaded area), the dashed line is the identity line (i.e. y = x). C_{MAX}: maximal cadence; OPT_{CADCT}: optimal cadence with constrained y-intercept; OPT_{CAD}: optimal cadence; ISO_{LIN}: isolinear sprint; ISO_{VEL}: isovelocity sprint; N·m: Newton-meters; $P_{MAX:}$ maximal power output; P_{MAXCT}: maximal power output with constrained intercept; r: Pearson product-moment coefficient of correlation; rpm: rotations per minute; T_{MAX}: maximal torque; W: watts.

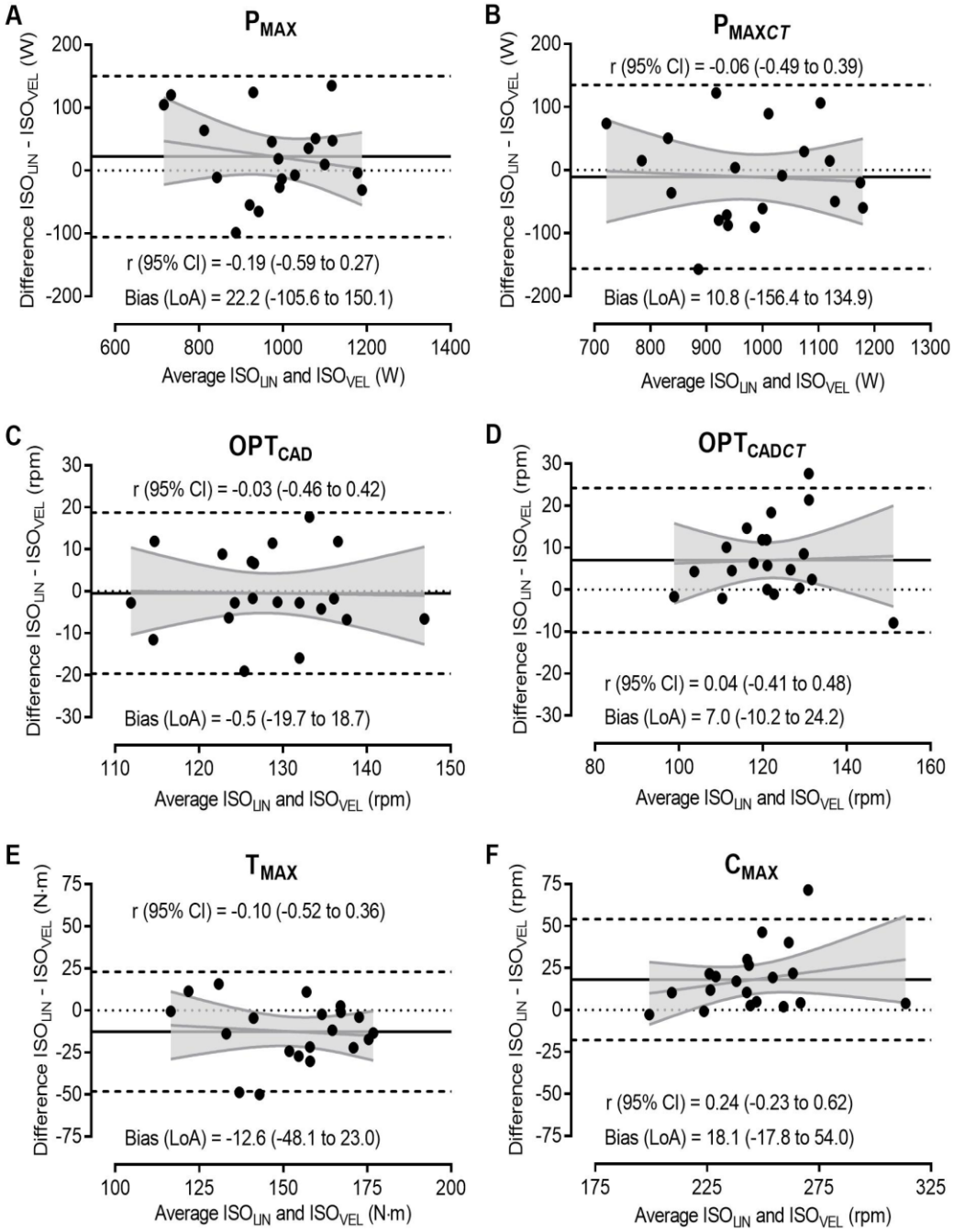

Figure 4. Agreement and a simple linear regression with the Pearson product-moment correlation (r) between isovelocity- and isolinear-derived parameters maximal power output with non-constrained (A) and constrained intercept (B), optimal cadence with non-constrained (C) and constrained intercept (D), maximal torque (E), and maximal cadence (F).

For all panels n = 20, points are the individual values, the dotted line represents the zero difference, the continuous line is the bias, and dashed lines are the upper and lower 95% limits of agreement bounds (i.e. the standard deviation of the differences × 1.96), and the gray shaded

area represents the 95% confidence interval for the linear regression between the bias and the average values to verify homoscedasticity or heteroscedasticity.

CI: confidence interval; C_{MAX}: maximal cadence; OPT_{CAD}*CT*: optimal cadence with constrained intercept; OPT_{CAD}: optimal cadence; ISO_{LIN}: isolinear sprint; ISO_{VEL}: isovelocity sprint; LoA: limits of agreement; N·m: Newton-meters; P_{MAX}: maximal power output; P_{MAX}*CT*: maximal power output with constrained intercept; r: Pearson correlation coefficient; rpm: rotations per minute; T_{MAX}: maximal torque; W: watts.

Reliability of isolinear-derived parameters

Table 2 presents the observed mean ± SD of each trial and the reliability results for ISOLIN-derived parameters. Overall, there were no systematic errors along the repeated trials ($p > 0.05$) for any parameter. Random errors presented acceptable absolute reliability based on CVTEM values for P_{MAX} (5.9%), OPT_{CAD} and OPT_{CADCT} (6.2% and 5.3%, respectively). Less acceptable reliability occurred for C_{MAX} (7.3%), T_{MAX} (9.1%), and P_{MAXCT} (11.5%). Relative reliability (i.e. ICC values) were very high and nearly perfect for P_{MAX} and P_{MAXCT} (0.84 and 0.77, respectively), high for T_{MAX} (0.66), and moderate for OPT_{CADCT}, OPT_{CAD}, and C_{MAX} (0.34 to 0.45).

Table 2. Reliability of derived parameters from isolinear sprints.

Parameter	Trial number					Mixed effects model	TEM*	CV (%)	ICC
	1	2	3	4	5				
P_{MAX} (W)	943 ± 152	975 ± 141	967 ± 129	994 ± 109	952 ± 119	$F_{(2,25)} = 0.885$; p = 0.423	52.4 (44.2 to 64.4)	5.9 (4.9 to 7.3)	0.84 (0.71 to 0.93)
P_{MAXCT} (W)	955 ± 139	1002 ± 136	979 ± 140	974 ± 124	948 ± 205	$F_{(3,35)} = 0.961$; p = 0.413	73.1 (61.9 to 89.3)	11.5 (9.7 to 14.2)	0.77 (0.60 to 0.90)
OPT_{CAD} (rpm)	128 ± 11	124 ± 10	125 ± 7	122 ± 10	123 ± 7	$F_{(3,41)} = 1.361$; p = 0.267	7.5 (6.3 to 9.2)	6.2 (5.2 to 77)	0.34 (0.12 to 0.63)
OPT_{CADCT} (rpm)	118 ± 3	116 ± 6	117 ± 8	116 ± 6	117 ± 7	$F_{(2,24)} = 0.354$; p = 0.681	6.3 (5.3 to 7.7)	5.3 (4.5 to 6.5)	0.45 (0.21 to 0.71)
T_{MAX} (N·m)	155 ± 22	166 ± 27	160 ± 24	170 ± 19	156 ± 22	$F_{(3,34)} = 2.507$; p = 0.082	13.7 (11.6 to 16.9)	9.1 (7.6 to 11.3)	0.66 (0.44 to 0.84)
C_{MAX} (rpm)	237 ± 27	231 ± 15	234 ± 13	223 ± 23	236 ± 17	$F_{(2,23)} = 1.169$; p = 0.324	15.4 (13.0 to 18.9)	7.3 (6.1 to 9.0)	0.42 (0.19 to 0.69)

Values are presented as mean ± standard deviation for observed data and point mean estimate (95% confidence interval) for TEM, CV, and ICC.

* same unit of measurement presented in the 'Parameter' column

C_{MAX}: maximal cadence; OPT_{CADCT}: optimal cadence with constrained intercept; OPT_{CAD}: optimal cadence; CV: coefficient of variation; ICC: intraclass correlation coefficient; N·m: Newton-meters; $P_{MAX:}$ maximal power output; P_{MAXCT}: peak power output with constrained intercept; rpm: rotations per minute; TEM: typical error of the measurement; T_{MAX}: maximal torque; W: watts.

3.1.4 Discussion

This study investigated the validity and reliability of a single ISO_{LIN} flying sprint for estimating the parameters from T-C and P-C relationships. A single sprint performed in ISO_{LIN} mode could help detect muscle fatigue during cycling exercises. The comparisons between ISO_{LIN} and ISO_{VEL} sprint cycling performance tested the validity. The main findings of the present study were that the parameters of T-C and P-C relationships were not different and well correlated between sprint modes. Although a significant difference was found in the extrapolations from the T-C relationship, there were significant correlations between ISO_{LIN} and ISO_{VEL} for T_{MAX} (large) and C_{MAX} (very large). Thus, while interchangeability between ISO_{LIN} and ISO_{VEL} could be assumed for OPT_{CAD} and P_{MAX}, the different extrapolations for the T-C relationship, despite being significantly correlated, suggest caution in determining T_{MAX} and C_{MAX} from single ISO_{LIN} tests. On the other hand, all parameters derived from the ISO_{LIN} sprint presented acceptable reliability, which would be the performance test's fundamental premise (Hopkins, 2000). The present study's findings demonstrated that a single ISO_{LIN} sprint test could be a suitable tool for quantifying the time course of muscle fatigue during and immediately after cycling tasks in well-trained male cyclists.

Traditionally, muscle fatigue has been assessed by measurement of peak torque during maximal isometric contractions (Place & Millet, 2020). Clearly, this technique presents limitations regarding the time necessary to prepare the subject with the equipment, especially after performing exercises on other ergometers, such as a treadmill or cycle ergometer (Place & Millet, 2020). The present study proposed a new strategy to measure fatigue directly during cycling exercises by using a mode that allows the participants to "flying start" the sprint at the preferred cadence. This mode could be more practical in quantifying muscle fatigue, mainly in the simulation of real-life cycling scenarios because of the difficulty of quickly stopping the freewheel. Moreover, delays could be critical to quantify the impact of a given fatiguing task accurately. Unlike ISO_{VEL}, which requires a set of tests in various cadences to extrapolate the T-C and P-C relationships, ISO_{LIN} allows a single sprint test to analyze the T-C spectrum. A point to highlight is that the "ISO_{LIN} flying sprint" at the preferred cadence ($\geq$ 70 rpm) and the specific criteria to include/exclude data points (more details in the Methods section) prevented the inclusion of low cadence values. Ideally, a balanced distribution of data points over the spectrum of the P-C relationship is desirable to obtain the most appropriate OPT_{CAD} and P_{MAX}. However, the present study had to deal with this limitation because the original idea was to propose a new strategy (with no delays) to measure fatigue during cycling exercises. Although it is worth noting that the results suggest that P-C relationship parameters were not different

and correlated between the two modes (ISO_{LIN} and ISO_{VEL}). One attempt to solve the possible right shift of the P-C parabola and, consequently, the estimate of P_{MAX} and OPT_{CAD} was to verify the reliability and validity of the constrained intercept, including the theoretical zero value.

The present study used the second-order polynomial equations with both constrained and non-constrained intercepts to extrapolate P_{MAX} and OPT_{CAD}. Concerning P_{MAX}, no significant difference was found ($p = 0.525$ and $p = 0.144$, respectively). However, a significant difference was found for OPT_{CAD} using the constrained intercept ($p = 0.002$). The goodness of fit (R^2) was also compared between constrained and non-constrained data as a parameter indicating estimate precision (Dorel, 2018; Wackwitz et al., 2021). Also, Wackwitz et al. (2021) verified that the second-order polynomial equations showed high R^2 values from P-C relationships derived in both ISO_{LIN} and ISO_{VEL} ($R^2 = 0.991 \pm 0.01$ and $R^2 = 0.991 \pm 0.01$, respectively). Significant differences were not found between ISO_{LIN} and ISO_{VEL} for both constrained and non-constrained intercepts ($p = 0.074$ and $p = 0.851$, respectively). In contrast to Wackwitz et al. (2021), the present study found a significant effect in the goodness of fit ($R^2 = p < 0.001$) for ISO_{LIN} and ISO_{VEL} by constraining the intercept. Moreover, there were significant differences in intra- and inter-methods for OPT_{CADCT} in ISO_{LIN}, with lower values than all the methods. The difference between the two methods for OPT_{CADCT} extrapolation was 7 rpm. Important to note that when the range of low cadences used is reduced, constraining the intercept to theoretical zero may shift the parabola to the left (Wackwitz et al., 2021). This seemed to be the case in the present study since ISO_{LIN} used a flying start that reduced the range of cadences in the left side of the P-C relationship, which possibly also affected the slope of the T-C relationship.

While the above-cited methodological aspects could have slightly affected the extrapolation of C_{MAX} and T_{MAX} from T-C relationships in ISO_{LIN}, this seems not to have influenced the reliability. In fact, TEM and CV determined in the present study were very similar to those reported by Kruger et al. (2019). The between-day CV observed in the present study demonstrates that T_{MAX}, C_{MAX}, and P_{MAX} have low day-to-day biological fluctuations. Notably, the average ICC for these parameters were 0.66, 0.42, and 0.84, suggesting low to good reliability. However, ICC is a relative measure of reliability, and its magnitude depends on the between-subjects variability (e.g., if participants differ little from each other, ICC values are small even if test-retest variability is small) (Weir, 2005). In the present study, the between-subjects variability was low, given the homogeneity of the sample in terms of sprint performance. Indeed, the homogeneity could hamper comparisons in a between-subjects design

(Weir, 2005). However, it is essential to note that torque or power could be reduced by fatigue in magnitudes up to 64%, depending on the fatiguing task (Iannetta et al., 2018). These results suggest that parameters derived by ISO_{LIN} have a high 'signal-to-noise' ratio (i.e. the change in measurement divided by the error of measurement) and, consequently, is sufficiently sensitive to detect clinically meaningful fatigue-induced changes.

ISO_{LIN} mode showed significantly higher T_{MAX} and lower C_{MAX} when compared to ISO_{VEL}. These results followed the values extrapolated by Kordi et al. (2019). On the other hand, the present study showed that both P_{MAX} and OPT_{CAD} were not different between the two sprints modes, corroborating the findings of Wackwitz et al. (2021), but contrasting with the results of Kordi et al. (2019). The reason for these discrepancies is unknown, but it could also be related to the differences in the sprint protocol affecting data points distribution over the T-C spectrum between the sprint's modes and studies. Thus, standardizing the amount and distribution of data points seems necessary for interchangeability between the different sprint modes. It could also be argued that the load used during ISO_{LIN} would be too low (i.e. no torque factor), affecting T-C and P-C relationships, especially when testing well-trained cyclists or stronger individuals. However, no significant correlations were found for the score changes between ISO_{LIN} and ISO_{VEL} with both P_{MAX} and T_{MAX}. This indicates that the most powerful individuals did not show higher differences between the sprint modes, which excludes any effect of a non-individualized sprint load. Therefore, all-out sprint tests without a torque factor using the Lode Excalibur ergometer could be the more generalized load when a single sprint evaluation is required.

A potential limitation of this study is the order of the sprints which could be considered a non-random order. While bias could be a consideration, one would argue that the power/torque vs velocity curves showed the typical behavior and a very good fitting. It is also important to point out, that the results of current study are restricted to the characteristic of the sample analysed (i.e. well-trained male cyclists). However, one could consider the present investigation as a preliminary report, since to the best of our knowledge this is the first study to compare the performance-derived parameters utilizing ISO_{LIN} and ISO_{VEL} modes, during cycling sprint tests. Thus, further studies are necessary to investigate if these results would be replicate in a broadly sample of cyclists and other sporstmen.

3.1.5 Conclusion

Using the P-C relationship, P_{MAX} and OPT_{CAD} could be detected similarly between the two sprint modes (ISO_{LIN} and ISO_{VEL}). Thus, the findings demonstrated that a single ISO_{LIN}

sprint test could be a suitable tool for quantifying the time course of muscle fatigue during and after cycling exercises in well-trained male cyclists. Therefore, the interchangeability of the modes should be extrapolated to different populations, situations, and conditions to understand better the implications of these similarities and differences between the modes.

4. CHAPTER THREE

4.1 STUDY TWO: The combined effect of caffeine and priming exercise on neuromuscular fatigue: isometric vs dynamic force assessments in trained cyclists

This second paper was submitted online in its first version on the European Journal of Applied Physiology. The current status is under review.

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Original research

Title: **The effect of caffeine chewing gum on muscle performance and fatigue after severe intensity exercise: isometric vs dynamic assessments in trained cyclists**

Running Head: **Caffeine chewing gum and muscle fatigue**

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Abstract

This study investigated the effect of caffeinated chewing gum (GUM_{CAF}) on muscle fatigue measures (isometric vs dynamic) after severe-intensity cycling bouts. Fifteen trained male cyclists participated in four visits. Each visit involved two severe-intensity cycling bouts (Δ_1 and Δ_2) lasting 6 minutes, separated by a 5-minute recovery period. Muscle fatigue was assessed by isometric maximal voluntary knee extension contraction (IMVC) with twitch interpolation technique and dynamically by 7 s *all-out* cycling sprints. Assessments were performed before GUM_{CAF} (Pre GUM) and after the cycling bouts (Post Exercise). GUM_{CAF} and placebo gum (GUM_{PLA}) were administered in a randomized double-blind procedure with participants receiving each gum type (GUM_{CAF} and GUM_{PLA}) during two separate visits. The results showed no significant interaction between gum type and time for both isometric and dynamic measurements ($p > 0.05$). The percentage change in performance from Pre GUM to Post Exercise showed no significant difference between GUM_{CAF} and GUM_{PLA} for either dynamic-derived T_{MAX} (~ -17.8% and -15.1%, respectively; $p = 0.551$) or isometric IMVC (~-12.3% and -17.7%, respectively; $p = 0.091$) measurements. Moderate to large correlations ($r = 0.31–0.51$) were found between changes in sprint maximal torque and maximal power output measurements and isometric force, for both gum conditions. GUM_{CAF} was not effective in attenuating muscle force decline triggered by severe-intensity cycling exercises, as measured by both isometric and dynamic methods. The correlations between IMVC and cycling maximal torque and power output suggest caution when interpreting isometric force as a direct measure of fatigue during dynamic cycling exercises.

Key Words: neuromuscular functions, fatigue, maximal power output, maximal isometric voluntary, sprinting, caffeine.

4.1.1 Introduction

Caffeine (CAF) ingestion is widely used as an ergogenic aid to improve sports performance (Burke, 2008; Grgic et al., 2020). Its ergogenic effects have been shown in various exercise modalities (Burke, 2008; Grgic et al., 2020), although the mechanisms appear to be diverse. Among the performance improvement mechanisms, it has been identified that CAF can act at the level of central nervous system, blocking adenosine receptors and enhancing neural drive to active muscles (Lima-Silva et al., 2021). Besides that, previous studies also have suggested that CAF could also act at a peripheral level by increasing muscle contractile force (Tarnopolsky et al., 2000; Tallis et al., 2012). Additionally, CAF ingestion might lead to increased nitric oxide production which increase tissue blood flow and oxygen supply to the exercising muscle during exercise (Ruiz-Moreno et al. 2020; Umemura et al., 2006), and consequently, can reduce exercise-induced fatigue, since potentially enhance muscle contractility (Lima-Silva et al., 2021).

The classical method to assess the neuromuscular function is performed by the measurement of force/torque from isometric maximal voluntary contractions (IMVC). Commonly, these measures are made together with transcranial magnetic stimulation (Aboodarda et al., 2018) or percutaneous electrical stimulation at nerve locations (Mira et al., 2018). Despite its widespread use, the IMVC method has limitations, particularly in the context of real-world sports. Considering that muscle fatigue is a transient event (Allen et al., 2008), a single measurement performed in only selected muscle groups may not reflect the total muscle fatigue developed in certain sports contexts. However, it remains the only reliable method to assess the combined neural and muscular contributions to performance decline. The intensity and duration of exercise, the engaged muscle mass, the mode and speed of muscle actions are factors that strongly influence the magnitude of decline in maximal voluntary force and the relative contribution of changes in contractile function (Behrens et al., 2023), along with environmental conditions (temperature, altitude) (Place & Millet, 2019). All these aspects

should be considered when measuring the muscle performance decline (i.e., fatigue). Another limitation is that IMVC relies on isometric contractions, which may not fully capture fatigue developed during dynamic sports activities. Finally, the timing of the IMVC measurement is crucial for accurate assessment of muscle performance and fatigue (Place & Millet, 2019). The duration between the completion of exercise and testing of muscle performance/fatigue, commonly takes 1 to 4 min, and this delayed assessment is problematic since recovery starts immediately after exercise has stopped (Place & Millet, 2019). Thus, this time delay can affect the proper detection of both neural and contractile properties. In this way, alternative methods that involve a more realistic approach are necessary, for a better understanding of fatigue induced by exercise.

Cycling sprint performance is a well-established method for measuring power output (Martin et al., 2007). This approach activates specific muscles involved in the exercise, allowing for the quantification of muscle performance and fatigue. However, a more comprehensive understanding of neuromuscular fatigue mechanisms during exercise might be gained by measuring muscle performance during whole-body high-intensity exercise. Research on the effects of CAF on fatigue in this context is still in its early stages (Couto et al., 2022; Black et al., 2015; Felippe et al., 2018; Santos et al., 2020).

Intriguingly, the effects of CAF ingestion on sprint performance are inconsistent. For example, a meta-analysis by Gonçalves Ribeiro et al. (2017) found no significant effects of CAF on maximal power output, whereas Grgic (2018) meta-analysis reported improvements in both mean and peak power during the Wingate sprint test after CAF. Similarly, studies on the effects of CAF on muscle force show mixed results. Some studies report positive effects (Grgic & Pickering, 2019; Grigic et al., 2018; Warren et al., 2010), while others found no significant effects on one-repetition maximum strength (Polito et al., 2016). To our knowledge, no research has examined the effect of CAF on muscle fatigue measures (isometric vs dynamic) in large

muscle groups after high-intensity cycling. It's important to note that assessing only dynamic or isometric force can provide an incomplete frame of fatigue development. Kruger et al. (2019) highlight the relevance of investigating fatigue through power output responses during dynamic exercises with large muscle mass (e.g., cycling) due to its functional connection to sports performance. Furthermore, the effect of CAF on muscle fatigue measures (isometric vs dynamic) after severe-intensity cycling bouts in trained cyclists remains unexplored, which is a common scenario in training and competition.

Another factor to consider with CAF supplementation is the route of ingestion (Wickham and Spriet, 2018). While capsule-delivered CAF takes about 60 minutes to reach peak plasma concentration, GUM_{CAF} and mouthwashes offer faster absorption (Kaminori et al., 2002; Wickham and Spriet, 2018). Although potential benefits of CAF on the central nervous system and skeletal muscle contraction have been demonstrated, no study has investigated the effects of GUM_{CAF} on neuromuscular responses.

Therefore, this study aimed to investigate the effect of GUM_{CAF} on muscle fatigue measures (isometric vs dynamic) induced by repeated bouts of high-intensity cycling exercises in trained subjects. We hypothesized that CAF ingestion would attenuate the decline in muscle performance after severe-intensity exercise, as assessed by a maximal cycling sprint test and IMVC. Additionally, we aimed to compare and correlate dynamic measures of fatigue, including maximal torque (T_{MAX}) and power output (P_{MAX}), with isometric measures obtained using IMVC after two bouts of severe-intensity cycling.

4.1.2 Methods

Participants

Fifteen trained male cyclists (mean ± standard deviation [SD]; age 26.5 ± 6.0 years, body mass 70.7 ± 4.7 kg, height 177.2 ± 5.4 cm; body fat 10.3% ± 3.8%; habitual caffeine consumption 85.5 ± 71.3 $mg \cdot day^{-1}$) participated in the study. The sample size calculation was

performed in the G*Power (Version 3.1.9.4, Germany) and was based on an intra-class correlation coefficient of 0.90, alpha of 5%, statistical power of 80%, and a small F effect size of 0.01. The calculation yielded a sample size of 13 participants.

All participants had at least two years of cycling training experience and competed at regional or national levels. They averaged 12 hours of cycling training per week and were advised to maintain a maximum of 8 hours per week during the study period. Participants were classified as "well-trained" cyclists (performance level 3) according to the proposal by De Pauw et al. (2013). They were informed of the experimental protocol and provided written voluntary informed consent, which was approved by the local University Institutional Ethics Committee (number 3.945.514) following the Declaration of Helsinki standards (Harriss & Atkinson, 2015).

Experimental Design

The study design was a randomized, cross-over, double-blind, and placebo-controlled trial. The experimental protocol is depicted in Figure 1. An environmentally-controlled laboratory (temperature: 22 ± 1 °C, relative humidity: 50% ± 1%) at a similar time of the day for each participant, was used for all the procedures. All cycling tasks were performed on an electromagnetic cycle ergometer (Excalibur Sport, Lode BV, Groningen, The Netherlands) with the pedal force measurement (PFM) system. Individual adjustment of seat and handlebar positions and pedaling frequency (rpm) was performed and registered at the first visit, which was kept constants for all subsequent tests. All isometric exercise tasks were performed on an isokinetic dynamometer (Biodex System 3, Biodex Medical Systems, Shirley, NY, USA) with individual adjustment of seat and the lever arm fixed to the anterior lower leg. Each equipment was calibrated according to the manufacturer's instructions, before each test session.

Participants performed five experimental sessions separated by an interval of at least 48 hours. In the first session, their anthropometric measures were obtained, and they performed an

incremental cycling test. After 15-20 min of the completion of incremental test, familiarization tests to maximal voluntary contraction and to *all-out* sprint was performed. In the four following visits, participants performed in each visit, two cycling bouts at Δ70 intensity (i.e., 70% of the difference between the pulmonary oxygen uptake [$\dot{V}O_2$] at first ventilatory threshold [VT_1] and maximal pulmonary oxygen uptake [$\dot{V}O_{2MAX}$]) with duration of 6 minutes each, separated by 5 minutes of passive rest (Figure 1). Muscle performance assessments were conducted before the cycling bouts, immediately after the second bout for the sprint test only, and one minute after the second bout for both the sprint and IMVC tests. The order of such four visits was randomized. Muscle performance assessments were performed dynamically in two visits (using either GUM_{CAF} or GUM_{PLA}) and isometrically in the other two visits (also using either GUM_{CAF} or GUM_{PLA}).

The choice of the cycling protocol consisting of two bouts of 6 min at severe intensity (i.e., Δ70) was made to guarantee an acute fatigue scenario allowing to use an iso time condition for both assessment protocols.

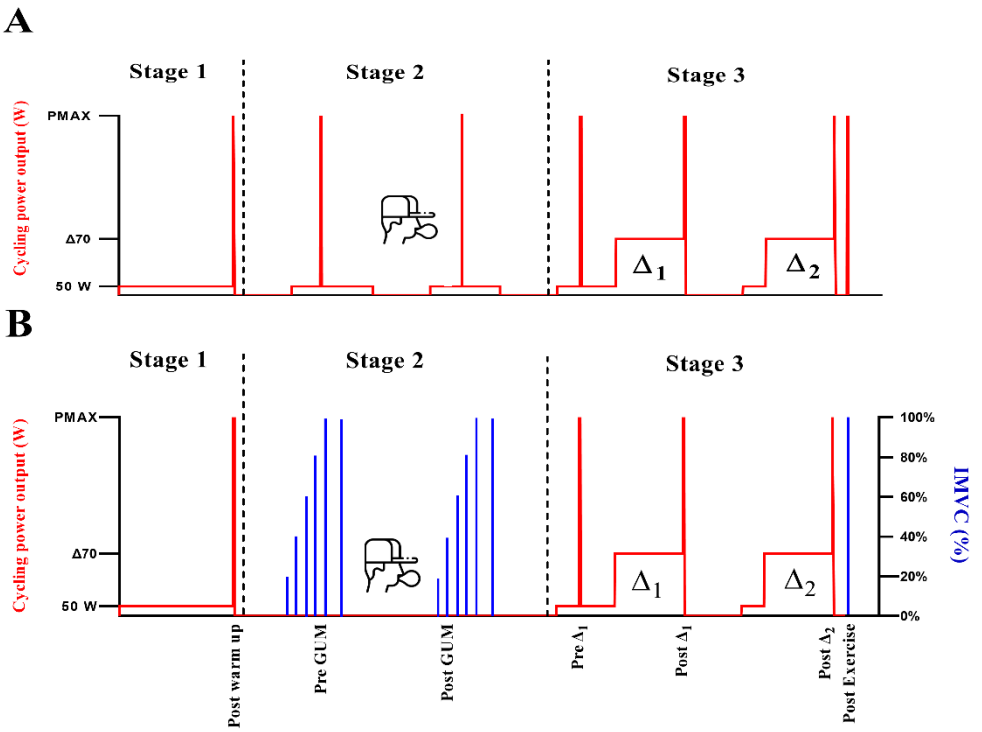

Figure 1. Design of protocols for dynamic (A) and isometric (B) trials.

Stage 1 = Warm up; Stage 2 = Pre and Post GUM dynamic and isometric exercise protocols and chewing gum ingestion; and Stage 3 = exercise task with two bouts (Δ_1 and Δ_2) at Δ70 intensity.

= chewing gum placebo or caffeine (GUM_{PLA} or GUM_{CAF}, respectively); P_{MAX} = maximal power output; IMVC = isometric maximal voluntary contraction.

Dietary Control

A protocol for dietary controlling was carried out with the intent to decrease the risk of bias regarding the effect of CAF and the participants' usual food intake. All participants were instructed to abstain from CAF intake for at least 24 hours before all the trials but to maintain their habitual dietary intake throughout the study. The 3-day dietary records completed in each trial period (one weekend day and two non-consecutive days during the week), were used to assess participants' usual food consumption during the period of study. In addition, the same

pattern of the pre-exercise meal of each subject was maintained over all the trials, to avoid within-subject differences in exercise performance. During the study period, the participants were instructed to discontinue using of any nutritional ergogenic aid. Habitual caffeine intake was assessed using three days of food records. The caffeine content of consumed foods and beverages was then quantified using the table proposed by Rocha et al. (2022).

Procedures

First visit - Incremental cycling test

The incremental cycling test was performed on a cycle ergometer, with an initial workload of 50 watts (W) and the workload was increased by 25 W every minute until voluntary exhaustion. Peak power output (PPO_{INC}), $\dot{V}O_{2MAX}$ and the VT_1 and second ventilatory threshold (VT_2) were determined from the test. The $\dot{V}O_{2MAX}$ was considered the highest moving average of 15 s of $\dot{V}O_2$ data and confirmed by following the secondary criteria of Howley et al. (1995). The VT_1 and VT_2 were identified by three experienced evaluators, using procedures described elsewhere (Meyer et al., 2005).

Severe exercise protocol

Stage 1 – Warm up

Each visit included a standardized warm-up protocol: 10 minutes of cycling at 50 watts using preferred cadence. Immediately following the warm-up, the Lode ergometer was programmed to switch from "hyperbolic" to "linear" mode, and then the participants performed a 7-second *all-out* sprint without torque factor (ISO_{LIN}) (‘Post WU’).

Stage 2 – Baseline assessment for dynamic and isometric measures

Five minutes following the warm-up, participants cycled for 2.5 minutes at 50 watts, then performed the ISO_{LIN} sprint (‘Pre GUM’). This was followed by another 4.5 minutes of cycling at 50 watts. Participants then chewed gum (either GUM_{CAF} or GUM_{PLA}) for 5 minutes

before discarding it. Following gum chewing, they repeated the same exercise sequence as before ('Pre GUM'), with the final sprint named 'Post GUM'. This 'Pre GUM' and 'Post GUM' testing procedure aligns with the isometric protocol described later.

The Isometric assessment protocol (conducted during two other visits) began 5 minutes after the cycling warm-up. Participants were seated in the dynamometer chair and first performed four submaximal IMVCs of the knee extensors at progressively increasing intensities (~20%, 40%, 60%, and 80% of their IMVC) for 5 seconds each, with 30 seconds of rest between contractions. Following the last submaximal contraction, there was another 30-second rest period before participants performed two IMVCs of the knee extensors from their dominant leg (right leg for all participants) for 5 seconds each, with 2 minutes of rest in between ('Pre GUM'). They then chewed gum (either GUM_{CAF} or GUM_{PLA}) for 5 minutes as previously described, followed by repeating the IMVCs ('Post GUM'). During the IMVCs, a high-frequency doublet electrical stimulation (100 Hz) was delivered to the femoral nerve at the peak force *plateau*. This was followed by three additional stimuli on the relaxed muscle, delivered 3 seconds apart: a paired pulse (100 Hz and 10 Hz), and a single pulse.

Stage 3 – Severe intensity bouts

Ten minutes following Stage 2, participants completed a 2-minute baseline cycle at 50 watts, followed by an ISO_{LIN} sprint ('Pre Δ1'). They then cycled again for 3 minutes at 50 watts before performing a 6-minute cycling bout at Δ70 intensity (Δ_1). Upon completion of the first cycling bout, they performed another ISO_{LIN} test ('Post Δ_1'). Following a 5-minute passive recovery period, participants resumed cycling for 2 minutes at 50 watts. This was followed by a 6-minute cycling bout at Δ70 intensity (Δ_2), and another ISO_{LIN} sprint ('Post Δ_2') at the end.

During the dynamic assessment visits, participants passively recovered for 50 seconds, then resumed pedaling at their preferred cadence for 10 seconds, followed by the final ISO_{LIN}

test (‘Post Exercise’). During isometric assessment visits, following the completion of the Δ_2 bout, participants returned to the isokinetic dynamometer one minute later to perform the IMVC test and complete the neuromuscular measures (‘Post Exercise’).

Specific protocols description

Cycling Sprints (ISO_{LIN})

For the *all-out* sprint assessments, the cycle ergometer was set to "iso-inertial linear mode" with a torque factor of 0 $Nm \cdot kg^{-1}$ (Nascimento et al., 2024). All 7-second sprints were performed immediately following a 5-second countdown. Before each sprint, participants were instructed to cycle at their preferred cadence. After the countdown, they were asked to perform the sprint with the highest possible cadence for the entire 7-second duration. The torque-angular velocity relationship for each sprint was determined according to the method described in Nascimento et al. (2024).

Isometric maximal voluntary contraction (IMVC)

To ensure stability during maximal contractions, participants were seated and secured in the dynamometer chair using Velcro straps. For IMVCs of the knee extensor muscles, a knee flexion angle of 70 degrees was used (with 0 degrees representing full knee extension). The lever arm was attached to the dominant leg just below the knee, with its axis of rotation aligned with the lateral femoral condyle. The test protocol consisted of two 5-second IMVCs of the knee extensors with a 2-minute rest period between contractions. All participants were familiarized with producing maximal force beforehand and were verbally encouraged during the IMVCs.

Femoral nerve stimulation

To assess both central and peripheral neuromuscular responses, the experiment employed voluntary contractions alongside electrically induced contractions of the femoral

nerve. An electrical stimulator (DS7AH Isolated HV Constant Current Stimulator, Digitimer, UK) delivered the electrical stimuli. For evaluation of the knee extensor muscles, electrode placement followed this protocol:

a) A cathode electrode (10 mm stimulating diameter; Meditrace 100, Covidien, Brazil) was secured with tape in the inguinal fold.

b) The anode, a larger rectangular electrode (50 mm × 90 mm; Durastick Plus, DJO Global, Vista, CA, USA), was placed on the gluteal fold. The stimulation protocol used single pulses delivered with increasing intensity until plateaus were reached in both twitch force and the amplitude of the maximal M-wave (the compound muscle action potential). To ensure supramaximal stimulation for eliciting maximal responses, an additional pulse at 120% of the previously determined intensity was delivered.

During the second IMVC, electrical stimulation of the femoral nerve was delivered at peak force (plateau) using a 100 Hz doublet. This was followed by three additional stimuli delivered to the relaxed muscle, separated by 3 seconds: a paired pulse at 100 Hz and 10 Hz, and a single pulse.

Chewing Gum

Participants chewed each type of gum for 5 minutes. After discarding the chewed gum into a tissue, they drank 250 mL of water. GUM_{CAF} contained 400 mg of CAF total, delivered in four pieces of commercially available gum (each containing 100 mg) (Military Energy Gum – Stay Alert, Arctic Mint flavor, Chicago, IL, USA). GUM_{PLA} consisted of four pieces of similar gum with a comparable flavor (Trident X Fresh Crystal Mint, Smaków, Poland). The chosen 400 mg caffeine dose resulted in an average body mass-adjusted intake of 5.7 $mg \cdot kg^{-1}$ (range: 5.2-6.4 $mg \cdot kg^{-1}$). This aligns well with the common practice in research of using a single caffeine dose of around 6 $mg \cdot kg^{-1}$ (Grgic et al., 2018). To account for peak plasma caffeine

concentration, exercise trials began 12 minutes after discarding the gum, as reported by Morris et al. (2019) where peak concentration occurred around 10 minutes after chewing.

Electromyography (EMG) signals

The electromyographic (EMG) activity of the *vastus lateralis* (VL) and *rectus femoris* (RF) muscles was monitored using bipolar surface electrodes (Kendall-Meditrace 100, Ag/AgCl, 20 mm diameter, 22 mm inter-electrode distance) placed according to Non-Invasive Assessment of Muscles Project – SENIAM recommendations. To minimize interference with the EMG signal, the skin over these muscles was shaved, abraded with fine sandpaper, and then cleaned with alcohol before electrode placement. A reference electrode was positioned on the anterior surface of the tibia. A four-channel Miotool EMG system (Miotec Equipamentos Biomédicos Ltda., Porto Alegre, Brazil) was used to acquire the EMG signals. This system has a common-mode rejection ratio of 126 dB and an input impedance of 10 GΩ. The EMG signal was amplified by a gain of 20 (resulting in a total gain of 2000 when combined with the preamplifier gain). A high-pass filter at 20 Hz and a low-pass filter at 500 Hz were applied to the signal. An analog-to-digital (A/D) converter board with an input range of -5 to +5 volts was used to convert the EMG signal into a digital signal for analysis. The acquired data were then analyzed using MatLab 6.5 software (The MathWorks Inc, Natick, Massachusetts, USA).

Data analysis

Parameters derived from dynamic assessment – Cycling sprint

The Pedal Force Measurement (PFM) system of Lode ergometer records data of torque and angular velocity every two degrees of each pedal stroke, totaling 180 information per pedal cycle. The PFM data was transferred to Excel software and analyzed to calculate the average torque, power output, and angular velocity per revolution. In this way, torque–velocity (T–V) and power output–velocity (P–V) relationships were obtained, allowing for the calculation of maximal theoretical torque (T_{MAX}) and maximal angular velocity (V_{MAX}), as well as the

extrapolation of maximal power output (P_{MAX}) and optimal cadence for peak power (OPT_{CAD}) (Samozino et al., 2007).

Additionally, the total work performed (in joules, J) during each sprint was calculated as the area under the curve of a power output (watts) *versus* time (seconds) plot. For comparative analysis between GUM_{CAF} and GUM_{PLA}, the work done during all sprints in each condition was summed.

Parameters derived from isometric assessment - Knee extensor contraction

Of the two isometric contractions performed, only the best measure of IMVC was used to quantify voluntary force. When there was a noticeable difference (>5%) in the IMVC, a third repetition was performed.

To quantify the peripheral fatigue the amplitudes of high-frequency doublet (Db_{100}), low-frequency doublet (Db_{10}), and peak twitch (Pt) were assessed from IMVC. Low-frequency fatigue was quantified by calculating the ratio between Db_{10} and Db_{100} ($Db_{10:100}$). To quantify the central fatigue the voluntary activation (VA) was calculated accordingly. M-wave was quantified as peak-to-peak amplitude and area derived from VL and RF muscles.

Statistics Analysis

Descriptive data are reported as the mean ± standard deviation (SD). Data were analyzed by using a mixed effects model. The model's fixed effects were the condition, i.e., gum (GUM_{CAF} and GUM_{PLA}), the time point of measurement, and the interaction gum × time point. The random effect of the model was the identity of each subject, to deal with repeated measurements. The model residuals normality was verified by the Shapiro–Wilk test. When appropriate, data were further explored using Tukey post hoc comparisons. Moreover, to compare fatigue between gum conditions and the force measurement (i.e., isometric or dynamic) at the end of Δ_2, a paired t-test was performed on the percentage change from 'Pre GUM' to at 'Post Δ_2' (only for sprints) and 'Pre Gum' to 'Post Exercise' (for sprints and

IMVC). A paired t-test was also used to compare the sum of work done during sprints between the GUM_{CAF} and GUM_{PLA} conditions. Pearson product-moment correlation was used to assess the relationships between the variables. The r values were interpreted as follows: |<0.10|, trivial; |0.10–0.29|, small; |0.30–0.49|, moderate; |0.50–0.69|, large; |0.70– 0.89|, very large; and |0.90–1.0|, almost perfect (Hopkins, 2015). Analysis was carried out using the software R (R Core Team, Viena, Austria) using the packages Rcmdr, lme4 and lsmeans. The statistical significance level was set as $p < 0.05$.

4.1.3 Results

The values obtained of PPO_{INC} and $\dot{V}O_{2MAX}$ were 350 ± 47 W and 4.1 ± 0.5 $L \cdot min^{-1}$ (57.3 ± 6.4 $mL \cdot kg^{-1} \cdot min^{-1}$), respectively. The VT_1 and VT_2 occurred at 65% ± 7% and 78% ± 4% of $\dot{V}O_{2MAX}$ and 51% ± 5% and 70% ± 4% PPO_{INC}, respectively. The Δ70 intensity occurred at 88% ± 3% and 78% ± 4% of $\dot{V}O_{2MAX}$ and PPO_{INC}, respectively. Also, Δ70 was located at 113% ± 5% and 112% ± 7% of VT_2 $\dot{V}O_2$ and power output, respectively.

The average daily CAF consumption of the participants was 71.9 ± 81.2 mg (range 0 to 219 mg).

Cycling Sprints Tests

Regarding the dynamic measures, there was no interaction effect between condition (gum) and time (sprint number) for all parameters ($p = 0.325$ to 0.979). There was a significant effect for GUM_{CAF} considering T_{MAX} ($p = 0.047$) and P_{MAX} ($p = 0.005$), but not for C_{OPT} ($p = 0.533$) and V_{MAX} ($p = 0.883$). On average across the seven sprints performed, P_{MAX} and T_{MAX} were higher for GUM_{CAF} than GUM_{PLA} condition ($p = 0.005$ and 0.047, respectively). All variables presented a significant effect for time ($p < 0.0001$) (Figure 2).

The comparisons between sprints revealed a reduction in P_{MAX} at both 'Post Δ_1' (percent mean ± SD changes of -28% ± 14% and -33% ± 14% for GUM_{CAF} and GUM_{PLA}, respectively)

and 'Post Δ_2' (-11% ± 12% and -36% ± 17% for GUM_{CAF} and GUM_{PLA}, respectively) compared to 'Pre GUM'. There was also a significant reduction in P_{MAX} between 'Pre GUM' and 'Post Exercise' (-11% ± 14% and -6% ± 16% for GUM_{CAF} and GUM_{PLA}, respectively). However, the magnitude of changes were smaller compared to the reductions observed between 'Pre GUM' and 'Post Δ_1' and 'Post Δ_2'. In other words, P_{MAX} was recovered somewhat within 1 minute after exercise. T_{MAX} was reduced at both 'Post Δ_1' (-15% ± 12% and -20% ± 15% for GUM_{CAF} and GUM_{PLA}, respectively), 'Post Δ_2' (-6% ± 20% and -25% ± 20% for GUM_{CAF} and GUM_{PLA}, respectively) and 'Post Exercise' (-18% ± 16% and -14% ± 19% for GUM_{CAF} and GUM_{PLA}, respectively) compared to 'Pre GUM'. However, the changes at 'Post Δ_1', 'Post Δ_2', and 'Post Exercise' were not different among them. V_{MAX} and OPT_{CAD} were reduced at 'Post Δ_1' and 'Post Δ_2' but augmented at 'Post Exercise' in relation to 'Pre GUM'. Individual percent changes to 'Pre GUM' of P_{MAX}, T_{MAX}, V_{MAX}, and OPT_{CAD} were presented in the Electronic Supplementary Material (File 1, Fig. S1).

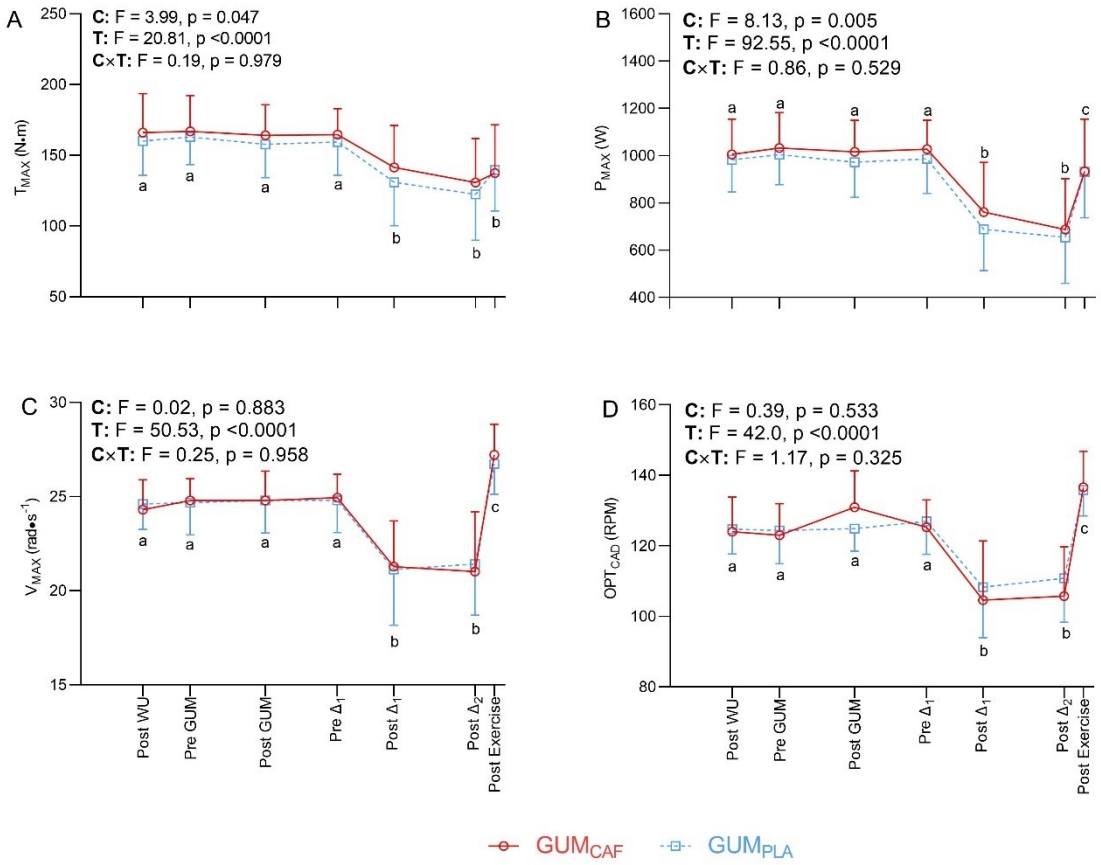

Figure 2. Time course of dynamic sprints-derived parameters maximal torque (T_{MAX}) (A), maximal power output (P_{MAX}) (B), maximal velocity (V_{MAX}) (C), and optimal cadence (OPT_{CAD}) (D), during GUM_{CAF} and GUM_{PLA} trials.

I: Ingestion mean effect, S: sprint number main effect, I × S: interaction between ingestion and sprint number effect. Sprints (mean of each sprint from GUM_{CAF} and GUM_{PLA}; i.e. sprint main effect) with the same letter are not statistically different ($p > 0.05$), and sprints with different letters are significantly different ($p < 0.05$).

Average values for the torque-velocity and power output-velocity relationships along with their derived parameters (T_{MAX} and V_{MAX}; P_{MAX} and OPT_{CAD}, respectively) at selected sprints 'Pre-GUM', 'Post Δ_1', 'Post Δ_2', and 'Post Exercise') are presented in Figure 3.

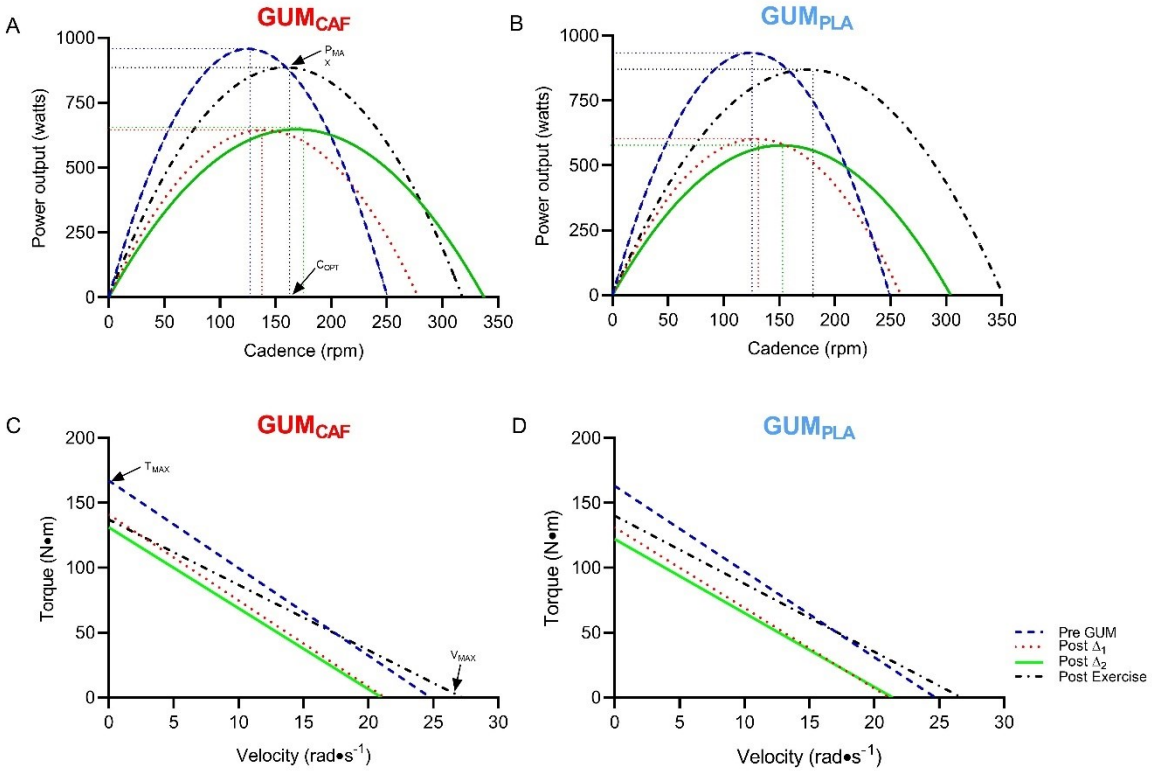

Figure 3. Power output-velocity (A and B) and torque-velocity relationships (C and D), along with their derived parameters P_{MAX} and OPT_{CAD} (A and B) and T_{MAX} and V_{MAX} (C and D) during GUM_{CAF} and GUM_{PLA} trials.

Note: data is the average of all subjects at each condition.

There was no significant difference in total work performed during sprints between GUM_{CAF} (18644 ± 4623 Joules) and GUM_{PLA} (18211 ± 4699 Joules; p = 0.523).

Knee extensors isometric contraction

Regarding the isometric measures (Figure 4), GUM_{CAF} was not effective in attenuating the fatigue when assessed by central (IMVC and VA) or peripheral measurements (Db100Hz, Db10Hz, single twitch, $Db_{10:100}$, Mwave of RF and VL muscles). No significant effect was detected for condition (p = 0.057 to 0.948), time (p = 0.051 to 0.893), or interaction condition × time (p = 0.120 to 0.684) in Db IMVC 100Hz, $Db_{10:100}$, VA, and Mwave of VL and RF muscles. The same absence of significant effect for condition was observed for IMVC,

Db100Hz, Db10Hz, and single twitch (p = 0.176 to 0.671) and condition × time interaction (p = 0.410 to 0.827), but there is significant effect for time in these variables (p <0.0001). Overall, for central (IMVC) or peripheral measurements (Db100Hz, Db10Hz, and single twitch), the partial comparisons reveled that 'Post Exercise' was different from the measurements during 'Pre GUM' and 'Post GUM' (p <0.0001). Individual percent changes to 'Post WU' of IMVC, Db100Hz, Db10Hz, single twitch, $IMVC_{Db100Hz}$, VA, $Db_{10:100}$, and Mwave of RF and VL muscles were presented in the Electronic Supplementary Material (File 1 Fig. S2).

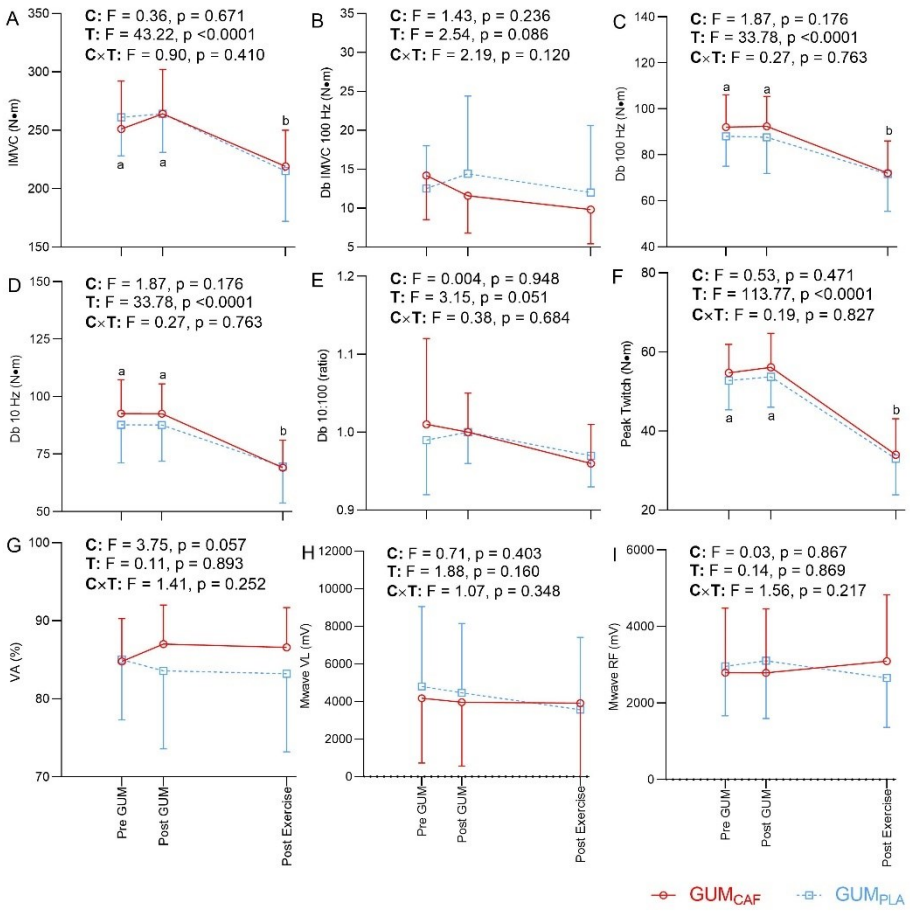

Figure 4. Time course of isometric maximal voluntary contration-derived parameters of isometric maximal voluntary contraction (A), high-frequency doublet during IMVC ($Db_{100Hz}IMVC$) (B), high-frequency doublet (Db_{100Hz}) (C), low-frequency doublet (Db_{10Hz}) (D), low- to high-frequency dublets ratio ($Db_{10:100}$) (E), peak twitch (F), voluntary activation (VA)

(G), and electromyograph signals for *vastus lateralis* (Mwave RF) (H) and *rectus femoris* (Mwave RF) (I), during GUM_{CAF} and GUM_{PLA} trials.
C: condition main effect, T: time main effect, I × C: interaction between condition and time. Isometric maximal voluntary contration (mean of each IMVC from GUM_{CAF} and GUM_{PLA}; i.e. isometric contraction main effect) with the same letter are not statistically different ($p > 0.05$), and IMVC with different letters are significantly different ($p < 0.05$).

Relashionship between isometric and sprint assessements

Figure 5 illustrates the relationship between isometric and sprint measures, i.e., the percent change of P_{MAX} and T_{MAX}, from 'Pre GUM' to 'Post Δ_2', along with the change in IMVC from 'Pre GUM' to 'Post Exercise'. It also shows the change of P_{MAX} and T_{MAX} from 'Pre GUM' to 'Post Exercise', in relation to the change in IMVC from 'Pre GUM' to 'Post Exercise'. "Moderate" to "large" correlations ($r = 0.31$ to 0.51) were observed, with the changes in GUM_{CAF} showing the highest values, but not significantly different from GUM_{PLA} ($p > 0.05$), correlations.

No differences occurred from 'Pre GUM' to 'Post Exercise', for sprint T_{MAX} between GUM_{CAF} and GUM_{PLA} (-17.8% ± 16.8% vs -15.1% ± 18.7%; $p = 0.551$) and IMVC (-12.3 ± 9.8% vs -17.7% ± 13.1%; $p = 0.091$), respectively. In the same way, no differences were found from 'Pre GUM' to 'Post Exercise' changes considering sprints T_{MAX} *versus* IMVC for both GUM_{CAF} ($p = 0.234$) and GUM_{PLA} ($p = 0.620$) conditions.

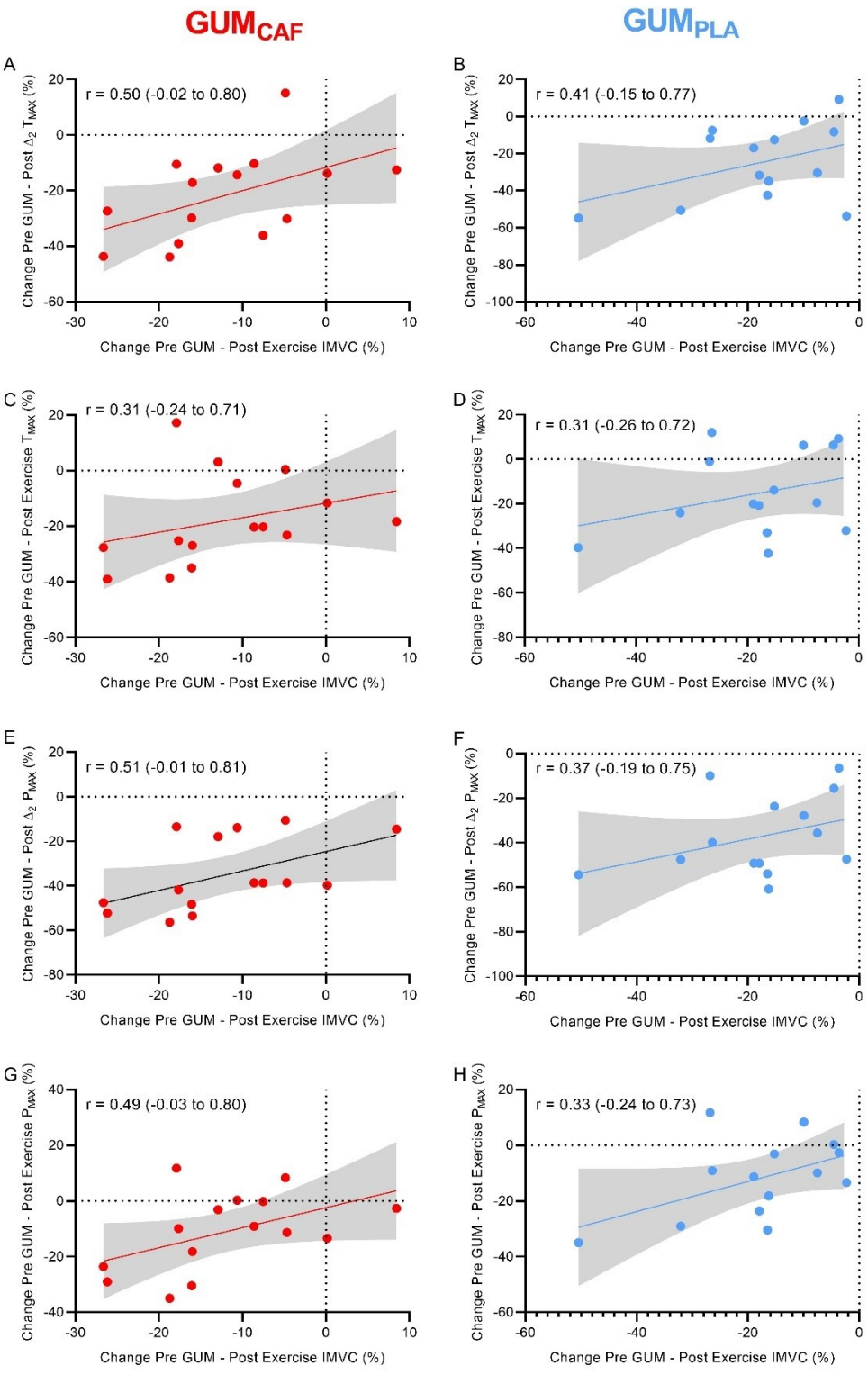

Figure 5. Association between the pecentual chance of P_{MAX}, T_{MAX}, V_{MAX}, and C_{OPT} from Pre GUM to Post Exercise and Pre GUM to Post Exercise, in relation to the change in IMVC from Pre GUM to Post Exercise.

Note: Each dot is the average change observed in each subject across the GUM_{CAF} and GUM_{PLA} trials from Isometric and Dynamic trials.

4.1.4 Discussion

According to our knowledge, this is the first investigation to examine the effects of GUM_{CAF} on muscle performance and fatigue using dynamic (cycling sprint) and isometric (knee extension) assessments. Our main finding revealed that GUM_{CAF} was not effective in mitigating the decrease in muscle force caused by repeated severe-intensity cycling exercises, regardless of the assessment method (isometric *versus* dynamic). However, on average, across all sprints, P_{MAX} and T_{MAX} were higher in the GUM_{CAF} group compared to the GUM_{PLA} group (Figure 2). Additionally, the percent change from 'Pre GUM' to 'Post Exercise' (i.e., 1 minute after the second bout) showed similar (no significant difference) between dynamic and isometric measurements, nor between the gum conditions.

Ergogenic effects of GUM_{CAF} over muscle performance/fatigue

This study investigated novel aspects of CAF delivery by administering gum 12 minutes before exercise onset. This timeframe aligns with previous reports indicating peak plasma CAF concentration occurs approximately 10 minutes after chewing GUM_{CAF} (Morris et al., 2019). Additionally, the cycling protocol employed two 6-minute bouts at severe-intensity (Δ70) to ensure identical exercise duration and intensity (unlike time-trial or time-to-exhaustion protocols). This approach induced an acute fatigue state, allowing us to assess neuromuscular performance at a consistent time point for both isometric and dynamic assessments.

The GUM_{CAF} was not effective in attenuating the muscle force decrease caused by these severe-intensity exercises. While the ergogenic effects of CAF on human performance are well-established (Grgic et al., 2018), data on the integrated (central and peripheral) effects of CAF on neuromuscular fatigue remains scarce (Lima Silva et al., 2021). No significant differences in T_{MAX} or P_{MAX} were observed between 'Post Δ_1' and 'Post Δ_2' for either GUM_{CAF} or GUM_{PLA} conditions. Interestingly, P_{MAX} did increase 'Post Exercise' compared to both 'Post Δ_1' and 'Post Δ_2', but these values remained lower than before Δ_1 and Δ_2 measurements. These findings

suggest that both exercise bouts effectively induced acute fatigue. This allows us to investigate the potential of CAF to alleviate neuromuscular fatigue.

Indeed, the measurement of neuromuscular performance and fatigue using a dynamic approach remains unexplored, particularly regarding the effect of CAF consumption on exercise. A key difference exists between isometric and dynamic assessments. Isometric assessments involve constant intramuscular pressure, while dynamic assessments involve contraction-relaxation cycles. This difference could affect muscle blood flow (Santarém et al., 2023; Kagaya & Ogita, 1992; Sadamoto et al., 1983). CAF is associated with increased nitric oxide production, which consequently leads to augmented tissue blood flow and oxygen supply to exercising muscles (Ruíz Moreno et al., 2020; Umemura et al., 2006). This could potentially enhance muscle contractility and reduce exercise-induced fatigue (Lima-Silva et al., 2021). Since these contrasting patterns are triggered by dynamic and isometric measures, they could have an impact on muscle blood flow. Therefore, using GUM_{CAF} offers a novel, integrative approach to investigate the impact of CAF on active muscles during a severe-intensity cycling exercise task. However, the results of the present study showed no significant ergogenic (performance-enhancing) effects of CAF on muscle performance when applied using the specific cycling sprint protocol proposed, compared to the isometric protocol, for studying fatigue and/or neuromuscular performance.

There was no interaction effect (condition × time) for any variable derived from the dynamic and isometric measures. This contrasts with previous studies where acute ingestion of GUM_{CAF} was shown to attenuate fatigue during repeated severe-intensity sprint exercise in competitive cyclists (Paton et al., 2010). Methodological differences between the studies likely limit our ability to definitively conclude on the ergogenic effect of GUM_{CAF} on cycling sprints. However, across all sprints in the present study, P_{MAX} and T_{MAX} were slightly higher for the GUM_{CAF} condition compared to the GUM_{PLA} condition. It's important to note that this

difference was only found in the dynamic measurements (cycling sprint), which are known to be more sensitive in detecting the ergogenic effect of CAF compared to isometric measurements. Additionally, the total work done during the sprints under each condition did not present difference.

For the isometric measurements (specifically, knee extension), there were significant effects of time observed in both the CAF and PLA conditions for IMVC when comparing 'Pre GUM' intake to 'Post Exercise' values (approximately -12.3% and -17.7% decrease, respectively). These findings align with previous research by Black et al. (2015). They demonstrated that both CAF and PLA capsules led to an improvement in IMVC (5.7% and 2.5% increase, respectively) 60 minutes after ingestion, when comparing pre- vs. post-supplement intake. Additionally, similar to the current study, Black et al. (2015) found that IMVC of the knee extensors was reduced to a similar extent for both CAF and PLA conditions after 30 minutes of cycling at 60% $\dot{V}O_{2MAX}$ followed by a 10-minute cycling time trial.

It's important to highlight that IMVC, a common indicator of overall fatigue (Thomas et al., 2015), showed a decrease in both CAF and PLA conditions after the severe-intensity cycling bouts in our trained cyclists. Interestingly, peripheral fatigue markers (Db100Hz, Db10Hz, and peak twitch) and the central fatigue index (VA) did not exhibit the same behavior. These findings regarding the central and peripheral fatigue markers partially agree with previous research (Santos et al., 2020), who reported no significant difference between CAF and PLA for the voluntary activation (i.e. central parameter). However, unlike our study, they found that CAF triggered a greater reduction in potentiated quadriceps twitch force compared to PLA, particularly in the group of subjects with a lower performance level relative to the group with a better performance level. The authors suggest that the development of central fatigue during a cycling time trial might be influenced by participants' performance levels. It's important to note that their study, similar to ours, involved a high-intensity exercise protocol.

While our results suggest no clear effect of CAF on central or peripheral fatigue markers besides IMVC, previous research suggests that fatigue during high-intensity exercise is primarily attributed to peripheral processes (Thomas et al., 2015; Felippe et al., 2018). Further investigation is needed to elucidate the specific effects of CAF on central and peripheral fatigue mechanisms in trained cyclists performing severe-intensity exercise.

Dynamic and isometric measurements of muscle fatigue, between the gum conditions.

The partial recovery of central and/or peripheral fatigue must be considered when assessing fatigue with a delay, such as the time it takes to move from the cycling ergometer to the neuromuscular assessment chair commonly used in prior studies (Black et al., 2015). Despite this potential recovery, we observed a similar degree of muscle fatigue in both the sprint and IMVC measurements (taken 1 minute after exercise completion) regardless of CAF ingestion. Additionally, we found "moderate" to "large" correlations between cycling P_{MAX} and IMVC, as well as between cycling T_{MAX} and IMVC, for both gum conditions. These results suggest a possible correlation between dynamic and isometric variables. However, the interchangeability of these measures is questionable because they assess different aspects of fatigue. Isometric assessments are helpful in evaluating peripheral and central components of neuromuscular fatigue, while dynamic measures represent more specific real-life scenarios and present less questionable applicability.

Therefore, our findings offer valuable insights for future research. To our knowledge, only Kruger et al. (2019) have previously assessed fatigue using both isometric and dynamic techniques. These authors pioneered the measurement of force parameters derived from cycling sprints (dynamic) and isometric contractions on a modified recumbent cycle ergometer, performed before and after three different fatiguing cycling exercises (30-second *all-out* effort, 10-minute bout, and 90-minute bout at corresponding intensities). They found distinct fatigue

responses based on isometric versus dynamic measurements following each exercise. Our data contradicts the results reported by Kruger et al. (2019), although methodological differences should be considered. Thus, our findings suggest that these specific isometric and dynamic measurements may not be interchangeable for detecting fatigue.

However, the decrease (percent change from 'Pre GUM' to 'Post Exercise') in T_{MAX} measured during the cycling sprint (-18% and -14% decrease for GUM_{CAF} and GUM_{PLA}, respectively) was very similar between our study and that reported by Kruger et al. (2019) (~-18% decrease). Interestingly, V_{MAX} in our study showed an increase compared to 'Pre GUM' (10% increase for GUM_{CAF} and 9% increase for GUM_{PLA}, whereas Kruger et al. (2019) reported a decrease (~8%) one minute after the intense exercise. Conversely, their values for P_{MAX} were higher (~-22% decrease) compared to the decreases found in our study for GUM_{CAF} (-11% decrease) and GUM_{PLA} (-6% decrease). Furthermore, in our study, "moderate" to "large" correlations were observed between P_{MAX} and IMVC ($r = 0.49$ and $r = 0.33$ for GUM_{CAF} and GUM_{PLA}, respectively) and between T_{MAX} and IMVC ($r = 0.31$ for both GUM_{CAF} and GUM_{PLA}). These correlation coefficients are higher than those reported by Kruger et al. (2019), who found much weaker "trivial" correlations between P_{MAX} and IMVC ($r = 0.079$) and between T_{MAX} and IMVC ($r = -0.070$).

Several key points need to be considered when comparing the findings of these studies. First, Kruger et al. (2019) employed a recumbent cycling ergometer, while our study used a conventional upright cycling ergometer with trained cyclists. Additionally, their criteria for "severe intensity" was defined as 5% above VT_2, whereas we used a $\Delta 70$ approach, which corresponds to approximately 12% above of power output at VT_2. Notably, the recumbent ergometer used by Kruger et al. (2019) might have limitations due to the participant's position affecting force application. This position can alter lever arms and consequently change the tangential force generated. While our study utilized a conventional upright cycling ergometer,

representing the specific exercise mode, Kruger et al. (2019) employed an innovative ergometer designed by Doyle-Baker et al. (2018) to assess neuromuscular function immediately after cycling. This innovative ergometer offers a distinct advantage by enabling the comparison of isometric measurements with data obtained from other equipment, not limited to the cycling ergometer itself. However, this approach comes at the cost of sacrificing specificity to the cycling modality and potentially introducing anatomical and biomechanical differences between participants due to the altered posture. Consequently, this combination of factors in Kruger et al.'s (2019) study may not accurately replicate the specific muscle fatigue mechanisms associated with upright cycling.

From a practical standpoint, the ISO_{LIN} sprint method offers several advantages over other methods. Firstly, it only requires a single repetition to measure T_{MAX} and V_{MAX}. Subsequently, P_{MAX} and OPT_{CAD} can be extrapolated from these values (Nascimento et al., 2024). Additionally, the ISO_{LIN} method reflects the specificity of the cycling modality because it engages all the muscle groups involved in actual cycling. Finally, measurements can be taken immediately after exercise without delay, which is a crucial advantage. Delays caused by transferring participants to other equipment can lead to an underestimation of exercise-induced neural and contractile impairments, potentially misinterpreting the etiology of fatigue (Place & Millet, 2019).

However, one limitation of this study is the restricted sample characteristic. It only included trained male cyclists and did not explore different exercise intensity domains. Therefore, further research is necessary to investigate the effect of GUM_{CAF} on neuromuscular fatigue in more diverse populations and across a wider range of exercise intensities.

4.1.5 Conclusion

GUM_{CAF} was not effective in attenuating the decrease in muscle force caused by the severe-intensity cycling exercise, regardless of the measurement method (isometric vs. dynamic). Interestingly, while neither GUM_{CAF} nor the GUM_{PLA} condition showed significant pre-to-post exercise changes, the dynamic assessment revealed a potential overall effect of GUM_{CAF} on peak mechanical variables during fatigued states. This is because P_{MAX} and T_{MAX} were, on average across all sprints, higher for the GUM_{CAF} compared to the GUM_{PLA} condition. Furthermore, although correlations were found between IMVC and both cycling P_{MAX} and T_{MAX} for both gum conditions, caution is advised regarding the interchangeability of dynamic and isometric variables. Therefore, the specific cycling sprint protocol employed in this study should be considered as a feasible tool for investigating fatigue and/or neuromuscular performance. This protocol could be applied to different populations, situations, and exercise conditions.

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Data availability The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of interest No potential conflict of interest was reported by the author(s).

References

The references of the paper are at “references section” page 107.

ELECTRONIC SUPPLEMENTARY MATERIAL FILE 1

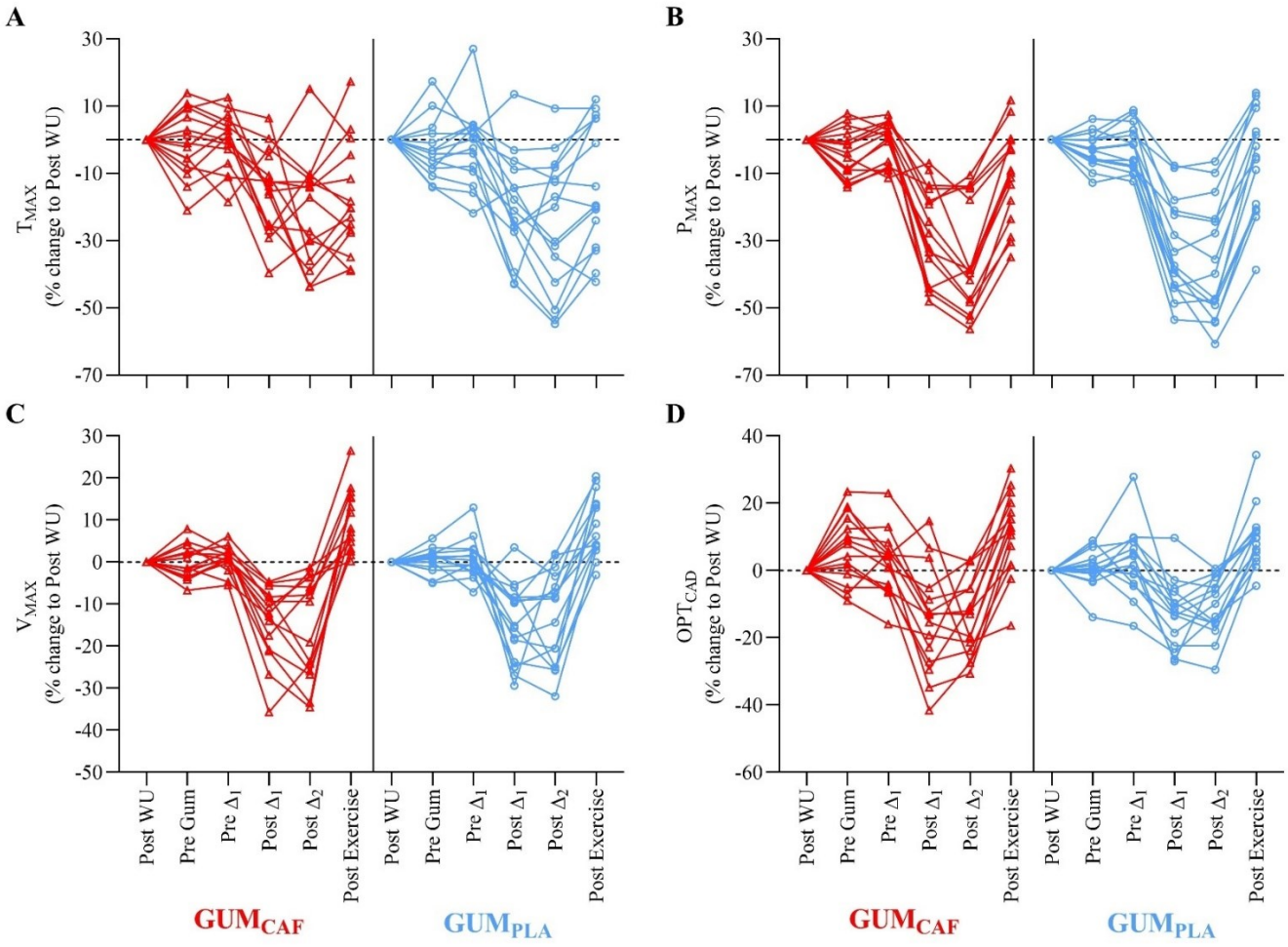

Figure S1 Individual percent changes to Post WU in maximal torque (A), maximal power output (B), maximal velocity (C), and optimal cadence (D), under caffeine (red triangles) and placebo (blue circles) chewing gum supplementation.

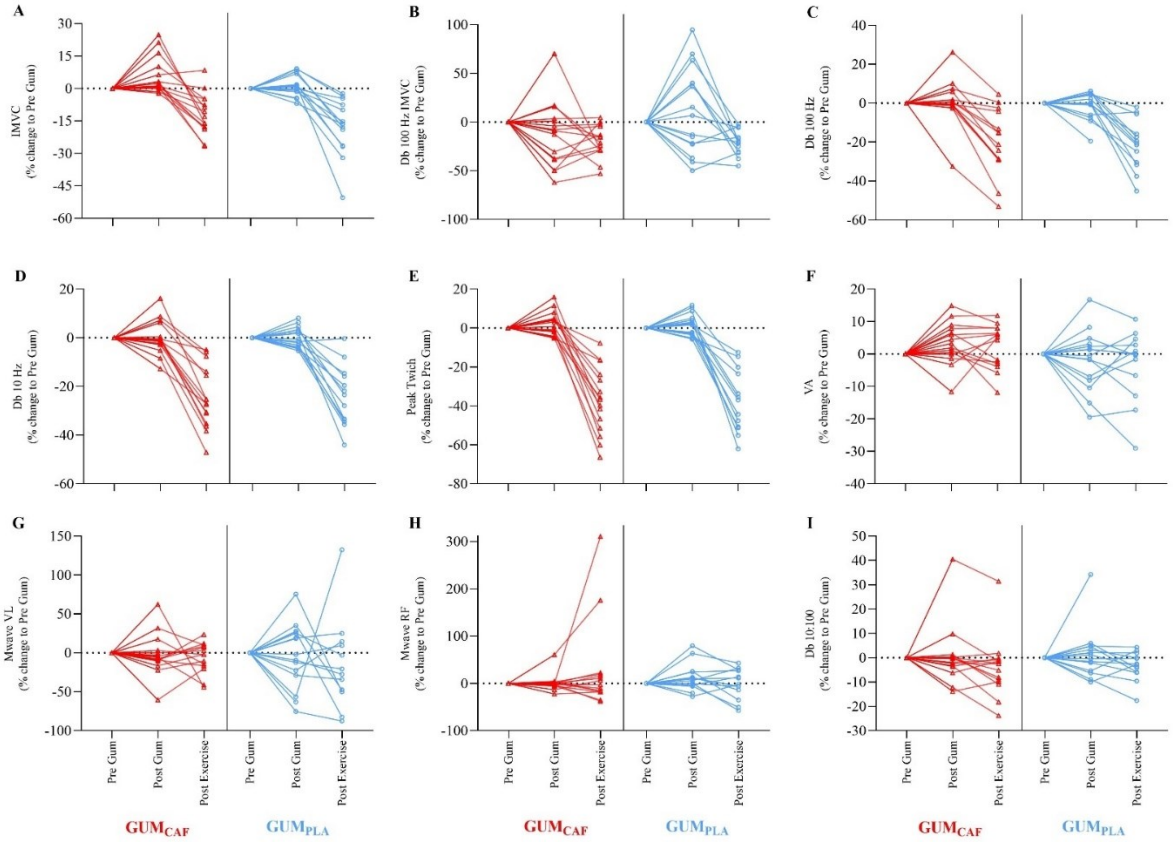

Figure S2 Individual percent changes to Pre GUM in isometric maximal voluntary contraction (IMVC) (A), high-frequency doublet during IMVC (Db_{100Hz}IMVC) (B), high-frequency doublet (Db_{100Hz}) (C), low-frequency doublet (Db_{10Hz}) (D), peak twitch (E), voluntary activation (VA) (F), Mwave of *vastus lateralis* (VL) muscle (G), Mwave of *rectus femoris* (RF) muscle (H), and low- to high-frequency doublets ratio ($Db_{10:100}$) (I), under caffeine (red triangles) and placebo (blue circles) chewing gum supplementation.

Table S1 Sprint-derived variables at Pre GUM and Post GUM and Post Exercise for caffeine or placebo chewing gum supplementation conditions.

GUM	Time point	T_{MAX} (N·m)	V_{MAX} (rad·s^{-1})	P_{MAX} (W)	OPT_{CAD} (rpm)
Caffeine	**Pre GUM**	166.9 ± 25.4	24.8 ± 1.2	1020.9 ± 144.4	123.1 ± 8.9
	Post GUM	164.1 ± 21.7	24.8 ± 1.6	976.4 ± 123.8	130.9 ± 10.4
	Post Exercise	137.4 ± 34.1	27.2 ± 1.6	912.9 ± 199.5	136.7 ± 10.2
Placebo	**Pre GUM**	162.9 ± 19.5	24.7 ± 1.7	984.1 ± 129.2	124.4 ± 9.4
	Post GUM	157.7 ± 23.4	24.8 ± 1.7	955.8 ± 140.2	124.9 ± 6.4
	Post Exercise	139.6 ± 29.1	26.7 ± 1.6	920.6 ± 181.7	135.8 ± 7.3

Data are expressed in means ± standard deviation. T_{MAX} = maximal torque, V_{MAX} = maximal velocity, P_{MAX} = maximal power output. OPT_{CAD} = optimal cadence.

Table S2 Neuromuscular function at Pre GUM and Post GUM and Post Exercise for caffeine or placebo chewing gum supplementation conditions.

GUM	Time point	IMVC (N·m)	Db_{100Hz}IMVC (N·m)	Db_{100Hz} (N·m)	Db_{10Hz} (N·m)	$Db_{10:100}$ (ratio)	Pt (N·m)	VA (%)
Caffeine	**Pre GUM**	251.3 ± 41.4	14.4 ± 6.1	92.9 ± 14.9	91.7 ± 14.2	0.99 ± 0.10	54.3 ± 7.5	84.6 ± 5.9
	Post GUM	264.3 ± 38.3	11.9 ± 4.9	92.3 ± 13.7	92.3 ± 13.7	0.99 ± 0.05	55.6 ± 8.9	86.9 ± 5.3
	Post Exercise	219.5 ± 30.7	9.6 ± 4.3	69.9 ± 11.6	67.4 ± 11.9	0.96 ± 0.05	32.2 ± 8.1	86.4 ± 5.2
Placebo	**Pre GUM**	261.1 ± 33.4	12.1 ± 5.7	90.2 ± 12.3	90.9 ± 13.7	1.00 ± 0.05	53.0 ± 7.9	86.1 ± 7.4
	Post GUM	263.9 ± 32.9	14.9 ± 10.7	90.6 ± 12.9	90.0 ± 12.9	0.99 ± 0.04	53.4 ± 8.3	83.7 ± 10.6
	Post Exercise	217.2 ± 43.9	12.1 ± 8.6	71.6 ± 16.2	69.5 ± 15.7	0.97 ± 0.04	33.1 ± 9.1	83.2 ± 10.2

Data are expressed in means ± standard deviation. IMVC = isometric maximal voluntary contraction. Db_{100hz}IMVC = High-frequency doublet during isometric maximal voluntary contraction. Db_{100} = High-frequency doublet. Db_{10} = Low-frequency doublet. $Db_{10:100}$ = Low-frequency fatigue. Pt = Peak twitch. VA = voluntary activation.

5. CHAPTER FOUR

5.1 STUDY THREE: No combined effect of caffeinated chewing gum and priming exercise on oxygen uptake and muscle NIRS-derived kinetics. A double-blind randomized crossover placebo-controlled trial in cyclists.

This first paper was accepted online on the International Journal of Sport Nutrition & Exercise Metabolism.

Original research

Title

No combined effect of caffeinated chewing gum and priming exercise on oxygen uptake and muscle NIRS-derived kinetics. A double-blind randomized crossover placebo-controlled trial in cyclists

Running Head: No combined effect caffeine and priming exercise.

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Abstract

This study aimed to investigate the effects of caffeine ingestion by chewing gum (GUM_{CAF}) combined with priming exercise on the pulmonary oxygen uptake ($\dot{V}O_2$) and NIRS-derived muscle oxygen extraction (HHb+Mb) kinetics, during cycling performed at severe-intensity domain. Fifteen trained cyclists completed four visits: two under a placebo gum (GUM_{PLA}) and two under GUM_{CAF} ingestion. Each visit consisted of two square-wave cycling bouts at Δ70 intensity (70% of difference between the $\dot{V}O_2$ at first ventilatory threshold and $\dot{V}O_{2MAX}$) with duration of 6 minutes each, and 5 minutes of passive rest between the bouts. The GUM_{PLA} or GUM_{CAF} (400 mg) were chewed for 5 minutes, 12 minutes before the first Δ70 bout in a randomized double-blind procedure. The fundamental phase and slow component of HHb+Mb and $\dot{V}O2$ kinetics were evaluated. For HHb+Mb kinetics, regardless of ingested gum, priming exercise effects occurred on the time constant (GUM_{CAF} 16.0 ± 4.0 vs 13.9 ± 2.9 s; GUM_{PLA} 15.7 ± 6.1 vs 13.2 ± 2.5 s), amplitude, slow component, time delay, and mean response time parameters ($p \leq 0.032$). For $\dot{V}O_2$ kinetics, there were significant effects of bouts on the amplitude, slow component, end $\dot{V}O_2$, and the gain kinetics parameters ($p < 0.017$). Baseline $\dot{V}O_2$ was higher during GUM_{CAF} than GUM_{PLA} ($p = 0.020$). No significant effects occurred for the interaction between gum and bout in any parameter of $\dot{V}O_2$ or HHb+Mb kinetics. Therefore, unlike the priming exercise in severe intensity exercise, GUM_{CAF} is not an effective strategy for improving $\dot{V}O_2$ or HHb+Mb kinetics acceleration.

Key words

Ergogenic aids; cycling; prior exercise

Key Points

- No additional effect of caffeinated chewing gum over priming exercise on $\dot{V}O2$ and HHb+Mb kinetics occurred during severe intensity exercise in trained male cyclists.
- The $\dot{V}O_2$ and HHb+Mb kinetics present similar behavior when combine caffeinated chewing gum and priming exercise, during double-blind randomized crossover placebo-controlled trial in cyclists.
- Unlike the priming exercise in severe intensity exercise, caffeinated chewing gum is not an effective strategy for speeding $\dot{V}O_2$ or HHb+Mb kinetics.

5.1.1 Introduction

Priming exercise has been widely used to explore the mechanistic bases of oxygen uptake ($\dot{V}O_2$) kinetics due to an enhanced oxidative energy turnover across the transition to exercise, which enables faster $\dot{V}O_2$ kinetics and lesser disturbance of intracellular homeostasis (Grassi et al. 1996; Burnley et al. 2002). In addition to the pulmonary $\dot{V}O_2$ kinetics, the use of near-infrared spectroscopy (NIRS) as a non-invasive method for muscle oxidative dynamics, suggests that priming exercise induce a slowing of muscle deoxygenation kinetics (Rossiter 2011). Of note, Fukuoka et al., (2015) suggested that there is a mechanistic link between priming exercise-induced increase in muscle hemoglobin [Hb] volume and a reduction of $\dot{V}O_2$ slow component ($\dot{V}O_{2SC}$), that serves to speed overall $\dot{V}O_2$ kinetics.

The use of caffeine (CAF) during exercises is a well-established ergogenic aid mainly for endurance performance (Burke, 2008; Grgic et al., 2020). However, the relationship between CAF ingestion and $\dot{V}O_2$ kinetics is controversial, since previous research suggest that the ergogenic effect of CAF at high-intensity endurance exercise may be partly mediated by an attenuation on the $\dot{V}O_{2SC}$ (Santalla et al., 2001). In contrast, other researchers have reported no changes in $\dot{V}O_2$ kinetics responses following CAF administration (Bell et al., 1999; Powers et al., 1986; Simmonds et al., 2010). Moreover, the effect of CAF on NIRS-derived muscle oxygen extraction kinetics (HHb+Mb) is not widely understood.

Most CAF studies typically use capsules ingested about 60 min before exercise (Wickham and Spriet, 2018). However, alternative CAF consumption methods, such as CAF mouth rinses and caffeinated chewing gum (GUM_{CAF}) (Wickham and Spriet, 2018), have gained attention due to faster absorption compared to capsules (Kamimori et al., 2002). Additionally, CAF might lead to increased tissue blood flow and oxygen supply to the exercising muscle during exercise (due to increased nitric oxide production) (Ruíz-Moreno et al. 2020), potentially enhancing muscle contractility and reducing exercise-induced fatigue (Lima-Silva et al. 2021). Investigating these concurrent mechanisms when using gum can shed light on CAF's impact on active muscles, blood flow redistribution, and oxygenation maintenance, as no previous research has explored the link between GUM_{CAF} intake and $\dot{V}O_2$ and HHb+Mb kinetics.

This study aimed to investigate the impact of combining GUM_{CAF} with priming exercises on $\dot{V}O_2$ and HHb+Mb kinetics during cycling bouts performed at the severe-intensity domain of exercise. Our main hypothesis posits that the effect of CAF could superimposed the priming exercise effect, resulting in faster $\dot{V}O_2$ and/or HHb+Mb kinetics, particularly during

the second bout. It is suggested that prior intense exercise may induce significant intracellular metabolic changes, including increased blood flow and reduced metabolic inertia, which could amplify the influence of CAF on $\dot{V}O_2$ and HHb+Mb kinetics.

5.1.2 Materials and methods

Participants

Fifteen trained male cyclists (mean ± standard deviation [SD]; age 26.5 ± 6.0 years; body mass 70.7 ± 4.7 kg, height 177.2 ± 5.4 cm; body fat 10.3% ± 3.8%) volunteered to participate in this study. The criteria for participation were having at least 2 years of experience with training in cycling endurance and were familiar with laboratory exercise testing procedures. The study protocol complied with the Declaration of Helsinki for human experimentation (Harriss & Atkinson, 2015) and the participants provided written voluntary informed consents, which were approved by the local Institutional Ethics Committee (number 3.945.514).

Experimental Design

The present investigation was a randomized cross-over and double-blind placebo-controlled trial. Allocation of the participants on GUM_{CAF} and GUM_{PLA} treatments order was made by a researcher who did not participate in the data collection. The experimental protocol was performed within 3-4 weeks with the tests being performed in the morning ~8:00 AM (± 2 h). All tests were carried out under laboratory-controlled conditions (temperature: 22 ± 1 °C and relative humidity: 50% ± 10%).

Participants completed five laboratory visits separated by a period of at least 48 h. In the first visit, the anthropometric measurements and the incremental cycling test were performed. In the four following visits, participants completed a warm-up followed by either GUM_{CAF} (in two visits) or GUM_{PLA} ingestion (in the other two visits). Each visit included two square-wave cycling bouts at Δ70 intensity (corresponding to 70% of the difference between $\dot{V}O_2$ at first ventilatory threshold and maximal oxygen uptake ($\dot{V}O_{2MAX}$)) with duration of 6 minutes each, separated by 5 minutes of passive rest (Figure 1). To decrease the risk of bias regarding the effect of GUM_{CAF} ingestion and the participants' usual food intake, a dietary control protocol was carried out. All participants were instructed to maintain their habitual dietary intake throughout the study, except to abstain from CAF intake for at least 48 h before all trials. The same pattern of the pre-exercise meal for each participant was maintained on all trial days to

avoid within-subject differences in exercise performance. Participants were instructed to discontinue the use of any nutritional ergogenic aid during the study period.

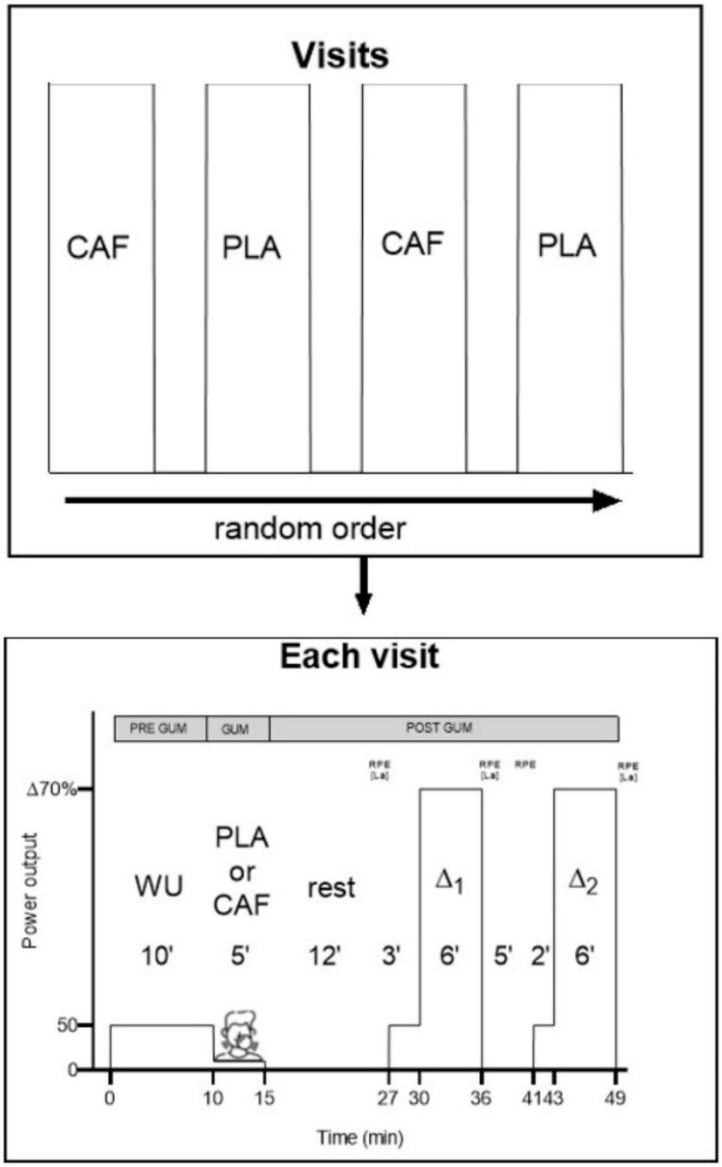

Figure 1 – Study design; Participants attended the laboratory on four occasions, during each visit, they performed two square-wave bouts, each lasting 6 minutes at an intensity of Δ70 (corresponding to 70% of the difference between $\dot{V}O_2$ at first ventilatory threshold and maximal oxygen uptake); in two visits, they ingested GUM_{CAF}, and in the other two visits, they ingested GUM_{PLA}.

RPE: rating of perceived exertion, [La]: blood lactate concentration, WU: warm-up.

Exercise testing

Incremental exercise test

The initial workload was 50 Watts (W) with increase of 25 W every minute (min) until voluntary exhaustion. The $\dot{V}O_{2MAX}$ was considered the highest moving average of 15 s of $\dot{V}O_2$ data and confirmed by following the criteria of Howley et al. (1995). The peak power output (PPO) was considered the final power output, while the maximal heart rate (HRMAX) was considered the highest individual value. The first ventilatory threshold was identified by three experienced evaluators and determined by following the criteria of Meyer et al. (2005).

Exercise bouts in the severe-intensity domain

The participants completed four visits, two under GUM_{CAF} and two under GUM_{PLA} ingestion. During each visit, they performed two bouts of square-wave exercise lasting 6 minutes each at an intensity of Δ70, with a 5-minute passive rest period between the bouts. A standardized warm-up was performed with 10 min at 50 W followed by caffeine chewing gum (see below) and 12 min of passive rest before the first bout. The first bout consisted of 3 min of baseline pedaling at 50 W, followed by 6 min at Δ70 (Δ1). Then, the participants recovered passively for 5 min and then resumed pedaling for 2 min at 50 W, followed by the second 6 min at Δ70 (Δ_2). The pilot data demonstrated steady state oxygen uptake being achieved within 2 minutes and the reason for the discrepancy, between the 3 minute and 2 minute timing prior to the square-wave transition, was intended solely to allow the familiarity with the face mask. $\dot{V}O2$ and NIRS data were collected throughout all trials. Blood lactate samples were collected from the earlobe during the rest and at the end of each exercise bout. At the end of the baseline and exercise bouts the rating of perceived exertion (RPE) was obtained. At the end of each experimental visit, the participants were asked to rate what gum (GUM_{CAF} or GUM_{PLA}) they thought they had chewed on that day.

Equipment and measures

Exercise testing was performed on an electronically braked cycle ergometer (Excalibur Sport PFM, Lode BV, Groningen, The Netherlands). Respiratory exchange data were measured by an automated open-circuit breath-by-breath gas analyzer (Quark CPET; COSMED), which was calibrated before each test according to the manufacturer's instructions. The blood lactate concentration ($[La^-]$) was assessed using an electro chemical analyzer (YSI 2700 STAT). The heart rate (HR) data set was continuously recorded (RS800CX Polar).

The NIRS-derived signal (Portamon, Artinis, Medical System, Zetten, The Netherlands) was measured by local oxygenation profiles of the vastus lateralis muscle. The NIRS probe was placed over the vastus lateralis muscle of the right leg, at least 10 cm above the knee joint along the vertical axis of the thigh, between the lateral epicondyle and the greater trochanter. This location was chosen for a single-site NIRS measurement taking into consideration the NIRS profile heterogeneity among and within the quadriceps muscles (Koga et al. 2007). The skinfolds at the site of NIRS application were determined (8.6 ± 3.7 mm) during the first visit. Differences in the absorption characteristics of light at two wavelengths (λ = 750 and 850 nm) were used to measure changes in tissue concentrations of oxyhemoglobin and/or myoglobin (HbO_2), deoxyhemoglobin and/or myoglobin (HHb).

Caffeine Chewing Gum

Participants chewed each type of gum during the 5 min of passive rest prior to the first Δ70 and were required to dispose of the chewed gum into a trash, then they drank 250 mL of water. The CAF administration consisted of four pieces of a commercially available gum(Military Energy Gum—Stay Alert, Arctic Mint) with 100 mg of CAF in each (total 400 mg), and the PLA consisted of four pieces of gum of similar characteristics (Trident XFresh Crystal Mint).

Data analyses

The breath-by-breath $\dot{V}O_2$ data analysis was processed and analysed as described previously (do Nascimento Salvador et al., 2023). Participants completed two visits under GUM_{CAF} ingestion and two visits under GUM_{PLA} ingestion. For each participant, data from the two GUM_{CAF} visits were time-aligned to the same Δ70 bout (i.e. Δ_1 or Δ_2) and averaged for analysis and presentation. The same procedure was applied to the data from the GUM_{PLA} visits. To identify key parameters, the model was 'fixed' to the baseline $\dot{V}O_2$ according to the monoexponential equation:

$$\dot{V}O_2(t) = \dot{V}O_{2base} + A_p \cdot [1 - e^{-(t - TDp / \tau p)}] \quad (1)$$

where $\dot{V}O_2$ (t) is the absolute $\dot{V}O_2$ at any given time (t), $\dot{V}O_{2base}$ is the average value of $\dot{V}O_2$ during the 1 min preceding the start of the exercise in the baseline period, Ap, TDp, and τp are the amplitude, delay time and time constant, respectively, characterizing the primary phase of $\dot{V}O_2$ kinetics.

The $\dot{V}O_{2SC}$ was determined following the procedures of previous study (Murgatroyd and Wylde, 2011). Thus, at the end of the cardio dynamic phase (20 s), monoexponential adjustments were performed in different time windows lasting up to 240 s, after the start of $\dot{V}O_{2SC}$. Then, the determination of the beginning of the slow phase was made according to criteria as follows: a) a breakpoint and a systematic increase in τ and Ap, respectively; b) a systematic decrease in TD; c) the smallest chi-square value; and d) the narrowest point in the 95% confidence interval. Thus, $\dot{V}O_{2SC}$ was calculated as follows:

$$\dot{V}O_{2SC} = \dot{V}O_{2END} - (\dot{V}O_{2base} + Ap) \quad (2)$$

Where $\dot{V}O_{2END}$ was considered the average $\dot{V}O_2$ of the last 30 s of exercise.

The slow component trajectory was determined (i.e. the rate of increase in $\dot{V}O_2$ during the second phase), as well as the functional gain of the fundamental phase with respect to work rate (in $ml \cdot W^{-1} \cdot min^{-1}$) and $\dot{V}O_{2SC}$ was expressed in percentage ($\%\dot{V}O_{2RES}$).

The HHb+Mb kinetics was modeled applying Equation (1) and (2) previously described.

Statistical analysis

A sample size calculation in G*Power (Version 3.1.9.4, Germany) was based on the intra-class correlation of 0.90, alpha of 5%, the statistical power of 80%, and a small F effect size (partial eta squared: 0.01); this yielded a sample size of 13 subjects. Descriptive data are presented as mean ± SD. A two-way repeated-measures ANOVA model was used to analyze the interaction between priming exercise and GUM_{CAF} (or GUM_{PLA}) administration. The model fixed effects were the ingestion (GUM_{CAF} and GUM_{PLA}), the bout (Δ_1 and Δ_2), and the interaction ingestion × bout. When appropriate, a Tukey post hoc test was used to determine the pairwise comparisons. The Shapiro-Wilk test was applied to check the normality of model residuals. Analysis was carried out using software R (R Core Team, Viena, Austria) with the packages 'Rcmdr', 'lme4', and 'lsmeans'. The statistical significance level was set as $p \leq 0.05$.

5.1.3 Results

Incremental cycling test

The values of $\dot{V}O_{2MAX}$, HR_{MAX}, and PPO obtained during the incremental test were 4.1 ± 0.5 $L \cdot min^{-1}$ (57.3 ± 6.4 $ml \cdot kg^{-1} \cdot min^{-1}$), 189 ± 7 bpm, and 350 ± 47 W, respectively. The $\dot{V}O_2$ at first ventilatory threshold and Δ70% occurred at 62% ± 4% and 88% ± 3% of $\dot{V}O_{2MAX}$, respectively; while first ventilatory threshold and Δ70% power outputs were located at 50% ± 4% and 78% ± 4% of PPO, respectively.

Exercise bouts at Δ70%

The average physiological (HR and $[La^-]$) and perceptual (RPE) responses obtained during baseline and the conditions at the end of the two bouts (Δ_1 and Δ_2) during GUM_{CAF} vs GUM_{PLA} are presented in Table 1. It was verified that there were significant effects on HR, RPE, and $[La^-]$ ($p < 0.001$) between first and second bouts regardless of the ingestion (i.e. ANOVA main effect of bout). A significant difference between GUM_{CAF} and GUM_{PLA} irrespective of the bout (ANOVA main effect of ingestion) was found for baseline HR ($p < 0.001$) and end exercise HR ($p = 0.017$); end exercise $[La^-]$ ($p < 0.001$), and baseline and end bout RPE ($p \leq 0.046$). There were no significant interaction effects for bout × ingestion ($p = 0.618$ to 0.709) for any variable.

Table 1. Physiological and perceptual responses during caffeine and placebo chewing gum ingestion for the first and second bout during exercise at Δ70.

Time measurement and variable	Δ_1		Δ_2		**ANOVA main effects**		
	GUM_{CAF} (mean ± SD)	**GUM_{PLA} (mean ± SD)**	**GUM_{CAF} (mean ± SD)**	**GUM_{PLA} (mean ± SD)**	**Bout**	**Ingestion**	**Bout × Ingestion**
Baseline HR (bpm)	118 ± 12	113 ± 12	128 ± 11	123 ± 11	$p < 0.001$	$p < 0.001$	$p = 0.709$
End exercise HR (bpm)	176 ± 5	174 ± 6	181 ± 5	180 ± 5	$p < 0.001$	$p = 0.017$	$p = 0.630$
Baseline [La^-] (mmol·L^{-1})	1.5 ± 0.2	1.4 ± 0.3	–	–	–	[a]$p = 0.384$	–
End exercise [La^-] (mmol·L^{-1})	9.8 ± 2.4	9.0 ± 1.2	13.1 ± 2.9	12.1 ± 1.8	$p < 0.001$	$p < 0.001$	$p = 0.687$
Baseline RPE (a.u.)	8.3 ± 2.1	8.7 ± 1.7	9.5 ± 2.2	10.1 ± 2.3	$p < 0.001$	$p = 0.046$	$p = 0.618$
End exercise RPE (a.u.)	15.2 ± 1.6	15.9 ± 1.3	17.2 ± 1.4	17.7 ± 1.5	$p < 0.001$	$p = 0.002$	$p = 0.693$

[a]: paired test-t, a.u.: arbitrary units, bpm: beats per minute, GUM_{CAF}: caffeine chewing gum condition, GUM_{PLA}: placebo chewing gum condition, HR: heart rate, Δ_1: first bout at Δ70, Δ_2: second bout at Δ70, [La^-]: blood lactate concentration, RPE: rating of perceived exertion.

Pulmonary $\dot{V}O_2$ kinetics

Figure 2A-D presents the $\dot{V}O_2$ behavior during the Δ_1 and Δ_2 exercises either during GUM_{CAF} and GUM_{PLA} conditions. The $\dot{V}O_2$ kinetics are presented in Figure 3, and it was verified that there were significant effects between first and second bout regardless of ingestion (i.e. ANOVA main effect of bout) on A, A_{TOTAL}, $\dot{V}O_{2SC}$, $\dot{V}O_{2END}$, and Gain $\dot{V}O_2$ parameters ($p \leq 0.001$ to 0.017). A significant difference between GUM_{CAF} and GUM_{PLA} irrespective of bout (ANOVA main effect of ingestion) was found only on Baseline $\dot{V}O_2$ ($p = 0.020$). There were no significant interaction effects on bout × ingestion ($p = 0.079$ to 0.992), suggesting no additional effect of GUM_{CAF} on priming effects of the prior exercise.

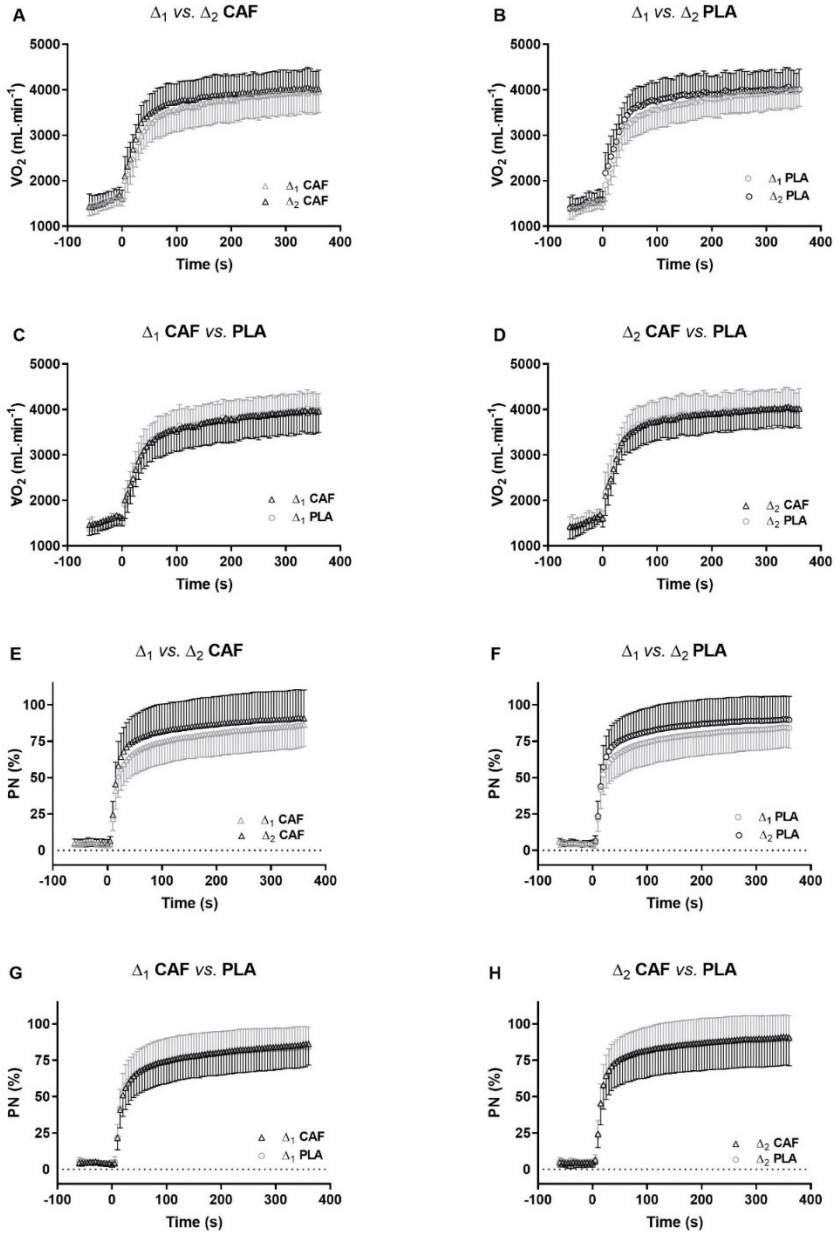

Figure 2 – Pulmonary oxygen uptake (A–D) and NIRS-derived (E–H) kinetics during caffeine and placebo chewing gum ingestion, during the two bouts of cycling exercise on severe intensity domain (Δ70).

Data are shown as means (triangles and circles) with standard deviations (error bars).

CAF: caffeine chewing gum condition, PLA: placebo chewing gum condition, PN: physiological normalization, $\dot{V}O_2$: oxygen uptake, Δ_1: first bout at Δ70, Δ_2: second bout at Δ70, Δ_1CAF: first bout at Δ70 during caffeine ingestion, Δ_2CAF: second bout at Δ70 during caffeine ingestion, Δ_1PLA: first bout at Δ70 during placebo ingestion, Δ_2PLA: second bout at Δ70 during placebo ingestion.

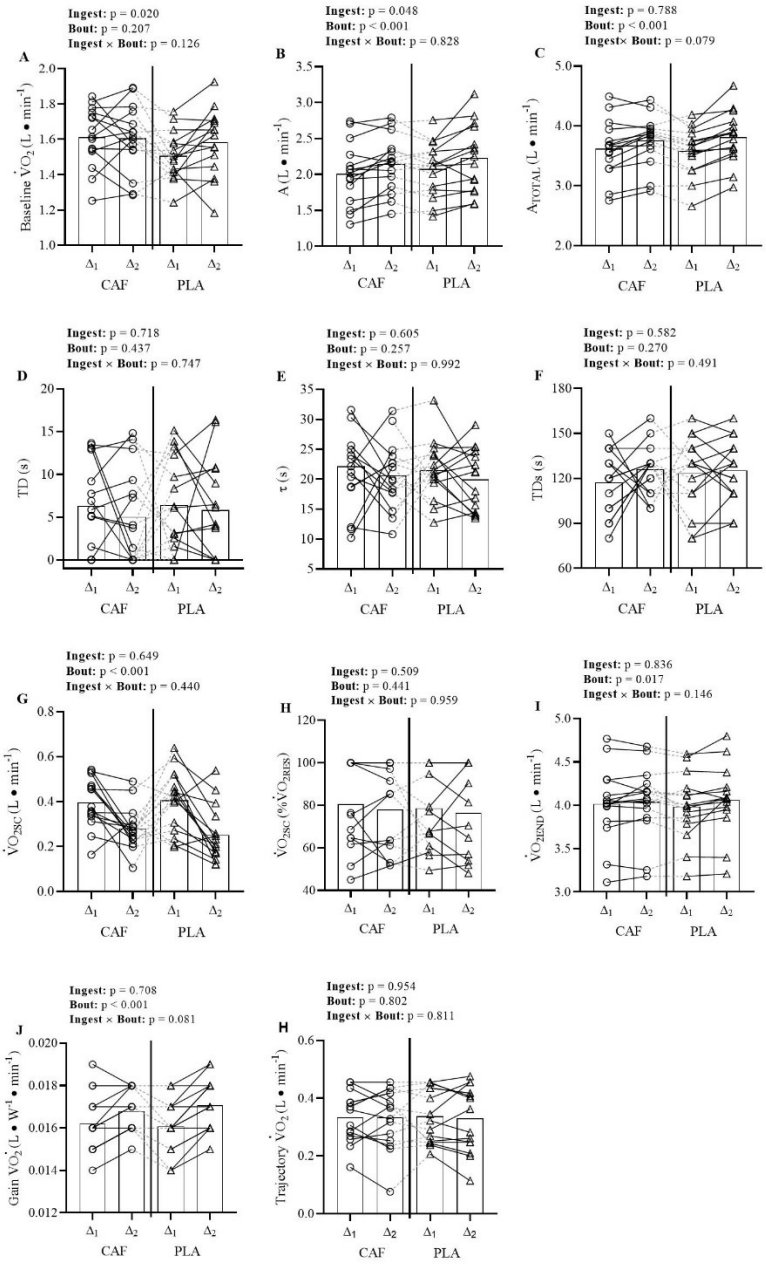

Figure 3 – Individual data (dots) and the sample means (bars) for the effect of caffeinated over placebo chewing gum and the priming exercise on the variables of the pulmonary oxygen uptake kinetics during the two constant bouts in the severe-intensity domain (Δ70).

Ingest: Ingestion ANOVA mean effect, Bout: bout ANOVA main effect, Ingest × Bout: interaction between ingestion and bout ANOVA effect, CAF: caffeine chewing gum condition, PLA: placebo chewing gum condition, $\dot{V}O_2$: oxygen uptake, Δ_1: first bout at Δ70, Δ_2: second bout at Δ70, A: Fundamental amplitude, A_{TOTAL}: amplitude + baseline, TD: time delay, τ: (tau) time constant, TD_S: time delay of slow phase, $\dot{V}O_{2SC}$: slow component of oxygen uptake, $\dot{V}O_{2END}$: mean $\dot{V}O_2$ in the last 30 s of exercise.

NIRS-derived kinetics

The NIRS-derived kinetics behavior during the Δ_1 and Δ_2 either during GUM_{CAF} and GUM_{PLA} conditions is shown in Figure 2E-H. The NIRS-derived kinetics parameters are presented in Figure 4, and it was verified that there were significant effects between first and second bout regardless of ingestion (ANOVA main effect of bout) on A, A_{TOTAL}, $HHb+Mb_{SC}$, $HHb+Mb_{END}$, TD, τ, and MRT parameters ($p \leq 0.001$ to 0.032). The comparison between GUM_{CAF} and GUM_{PLA} irrespective of the bout (ANOVA main effect of ingestion) revealed no significant difference in any parameter of NIRS-derived kinetics ($p = 0.119$ to 0.895). Also, there were no significant effects on interaction bout × ingestion ($p = 0.215$ to 0.981).

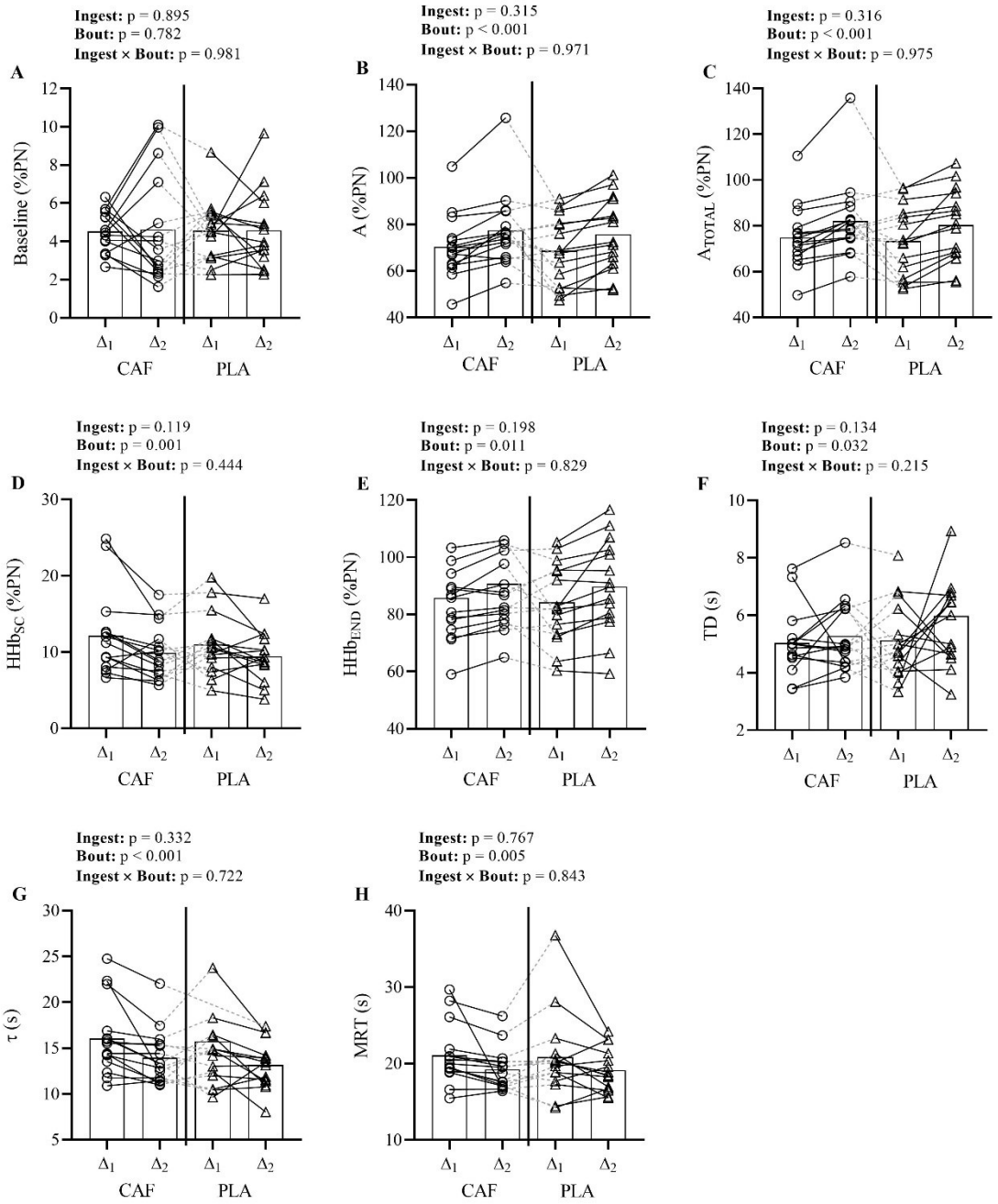

Figure 4 – Individual data (dots) and the sample means (bars) for the effect of caffeinated over placebo chewing gum and priming exercise on the variables of the deoxyhemoglobin and/or myoglobin (HHb) kinetics during the two constant bouts in the severe-intensity domain (Δ70). Ingest: Ingestion ANOVA mean effect, Bout: bout ANOVA main effect, Ingest × Bout: interaction between ingestion and bout ANOVA effect, CAF: caffeine chewing gum condition, PLA: placebo chewing gum condition, PN: physiological normalization, Δ_1: first bout at Δ70,

Δ_2: second bout at Δ70, A = fundamental amplitude. A_{TOTAL} = amplitude + baseline. HHb_{SC} = subtraction of END - A_{TOTAL}. HHb_{END} = mean of the last 30 s of exercise. TD = time delay. τ = time constant. MRT = mean response time (TD + τ).

Supplement blinding efficacy

At the end of each daily experiment, the participants were asked what gum they believed had chewed. The rates of correct guesses were 40%, 73%, 80%, and 93% on the first to fourth visit, respectively (average of 72% with a 95% CI of 49% to 94%). A binomial test was used for comparing the percentage of correct guesses with a probability of 50% and confirmed that the rate of correct guesses did not differ significantly from the 50% rate (p = 0.120).

5.1.4 Discussion

The main findings of the present study were that no additional effect of GUM_{CAF} over prior exercise on $\dot{V}O_2$ and HHb+Mb kinetics occurred during severe intensity exercise in trained male cyclists. Also, a similar behavior was observed at pulmonary and muscular level responses, being that GUM_{CAF} did not alter any $\dot{V}O_2$ or HHb+Mb kinetics parameters, except the baseline $\dot{V}O_2$ for the first exercise. Thus, the main hypothesis was not supported as the GUM_{CAF} failed to potentiate the effects of priming exercise on $\dot{V}O_2$ and HHb+Mb kinetics during an intense exercise in trained cyclists, particularly in the second bout of exercise.

In the current study, novel aspects were investigated with gum delivery CAF method. We used 12 minutes separating CAF ingestion and the exercise commencement, based on previously reporting by Morris et al. (2019), where the peak plasma CAF concentration occurred at approximately 10 minutes after chewing GUM_{CAF}. In addition, the measurement of HHb+Mb at a selected muscle provided a more comprehensive view than previous studies that had analysed the effects of CAF on $\dot{V}O_2$ kinetics (Bell et al., 1999; Simmonds et al., 2010; Santalla et al., 2001). Although, there were no observed effect of CAF on the fast component of pulmonary $\dot{V}O_2$ kinetics during a single cycling exercise (Bell et al., 1999; Simmonds et al., 2010), Santalla et al., (2001) found an attenuation on the amplitude of $\dot{V}O_2$ slow component during running exercise at severe intensity. It is important to highlight that these authors used a limited method to detect the $\dot{V}O_{2SC}$, that is, the difference between end and 3-min values. In turn, we have used a more robust technique to detect $\dot{V}O_{2SC}$ (i.e. slow component trajectory) during cycling exercise, which implies different responses of $\dot{V}O_2$ and HHb+Mb kinetics due to the recruitment of different groups and muscle fibers. However, despite the differences in

the methodological approaches, the data of present study supports the fact that CAF does not accelerate the $\dot{V}O_2$ kinetics.

The effect of prior exercise on the acceleration of primary $\dot{V}O_2$ kinetics and on attenuation of $\dot{V}O_2$ slow component is widely known (Burnley et al., 2000; DeLorey et al., 2007; Fukuoka et al., 2015). Based on some neurophysiological effects of CAF, we tested whether both conditions (i.e. priming exercise and CAF) could trigger a synergistic effect on the $\dot{V}O_2$ kinetics. For instance, there is evidence that CAF augments endothelium-dependent vasodilation due to an increase on nitric oxide production. Thus, CAF might lead to increased tissue blood flow and oxygen supply to the exercising muscle during exercise, which partly supports our hypothesis that HHb+Mb kinetics could be accelerated. Indeed, the effect of CAF on muscle O_2 saturation (measured by NIRS) has been shown at intensities below 70% of $\dot{V}O_{2MAX}$ during an incremental exercise test (Ruíz-Moreno et al. 2020). In this sense, the different intensities could explain the absence of effect in our experimental model. As expected, the magnitude of $\dot{V}O_{2SC}$ decreased during the second bout of exercise. However, CAF did not potentiate this effect, as could be observed by absence of significant interaction effect. Therefore, the results suggest that unlike the priming exercise in severe intensity exercise, GUM_{CAF} is not an effective strategy for accelerating $\dot{V}O_2$ or HHb+Mb kinetics or decreasing the $\dot{V}O_{2SC}$, and consequently would be unlikely to attenuate muscle fatigue during severe intensity exercise in trained male cyclists.

The study found that GUM_{CAF} had similar effects on $\dot{V}O_2$ and HHb+Mb kinetics responses both centrally and peripherally. To sustain a constant work rate during exercise, additional muscle fibers must be recruited (Murgatroyd and Wylde, 2011). However, it is not totally understood whether this contributes directly to increasing energy and oxygen costs reflected in $\dot{V}O_{2SC}$, or if peripheral fatigue itself reduces efficiency, or if both factors were at play (Murgatroyd and Wylde, 2011). In this study, HHb+Mb kinetics offer valuable insights into comparison between Δ_1 and Δ_2, specifically for the time constant (τ), in both gum conditions, which were different from the $\dot{V}O_2$ kinetics. The difference observed for τ between $\dot{V}O_2$ and HHb+Mb kinetics parameters might be attributed to differences in acquisition rates (i.e. higher for NIRS) and the location of measurements. Additionally, it's important to note that our findings contrast with those of Fukuoka et al. (2015). Their study demonstrated that priming (heavy) exercise significantly slowed the [deoxy (Hb + Mb)] kinetics (i.e. increased τ), although the coefficient of variation for the primary time constant decreased. These discrepancies in results may arise from differences not only in the participant training levels but also in data modeling procedures, as Fukuoka et al. (2015) employed a three-term exponential fitting.

Furthermore, Fukuoka et al. (2015) presented [deoxy (Hb + Mb)] kinetics parameters at four distinct sites, including *vastus lateralis* and *rectus femoris* muscles at both distal and proximal locations. This differs from our study, where we focused on a single distal site in the *vastus lateralis*.

Although no significant ingestion/bout interactions occurred in any variable of $\dot{V}O_2$ and HHb+Mb kinetics, significant differences were found in magnitude of $\dot{V}O_{2SC}$ between Δ_1 and Δ_2 regardless of chewing gum being administered (i.e. GUM_{CAF} or GUM_{PLA}). Fukuoka et al. (2015) showed no changes occurred in the end-exercise $\dot{V}O_2$ and time-constant τ, consistent with the results of the present study, which reduced $\dot{V}O_{2SC}$ without altering $\dot{V}O_{2END}$ and τ in CAF condition.

For other physiological measurements, significant differences were observed between GUM_{CAF} (i.e. greater values) and GUM_{PLA} for baseline HR (at Δ_1 and Δ_2); baseline and end bout RPE; and $[La^-]$ at the end of the second exercise bout. Since no significant ingestion/bout interactions occurred for any variable of HHb+Mb kinetics, one could observe an interesting disconnect between HR and rate of muscle oxygen delivery. There is evidence that several factors associated with CAF, as adenosine-receptor antagonism and enhanced sympathetic activity, induces effect on cardiovascular regulation, which can result in either central or peripheral actions (Graham, 2001). The results suggest that for exercises similar (i.e. duration and intensity) to that used in the present study, CAF had a tendency to affect more the central (HR, baseline $\dot{V}O_2$ and RPE) than peripheral (HHb+Mb kinetics) responses.

There are likely physiological mechanisms linked to the effects of priming exercise, which could induce a beneficial influence on the amount of work-to-energy demand ratio. One of them could possibly be the increase in the recruitment of motor unit profiles, which could represent an optimization of the adaptation to sustain the exercise with the same external load. In this way, less intracellular disturbance could be generated and exercise tolerance could be improved (DeLorey et al., 2007; Grassi, 1996). In addition, another factor could be related to O_2 delivery to the active tissues and an increase in mitochondrial enzymatic activities after the priming exercise, which could attenuate the rate of fatigue development, since these aspects play a critical role as a rate-limiting factor (Murias, Spencer, & Paterson, 2014).

One limitation of this study is the restriction in the characteristic of the sample (i.e. trained male cyclists). We recognize that the present study could be included female cyclists in the sample, which would produce a more comprehensive data. Recent and strong evidence have shown that physiological responses are not significantly influenced by their menstrual cycle phase or hormonal contraceptive use (Taipale-Mikkonen et al., 2021). In addition, it could also

be argued that, rather than 70% delta, a fixed percentage above critical power (measured independently) would have ensured all subjects are in the same (severe) intensity domain. However, all the participants achieved $\dot{V}O_{2MAX}$ during the two bouts in all visits, showing that subjects exercised at the severe domain. Finally, it is also worth mentioning the individual sensitivity to CAF, given that caffeinated gum and mouth rinse delivery appears to act via central rather than peripheral mechanisms, which may account, in part, for some of the variability on findings. In recent years, different genetic polymorphism which are responsible for the CAF absorption and metabolization have been identified, such as ADORA2A and CYP1A2 (Nehlig 2018). Although it is out-of-scope of the current study, this should be further investigating especially regarding the effects of GUM_{CAF}.

5.1.5 Conclusion

GUM_{CAF} did not potentiate the effects of priming exercise on $\dot{V}O_2$ and HHb+Mb kinetics during exercise at a severe intensity domain in trained male cyclists. Therefore, unlike the priming exercise in severe intensity exercise, GUM_{CAF} is not an effective strategy for accelerating $\dot{V}O_2$ or HHb+Mb kinetics.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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References

The references of the paper are at “references section” page 107.

6. CONCLUSION

Study1: From the power-cadence hyperbolic relationship, the parameters P_{MAX} and OPT_{CAD} could be detected similarly between the ISO_{VEL} and ISO_{LIN} sprint cycling modes. The findings also demonstrated that the single 7-second sprint test (i.e. isolinear) could be a suitable tool for quantifying the time course of muscle fatigue during and after cycling exercises in well-trained male cyclists. Therefore, the interchangeability of the modes should be extrapolated to different populations, situations, and conditions to understand better the implications of the agreement between the modes.

Study 2: GUM_{CAF} was not effective to attenuate the muscle force decrement triggered by the severe intensity cycling exercises, when measured by both methods (isometric vs. dynamic). However, some variables derived from all sprints measure, namely P_{MAX} and T_{MAX}, were higher for GUM_{CAF} than GUM_{PLA}. In addition, the percentage decrement from before and after the exercise showed no difference for dynamic and isometric measurements, between the gum conditions, although the correlations between isometric force and cycling peak torque/power for both gum conditions suggest interchangeability between dynamic and isometric variables. Thus, the sprint cycling protocol proposed in the present study should be considered for studying fatigue and/or neuromuscular performance.

Study 3: GUM_{CAF} did not provide a synergic effect with priming exercise on $\dot{V}O_2$ and HHb+Mb kinetics during exercise at the severe intensity domain in trained male cyclists. Therefore, unlike the priming exercise, GUM_{CAF} is not an effective strategy for accelerating $\dot{V}O_2$ or HHb+Mb kinetics.

REFERENCES

ABOODARDA, S. J. et al. Effects of endurance cycling training on neuromuscular fatigue in healthy active men. Part II: Corticospinal excitability and voluntary activation. **European Journal of Applied Physiology**, 118, p. 2295–2305, 2018.

AUSTRALIAN INSTITUTE OF SPORT (AIS). **The AIS Sports Supplement Framework**. 2022 August; Retrieved from https://www.sportaus.gov.au/ais/nutrition/supplements https://www.ais.gov.au/__data/assets/pdf_file/0014/1000841/Position-Statement-Supplements-and-Sports-Foods.pdf

ALLEN DG, LAMB GD, WESTERBLAD H. Skeletal muscle fatigue: cellular mechanisms. Physiol Rev. 2008 Jan;88(1):287-332. doi: 10.1152/physrev.00015.2007. PMID: 18195089.

ALTIMARI, L.R. et al. Cafeína e performance em exercícios anaeróbios. **Revista Brasileira de Ciências Farmacêuticas**, São Paulo, v. 42, n. 1, p.17-27, 2006.

AMANN M (2011) Central and peripheral fatigue: Interaction during cycling exercise in humans. **Medicine Science Sports Exercise**, 43(11):2039–2045. doi:10.1249/MSS.0b013e31821f59ab

ANSELME, F. et al. Caffeine increases maximal anaerobic power and blood lactate concentration. **European Journal Applied Physiology Occupational Physiology**, v. 65, n. 2, p. 188-91, 1992.

BARON, R. et al. Measurement of maximal power output in isokinetic and non-isokinetic cycling. A comparison of two methods. **International Journal Sports Medicine**, v.20, p.532-537, 1999.

BARRETO, G. et al. Effects of caffeine chewing gum supplementation on exercise performance: A systematic review and meta-analysis. **European Journal Sport Science**, v. 27, p. 1-12, 2022. doi: 10.1080/17461391.2022.2049885.

BARSTOW, T. J. et al. Influence of muscle fiber type and pedal frequency on oxygen uptake kinetics of heavy exercise. **Journal Applied Physiology**, v. 81, n. 4, p. 1642–1650, 1996.

BAZZUCCHI, I. et al. Caffeine improves neuromuscular function during maximal dynamic exercise., **Muscle nerve**, v. 43, n. 6, p. 839-844, 2011.

BECK, T. W. et al. The acute effects of a caffeine-containing supplement on strength, muscular endurance, and anaerobic capabilities. **Journal Strength Condition Research**. v. 20, n. 3, p. 506-10, 2006.

BEEDIE, C. J. et al. Placebo effects of caffeine on cycling performance. **Medicine Science Sports Exercise,** v. 38, p. 2159–2164, (2006). doi:10.1249/01.mss.0000233805.56315.a9

Beelen A, Sargeant AJ. Effect of fatigue on maximal power output at different contraction velocities in humans. **Journal Applied Physiology** (1985), v. 71, p. 2332–2337, 1991.

BEHM, D.G.; ST-PIERRE, D.M.; PEREZ, D. Muscle inactivation: assessment of interpolated twitch technique. **Journal Applied Physiology**, v. 81, p. 2267–2273, 1996.

BEHRENS M, GUBE M, CHAABENE H, PRIESKE O, ZENON A, BROSCHEID KC, SCHEGA L, HUSMANN F, WEIPPERT M. Fatigue and Human Performance: An Updated Framework. **Sports Medicine,** v. 53(1), p. 7-31, 2023. doi: 10.1007/s40279-022-01748-2.

BELL, C. et al. The effects of caffeine on the kinetics of O2 uptake, CO2 production and expiratory ventilation in humans during the on-transient of moderate and heavy intensity exercise. **Experimental Physiology**, v. 84, n. 4, p. 761–774, 1999.

BENEDETTI, F. Mechanisms of placebo and placebo-related effects across diseases and treatments. **Annual Review of Pharmacology and Toxicollogy, v.** 48, p. 33–60, 2008

BENOWITZ, N. L., JACOB, P.; SAVANAPRIDI, C. Determinants of nicotine intake while chewing nicotine polacrilex gum. **Clinical Pharmacology Therapeutics**, v. 41, p. 467–473, 1998.

BERTUCCI, W. et al. Effects on the crank torque profile when changing pedalling cadence in level ground and uphill road cycling. **Journal Biomech**, v. 38, n.5, p.1003–1010, 2005.

BLACK, M. I. et al. Self-pacing increases critical power and improves performance during severe-intensity exercise. **Applied Physiology, Nutrition, and Metabolism**, v. 9, n. October 2014, p. 1–9, 2015.

BOUSQUET, E. et al. Bioavailability of two formulations of acetylsalicylic acid gums. **Pharmazie**, v.47, p.607–609, 1992.

BRIDGE, C. A.; JONES, M. A., The effect of caffeine ingestion on 8 km run performance in a field setting., **Journal of Sports Sciences**, v. 24, n. 4, p. 433-439, 2006.

BRISSWALTER, J. et al. Energetically optimal cadence vs. freely-chosen cadence during cycling: effect of exercise duration. **International Journal Sports Medicine**, v.21, n. 1, p. 60–64, 2000. doi:10.1055/s-2000-8857

BURKE, L. M. Caffeine and sports performance. **Applied Physiology, Nutrition, and Metabolism**, v. 33, n. 6, p. 1319-34, 2008. doi: 10.1139/H08-130. PMID: 19088794.

BURNLEY, M., et al. Effects of prior heavy exercise on phase II pulmonary oxygen uptake kinetics during heavy exercise. **Journal of Applied Physiology**, v. 89, n. 4, pag. 1387–1396, 2000.

BURNLEY, M. et al. Effects of prior heavy exercise on VO(2) kinetics during heavy exercise are related to changes in muscle activity. **Journal of Applied Physiology**, (Bethesda, Md. : 1985), v. 93, n. 1, p. 167–74, jul. 2002.

BURNLEY, M.; JONES, A. M. Oxygen uptake kinetics as a determinant of sports performance. **European Journal Sport Science**, v. 7, pag. 63–79, 2007.

BUŚKO K. Power output and mechanical efficiency of human muscle in maximal cycle ergometer efforts at different pedalling rates. **Biology of Sport**, v.22, n. 1, p. 35–51, 2005.

CANNON, D. T. et al. Skeletal muscle fatigue precedes the slow component of oxygen uptake kinetics during exercise in humans. **The Journal of physiology**, v. 589, n. Pt 3, p. 727–739, 1 fev. 2011.

CAPUTO, F. et al. Cafeína e desempenho anaeróbio. **Revista Brasileira de Cineantropometria e Desempenho Humano**, Florianópolis, v. 14, n. 5, p. 602-614, 2012.

CHRISTENSEN P.M, SHIRAI Y, RITZ C, et al. Caffeine and bicarbonate for speed. a metaanalysis of legal supplements potential for improving intense endurance exercise performance. **Frontiers Physiology**, v. 9, n. 8, p. 240, 2017.

CHRISTURP, L. L. et al. Relative bioavailability of methadone administered in chewing gum and tablets. **Acta Pharm. Nord**, v. 2, p. 83–88, 1990.

COLOSIO, A. L, et al. Metabolic instability vs fibre recruitment contribution to the [Formula: see text] slow component in different exercise intensity domains. **Pflügers Archiv: European Journal of Physiology**, v. 473, n. 6, p. 873-882, 2021doi: 10.1007/s00424-021-02573-8.

COUTO, P. G, et al. Effects of caffeine on central and peripheral fatigue following closed- and open-loop cycling exercises. **Brazilian Journal of Medical Biological Research**, Feb 28;55:e11901, 2022. doi: 10.1590/1414-431X2021e11901.

COYLE, E. F. et al. Determinations of endurance in well-trained cyclists. **Journal Applied Physiology**, v. 64, p. 2622-30, 1988.

COYLE, E. F. et al. Physiological and biomechanical factors associated with elite endurance cycling performance. **Medicine Science Sports Exercise**, v. 23, n.1, p. 93-107, 1991.

CRUZ, R. S. et al. Caffeine Affects Time to Exhaustion and Substrate Oxidation during Cycling at Maximal Lactate Steady State. **Nutrients**, v. 30, n.7(7), p. 5254-64. 2015. doi: 10.3390/nu7075219.

DALY, J. W. et al. The role of adenosine receptors in the central action of caffeine. **Pharmacopsychoecologia**, v. 7, n. 2, p. 201-213, 1994.

DAVIS, J. K.; GREEN, J. M. Caffeine and anaerobic performance: ergogenic value and mechanisms of action. **Sports Medicine**, v. 39, p. 813–32. 2009.

DAVIS R. R.; HULL, M. L. Measurement of pedal loading in bicycling: II. Analysis and results. **Journal of Biomechanics**, v. 14, n. 12, p. 857-72, 1981. doi: 10.1016/0021-9290(81)90013-0.

DAVOLI, E. et al. 1998. Rapid solid-phase extraction method for automated gas chromatographic-mass spectrometric determination of nicotine in plasma. **Journal Chromatography B, Biomedical Science Application**, v. 707, p. 312–316, 1998.

DEL COSO, J.; MUÑOZ, G.; MUÑOZ-GUERRA, J. Prevalence of caffeine use in elite athletes following its removal from the World Anti-Doping Agency list of banned substances. **Applied Physiology, Nutrition, and Metabolism**, v. 36, p. 555–61, 2011.

DELOREY, D.S., KOWALCHUK, J.M., HEENAN, A.P., DUMANOIR, G.R., PATERSON, D.H. (2007). Prior exercise speeds pulmonary O2 uptake kinetics by increases in both local muscle O2 availability and O2 utilization. **Journal of Applied Physiology**, (1985). 103(3), 771-8. doi: 10.1152/japplphysiol.01061.2006.

DE PAUW, K, ROELANDS, B, CHEUNG, SS, DE GEUS, B, RIETJENS, G, MEEUSEN, R. Guidelines to classify subject groups in sport-science research. **International Journal Sports Physiology Performance,** 2013 Mar;8(2):111-22. doi: 10.1123/ijspp.8.2.111.

DOHERTY, M.; SMITH, P. M. Effects of caffeine ingestion on exercise testing: a meta-analysis. **International Journal Sport Nutrition Exercise Metabolism**, Dec;v. 14, n. 6, p. 626-46, 2004. doi: 10.1123/ijsnem.14.6.626.

DOHERTY, M.; SMITH, P. M. Effects of caffeine ingestion on rating of perceived exertion during and after exercise: a meta-analysis**. Scandinavian Journal of Medicine & Science in Sports**, v. 15, n. 2, p. 69-78, 2005.

do NASCIMENTO SALVADOR, P. C. et al. The effects of priming exercise on the V̇O2 slow component and the time-course of muscle fatigue during very-heavy-intensity exercise in humans. **Applied Physiology, Nutrition, and Metabolism**, v. 43, n. 9, p. 909-919. 2018 doi: 10.1139/apnm-2017-0769.

DOREL, S. Mechanical effectiveness and coordination: new insights into sprint cycling performance. In J. B. Morin, & P. Samozino (Eds.), **Biomechanics of training and testing**, Cham: Springer. p. 33–62, 2018a. https:// doi.org/10.1007/978-3-319-05633-3

DOREL, S. Maximal force-velocity and power-velocity characteristics in cycling: Assessment and relevance. In J. B. Morin, & P. Samozino (Eds.), **Biomechanics of training and testing**, Cham: Springer, p. 7–31, 2018b. https:// doi.org/10.1007/978-3-319-05633-3_2 D

DOREL S, et al. Influence of two pedalling rate conditions on mechanical output and physiological responses during all-out intermitente exercise. **European Journal Applied Physiology**, v. 89, n. 2, p. 157–165, 2003.

DOREL, S. et al. Torque and power-velocity relationships in cycling: relevance to track sprint performance in world-class cyclists. **International Journal Sports Medicine**, v. 26, p. 739–746, 2005.

DOYLE-BAKER, D. et al. An innovative ergometer to measure neuromuscular fatigue immediately after cycling. **Medicine Science Sports Exercise**, 50:375-387, 2018.

DRISS, T.; VANDEWALLE, H. The measurement of maximal (anaerobic) power output on a cycle ergometer: a critical review. **Biomed Research International**, 2013;2013:589361. doi:10.1155/2013/589361

ENOKA RM, DUCHATEAU J. Muscle fatigue: what, why and how it influences muscle function. Journal Physiology, v. 1(1), p.11-23, 2008. doi: 10.1113/jphysiol.2007.139477.

FARHADI, H.; HADI, H.; SABEGH, M.A. Effect of caffeine gum ingestion on blood lactate and glucose during 1500-m running, **Annals of Biological Research**, v. 2, n. 5, p. 252-257, 2011.

FARHADI, H.; HADI, H. Effect of different dosages caffeine gum ingestion on mid-endurance performance, **Annals of Biological Research**, v. 2, n. 6, p. 681-686, 2011.

FELIPPE LC, FERREIRA GA, LEARSI SK, BOARI D, BERTUZZI R, LIMA-SILVA AE (2018) Caffeine increases both total work performed above critical power and peripheral fatigue during a 4-km cycling time trial. **Journal Applied Physiology**, p. 124:1491–1501, 2018. doi: 10.1152/japplphysiol.00930.2017

FERNÁNDEZ-GARCÍA, B., PÉREZ-LANDALUCE, J., RODRÍGUEZ-ALONSO, M., & TERRADOS, N. Intensity of exercise during road race pro-cycling competition. **Medicine and Science in Sports and Exercise**, 32(5), 1002–1006, 2000. https://doi.org/10.1097/00005768-200005000-00019

FOAD, A. J.; BEEDIE, C. J.; COLEMAN, D. A. Pharmacological and psychological effects of caffeine ingestion in 40-km cycling performance. **Medicine Science Sports Exercise**, v. 40, p. 158–165, 2008. doi: 10.1249/mss.0b013e3181593e02

FONDA, B.; SARABON, N. Biomechanics of Cycling. **Sport Science Review**, XIX(1-2), 2010. doi:10.2478/v10237-011-0012-0

FRC RESEARCH CORPORATION. “The Impact of Chewing Gum on Consumers’ Stress Levels.” Survey conducted in June, 2006 in 280 male and female respondents aged 18-49 for the Wrigley Science Institute

FROYD, C., MILLET, G.Y., NOAKES, T.D. The development of peripheral fatigue and short-term recovey during self-paced high-intensity exercise. **The Journal of Physiology**, 2013; 591:1339Y46.

FUKUOKA, Y. et al. Reduction of V̇O2 slow component by priming exercise: novel mechanistic insights from time-resolved near-infrared spectroscopy. **Physiological Reports**, v. 3(6), 2015. e12432, 2015. doi: 10.14814/phy2.12432.

GANDEVIA, S. C. Spinal and Supraspinal Factors in Human Muscle Fatigue. Physiology Reviews, v. 81(4), p.1725–1789, 2001. doi:10.1152/physrev.2001.81.4.1725

GARDNER, A. S. et al. Maximal torque- and power-pedaling rate relationships for elite sprint cyclists in laboratory and field tests**. European Journal Applied Physiology**, v. 101, n. 3, p. 287-292, 2007. doi:10.1007/s00421-007-0498-4

GEERS, A. L. et a. Reconsidering the role of personality in placebo efects: dispositional optimism, situational expectations, and the placebo response. **Journal of Psychosomatic Research**, v. 58, p. 121–7, 2005.

GLAISTER, M. et al. Caffeine supplementation and peak anaerobic power output. **European Journal Sport Science**, v. 15, n. 5, p.400-6, 2015.

GLAISTER, M. et al. Caffeine and sprinting performance: dose responses and efficacy. **Journal Strength Condition Research**, v. 26, n. 4, p. 1001-1005, 2012. doi:10.1519/JSC.0b013e31822ba300

GLAISTER, M. et al. Caffeine and Sprint Cycling Performance: Effects of Torque Factor and Sprint Duration**. International Journal Sports Physiology Performance**. 2019 v. 14, n. 4, p. 426-431, 2019. doi: 10.1123/ijspp.2018-0458.

GONÇALVES, E. M. et al. Neuromuscular fatigue threshold, critical power and anaerobic work capacity under caffeine ingestion. **International Sportmed Journal**, [s.l.], v. 11, n. 4, p.380-388, 2010.

GONÇALVES RIBEIRO, R. et al . Acute effects of caffeine intake on athletic performance: A systematic review and meta-analysis. **Revista Chilena de Nutrición,** Santiago, v. 44, n. 3, p. 283-291, 2017. doi.org/10.4067/s0717-75182017000300283.

GRAHAM, T. E. Caffeine and exercise: metabolism, endurance and performance. **Sports Medicine**, v. 3, n. 11, p. 785-807, 2001. doi: 10.2165/00007256-200131110-00002

GRAHAM, T.E.; SPRIET, L. L. Performance and metabolic responses to a high caffeine dose during prolonged exercise. **Journal Applied Physiology**, v. 71, n. 6, p. 2292-8, (1985). 1991. doi: 10.1152/jappl.1991.71.6.2292.

GRASSI, B. Regulation of oxygen consumption at exercise onset: Is it really controversial? **Exercise and Sport Sciences Reviews**, v. 29, n. 3, pag. 134–138, 2001. doi:10.1097/00003677-200107000-00009

GRASSI, B. Delayed metabolic activation of oxidative phosphorylation in skeletal muscle at exercise onset. **Medicine and Science in Sports and Exercise**, 37(9), 1567–1573, 2005.

GRASSI, B., QUARESIMA, V. Near-infrared spectroscopy and skeletal muscle oxidative function in vivo in health and disease: a review from an exercise physiology perspective. **Journal of Biomedical Optics**, v. 21, n. 9, p. 2016. doi: 10.1117/1.JBO.21.9.091313.

GRASSI, B., POOLE, D.C., RICHARDSON, R.S., KNIGHT, D.R., ERICKSON, B.K., AND WAGNER, P.D. (1996). Muscle O2 uptake kinetics in humans: implications for metabolic control. **Journal of Applied Physiology**, 80, 988–998. doi: 10.1152/jappl.1996.80.3.988

GRASSI, B., ROSSITER, H.B., ZOLADZ, J.A. Skeletal muscle fatigue and decreased efficiency: two sides of the same coin? **Exercise and port Sciences Reviews**. 2015 Apr;43(2):75-83. doi: 10.1249/JES.0000000000000043.

GREER, F.; MORALES, J.; COLES, M. Wingate performance and surface EMG frequency variables are not affected by caffeine ingestion. **Applied Physiology Nutrition Metabolism**, v. 31, n. 5, p. 597-603, 2006.

GRGIC, J. Caffeine ingestion enhances Wingate performance: a meta-analysis. **European Journal Sport Science**, v. 18, p. 219–25, 2018.

GRGIC J, DEL COSO J. Ergogenic Effects of Acute Caffeine Intake on Muscular Endurance and Muscular Strength in Women: A Meta-Analysis. **Int J Environ Res Public Health**. 2021 May 27;18(11):5773. doi: 10.3390/ijerph18115773.

GRGIC, J. et al. Wake up and smell the coffee: caffeine supplementation and exercise performance-an umbrella review of 21 published meta-analyses. **British Journal Sports Medicine**, v. 54, n. 11, p. 681-688, 2020. doi: 10.1136/bjsports-2018-100278.

GRGIC, J.; MIKULIC, P. Caffeine ingestion acutely enhances muscular strength and power but not muscular endurance in resistance-trained men. **European Journal Sport Science**, v. 17, p.1029–36, 2017.

GRGIC, J.; PICKERING, C. The effects of caffeine ingestion on isokinetic muscular strength:A meta-analysis. **Journal Science Medicine Sport**, v. 22, p. 353–60, 2019.

GRGIC, J. et al. Effects of caffeine intake on muscle strength and power: a systematic review and meta-analysis. **Journal International Society of Sports Nutrition**, v. 5, p. 15-11, 2018

GUERRA, R. O.; BERNARDO, G.C.; GUTIÉRREZ, C. V. Cafeína e esporte. **Revista Brasileira de Medicina do Esporte**, v. 6, n. 2, p.60-62, 2000.

GURD, B. J. et al. Prior heavy exercise elevates pyruvate dehydrogenase activity and speeds O2 uptake kinetics during subsequent moderate-intensity exercise in healthy young adults. **Journal Physiology**, v. 15, n.577, p. 85-96, 2006. doi:10.1113/jphysiol.2006.112706.

HARRISS, D.J.; ATKINSON, G. Ethical standards in sport and exercise science research: 2016 Update. **International Journal of Sports Medicine**, 36(14), 1121-4, 2015. doi: 10.1055/s-0035-1565186.

HOWLEY, E.T., BASSETT, JR., B.D., & WELCH, H.G. (1995). Criteria for maximal oxygen uptake: Review and commentary. **Medicine & Science in Sports & Exercise**, 27(9), 1292–1301.

HOPKINS, W. G. "Spreadsheets for analisys of validity and reliability," **Sportscience**, vol. 19, pp. 36–42, 2015.

HURST, P. et al. Athletes intending to use sports supplements are more likely to respond to a placebo. **Medicine Science Sports Exercise**, v. 49, p. 1877–83, 2017.

HUXLEY, A. F. Muscle structure and theories of contraction. **Progress in Biophysics and Biophysical Chemistry**, n. 7, p. 255–318, 1957.

IANNETTA, D, INGLIS, EC, FULLERTON, C, PASSFIELD, L, MURIAS, JM. Metabolic and performance-related consequences of exercising at and slightly above MLSS. **Scandinavian Journal Medicine Science Sports**, v. 28(12), p. 2481-2493, 2018. doi:10.1111/sms.13280

JONES, A. M.; FULFORD, J.; WILKERSON, D. P. Influence of prior exercise on muscle [phosphorylcreatine] and deoxygenation kinetics during high-intensity exercise in men. **Experimental Physiology**, v. 93, n. 4, p. 468–478, 2008.

KAGAYA, A, OGITA, F. Blood flow during muscle contraction and relaxation in rhythmic exercise at different intensities. **The Annals of Physiology Anthropology**, v. 11(3), p. 251-6, 1992. doi: 10.2114/ahs1983.11.251

KALMAR, J. M.; CAFARELLI, E. Effects of caffeine on neuromuscular function. **Journal of Applied Physiology**, v. 87, n. 2, p. 801-808, 1999.

KALMAR, J. M.; CAFARELLI, E. Caffeine: a valuable tool to study central fatigue in humans? **Exercise and Sport Sciences Reviews**, v. 32, n. 4, p. 143-147, 2004.

KAMIMORI, G. H. et al. The rate of absorption and relative bioavailability of caffeine administered in chewing gum versus capsules to normal healthy volunteers. **International Journal of Pharmaceutics**, v. 234, n. 1-2, p. 159-167, 2002.

KAMIMORI, G. H. et al. Effect of three caffeine doses on plasma catecholamines and alertness during prolonged wakefulness. **European Journal of Clinical Pharmacology**, v. 56, n. 8, p. 537-44, 2000. doi: 10.1007/s002280000186.

KAPLAN G. B, et al. Dose dependent pharmacokinetics and psychomotor effects of caffeine in humans. **Journal Clinical Pharmacology**, v. 37, p. 693–703, 1997.

KIRSCH, I.; & WEIXEL, L. J. Double-blind versus deceptive administration of a placebo. **Behavioral Neuroscience**, v. 102, p. 319–323, 1998. doi: 10.1037/0735-7044.102. 2.319

KLEIN, M. G, SIMON BJ, SCHNEIDER MF. Effects of caffeine on calcium release from the sarcoplasmic reticulum in frog skeletal muscle fibres. **Journal Physiology**, v. 425, p. 599-626, 1990. doi: 10.1113/jphysiol.1990.sp018120.

Koga, S., et al. Spatial heterogeneity of quadriceps muscle deoxygenation kinetics during cycle exercise. **Journal of Applied Physiology**, (1985), v. 103(6), p. 2049-56, 2007. doi: 10.1152/japplphysiol.00627.2007.

KORDI, M. et al. Isovelocity vs. Isoinertial Sprint Cycling Tests for Power- and Torque-cadence Relationships. **International Journal Sports Medicine**, v. 40, n. 14, p. 897-902, 2019. doi: 10.1055/a-0989-2387. Epub 2019 Oct 7. PMID: 31590190.

KRÜGER, R. L. et al. Fatigue and recovery measured with dynamic properties versus isometric force: effects of exercise intensity. **Journal Experimental Biology**, 2019 v. 9, p. 222. doi: 10.1242/jeb.197483.

KRUSTRUP, P. et al. Neuromuscular blockade of slow twitch muscle fibres elevates muscle oxygen uptake and energy turnover during submaximal exercise in humans. **The Journal of Physiology**, v. 586, n. Pt 24, p. 6037–6048, 2008.

JONES, A. M.; FULFORD, J.; WILKERSON, D. P. Influence of prior exercise on muscle [phosphorylcreatine] and deoxygenation kinetics during high-intensity exercise in men. **Experimental physiology**, v. 93, n. 4, p. 468–478, 2008.

LANE, S. C. et al. Single and combined effects of beetroot juice and caffeine supplementation on cycling time trial performance. **Applied Physiology Nutrtition Metabolism**, v. 39, p. 1050–7, 2014.

LANE, J. D. et al. Menstrual cycle effects on caffeine elimination in the human female. **European Journal of Clinical Pharmacology**, v. 43, p. 543–546, 1992. doi: 10.1007/BF02285099

LARA, B. et al. Acute caffeine intake increases performance in the 15-s Wingate test during the menstrual cycle. British Journal of Clinical Pharmacology, v. 86(4), p. 745-752, 2020. doi: 10.1111/bcp.14175.

LIU, T. Route of placebo administration: Robust placebo effects in laboratory and clinical settings. **Neuroscience & Biobehavioral Reviews**, v. 83, p. 451–457, 2017. doi:10.1016/j.neubiorev.2017.09.018

LIMA-SILVA, A. E. et al. Caffeine during high-intensity whole-body exercise: an integrative approach beyond the central nervous system. **Nutrients**, v. 13(8), 2503, 2021. doi: 10.3390/nu13082503

LOPES-SILVA, J. P.; SANTOS, J. F. DA S.; & FRANCHINI, E. Can caffeine supplementation reverse the effect of time of day on repeated sprint exercise performance? **Applied Physiology, Nutrition, and Metabolism**, v. 44, n. 2, p. 187-193, 2019. doi:10.1139/apnm-2018-0373

LUDBROOK, J. Confidence in Altman–Bland plots: a critical review of the method of differences. **Clinical and Experimental Pharmacology & Physiology**, v. 37(2), p.143-149, 2010.

MALCATA RM, HOPKINS WG. Variability of competitive performance of elite athletes: a systematic review. Sports Medicine, v. 44(12), p. 1763-74, 2014. doi: 10.1007/s40279-014-0239-x.

MARTIN, J. C. et al. Validation of a mathematical model for road cycling power. **Journal Applied Biomech,** v. 14, p. 276 – 291, 1998.

MARTIN, J. C.; DAVIDSON, C. J.; & PARDYJAK, E. R. Understanding sprint-cycling performance: the integration of muscle power, resistance, and modeling. **International Journal Sports Physiology and Performance**, v. 2(1), p. 5-21, 2007.

MARTINS, G. L. et al. Caffeine and Exercise Performance: Possible Directions for Definitive Findings. **Frontiers in Sports Active Living**. 2020 Dec 11;2:574854. doi: 10.3389/fspor.2020.574854.

MARTIN, J. C.; WAGNER, B. M.; COYLE, E. F. Inertial-load method determines maximal cycling power in a single exercise bout. **Medicine Science Sports Exercise**, v. 29, n. 11, p. 1505-1512, 1997. doi:10.1097/00005768-199711000-00018

MATTIONI MATURANA F. et al. Faster V̇O2 kinetics after priming exercises of different duration but same fatigue. **Journal Sports Science**, v. 36, n. 10, p. 1095-1102, 2018. doi: 10.1080/02640414.2017.1356543.

MAUGHAN et al. IOC consensus statement: dietary supplements and the high-performance athlete. **British Journal of Sports Medicine**, v. 52(7), p. 439-455, 2018. doi: 10.1136/bjsports-2018-099027.

MENASPÀ, P, ABBISS, C, & MARTIN, D. Performance analysis of a world-class sprinter during cycling grand tours. **International Journal of Sports Physiology and Performance**, v. 8(3), p. 336–340, 2012. https://doi.org/10. 1123/ijspp.8.3.336

MEYER, T. et al. A conceptual framework for performance diagnosis and training prescription from submaximal gas exchange parameters – theory and application. **International Journal Sports Medicine**, v. 26, n. 1, pS38-48, 2005.

MIRA, J, et al. Effects of endurance training on neuromuscular fatigue in healthy active men. Part I: Strength loss and muscle fatigue. **European Journal of Applied Physiology**, v. 118(11), p. 2281-2293, 2018. doi: 10.1007/s00421-018-3950-8

MONTGOMERY, G. H.; KIRSCH, I. Classical conditioning and the placebo efect. **Pain**. v. 72, p. 107–13, 1997.

MORRIS, C. et al. Caffeine release and absorption from caffeinated gums. **Food Function**, v.1, n. 10(4), p. 1792-1796, 2019. doi: 10.1039/c9fo00431a.

MURGATROYD, S. R.; and L. A. WYLDE. 2011. The power-duration relationship of high-intensity exercise: from mathematical parameters to physiological mechanisms. **Journal Physiology**, v. 589, p. 2443–2445, 2011.

MURIAS, J. M., & PATERSON, D. H. Slower V̇O2 kinetics in older individuals: Is it inevitable? **Medicine & Science in Sports & Exercise**, v. 47, n. 11, pag. 2308–2318, 2015. doi:10.1249/MSS.0000000000000686

MURIAS, J. M., SPENCER, M. D., & PATERSON, D. H. The critical role of O2 provision in the dynamic adjustment of oxidative phosphorylation. **Exercise and Sport Sciences Reviews**, v. 42, n. 1, pag. 4–11, 2014. doi:10.1249/ JES.0000000000000005

NASCIMENTO, E. M. F. et al. Reliability and Validity of Cycling Sprint Performance at Isolinear Mode Without Torque Factor: A Preliminary Study in Well-Trained Male Cyclists. **Research Quartely for Exercise and Sport**, v. 6, p. 1-8, 2024. doi: 10.1080/02701367.2023.2298752

NEHLIG, A. Interindividual Differences in Caffeine Metabolism and Factors Driving Caffeine Consumption. **Pharmacological Reviews**, v. 70(2), p. 384-411, 2018. doi: 10.1124/pr.117.014407.

OBERLIN-BROWN, K. T. et al. Oral presence of carbohydrate and caffeine in chewing gum: independent and combined effects on endurance cycling performance. **International Journal Sports Physiology Performance**, 11, p. 164–71, 2016.

PAINELLI, V. S. et al. Comment on: "Caffeine and Exercise: What Next?". **Sports Medicine**, v. 50, n. 6, p. 1211-1218, 2020. doi: 10.1007/s40279-020-01278-9. PMID: 32125669.

PATON, C.; COSTA, V.; GUGLIELMO, L. G. A. Effects of caffeine chewing gum on race performance and physiology in male and female cyclists. **Journal Sports Science**, v. 33, p. 1076–83, 2015.

PATON, C. D.; LOWE, T.; IRVINE, A. Caffeinated chewing gum increases repeated sprint performance and auments increase in testosterone in competitive cyclists. **European Journal Applied Physiology**, n. 110, p.1243-1250, 2010.

PENETAR, D. et al. Caffeine reversal of sleep deprivation effects on alertness and mood. **Psychopharmacology**, v. 112, p. 359–65, 1993.

PICKERING, C.; GRGIC, J. Caffeine and Exercise: What Next? **Sports Medicine**, v. 49(7), p. 1007-1030, 2019. doi: 10.1007/s40279-019-01101-0.

PIRES, F. O. et al. Caffeine and placebo improved maximal exercise performance despite unchanged motor cortex activation and greater prefrontal cortex deoxygenation. **Frontiers in Physiology**, v. 9, pag. 1144, 2018.

PLACE, N.; MILLET, G. Y. Quantification of Neuromuscular Fatigue: What Do We Do Wrong and Why? **Sports Medicine**, v. 50(3), p. 439-447, 2020. doi: 10.1007/s40279-019-01203-9

PLASKETT, C. J.; CAFARELLI, E. Caffeine increases endurance and attenuates force sensation during submaximal isometric contractions. **Journal Applied Physiology** (1985), v. 91, n. 4, p. 1535-44, 2001. doi: 10.1152/jappl.2001.91.4.1535.

POOLE, D. C., et al. Control of oxygen uptake during exercise. **Medicine & Science in Sports & Exercise**, v. 40, n. 3, pag. 462–474, 2008. doi:10.1249/MSS.0b013e31815ef29b

POLITO M. D. et al. Acute effect of caffeine consumption on isotonic muscular strength and endurance: a systematic review and meta-analysis. **Science Sports**, v. 31, p. 119–28, 2016.

POWERS, S. K. et al. Caffeine alters ventilatory and gas exchange kinetics during exercise. **Medicine Science Sports Exercise**, v. 18, n. 1, p. 101-6, 1986.

RAYA-GONZÁLEZ J. et al. Acute Effects of Caffeine Supplementation on Movement Velocity in Resistance Exercise: A Systematic Review and Meta-analysis. **Sports Medicine,** v. 50(4), p. 717-729, 2020. doi: 10.1007/s40279-019-01211-9.

REISER, R. F. et al. Standing and seated Wingate protocols in human cycling. A comparison of standard parameters. **European Journal of Applied Physiology**, v. 88(1-2), p. 152-157, 2002. doi:10.1007/s00421-002-0694-1

RIETVELD, E. C. et al. Rapid onset of an increase in caffeine residence time in young women due to oral contraceptive steroids. **European Journal of Clinical Pharmacology**, v. 26, p. 371–373, 1984. doi: 10.1007/BF00548769

RIVERS, W. H.; WEBBER, H. N. The action of caffeine on the capacity for muscular work. **Journal Physiology**, v. 27, n. 36(1), p. 33-47, 1907. doi: 10.1113/jphysiol.1907.sp001215. PMID: 16992882; PMCID: PMC1533733.

ROCHA, P. L. A.; LIMA, A. L. C.; SAUNDERS, B.; REIS, C. E. G. Development of a Caffeine Content Table for Foods, Drinks, Medications and Supplements Typically Consumed by the Brazilian Population. Nutrients. Oct 21;14(20):4417, (2022). doi: 10.3390/nu14204417.

ROSSITER, H. B. Exercise: Kinetic considerations for gas exchange. **Comprehensive Physiology**, v. 1, n. 1, pag. 203–244, 2011. doi:10.1002/cphy.c090010

RUÍZ-MORENO, C. et al. Acute caffeine intake increases muscle oxygen saturation during a maximal incremental exercise test. **British Journal of Clinical Pharmacology**, v. 86, p. 861–867, 2020. doi: 10.1111/bcp.14189

RYAN, E. J. et al. Caffeine Gum and Cycling Performance: A Timing Study. **The Journal of Strength & Conditioning Research**, v. 27, n. 412, p. 1, 2013.

RYAN, E. J. et al. Low-dose caffeine administered in chewing gum does not enhance cycling to exhaustion. **Journal Strength Condition Research**, v. 26, p. 844–50, 2012

SADAMOTO, T.; BONDE-PETERSEN, F.; SUZUKI, Y Skeletal muscle tension, flow, pressure, and EMG during sustained isometric contractions in humans. European Journal of Applied Physiology Occupational Physiology, v. 51(3), p. 395-408, 1983. doi: 10.1007/BF00429076

SAHLIN, K. et al. Prior heavy exercise eliminates VO2 slow component and reduces efficiency during submaximal exercise in humans. **Journal Physiology**, v. 1, n. 564(Pt 3), p. 765-73, 2005. doi: 10.1113/jphysiol.2005.083840.

SAMOZINO, P., HORVAIS, N. and HINTZY, F. Why does power output decrease at high pedaling rates during sprint cycling? **Medicine Science Sports Exercise**, v. 39, p. 680-687, 2007.

SANTALLA, A.; LUCÍA, A.; PÉREZ, M. Caffeine ingestion attenuates the VO2 slow component during intense exercise. **The Japanese journal of physiology**, v. 51, n. 6, p. 761–764, 2001.

SANTARÉM, D. et al. Comparing the effects of dynamic and holding isometric contractions on cardiovascular, perceptual, and near-infrared spectroscopy parameters: A pilot study. **PLoS One**, v. 16;18(2):e0281885, 2023. doi: 10.1371/journal.pone.0281885

SANTOS P. S., et al. Caffeine increases peripheral fatigue in low-but not in high-performing cyclists. **Applied Physiology Nutrition Metabolism**, v.45, p. 1208–1215, 2020. doi: 10.1139/apnm-2019-0992.

SARGEANT, AJ. Human power output and muscle fatigue. **International Journal Sports Medicine**, v. 15(3), p. 116-21, 1994. doi: 10.1055/s-2007-1021031. PMID: 8005722.

SARGEANT, A. J.; HOINVILLE, E.; YOUNG, A. Maximum leg force and power output during short-term dynamic exercise. **Journal of Applied Physiology**: **Respiratory, Environmental and Exercise Physiology**, v. 51, n. 5, p. 1175-82, 1981. doi: 10.1152/jappl.1981.51.5.1175. PMID: 7298457.

SAUNDERS, B., et al. Placebo in sports nutrition: a proof-ofprinciple study involving caffeine supplementation**. Scandinavian Journal of Medicine & Science in Sports**, v. 27, p. 1240–1247, 2016. doi:10.1111/sms.12793

SHABIR A, et al. The Influence of Caffeine Expectancies on Sport, Exercise, and Cognitive Performance. **Nutrients**, v. 17, n. 10(10), p. 1528, 2018. doi: 10.3390/nu10101528.

SHARGEL, L.; YU, A. B. C. Applied biopharmaceutics and pharmacokinetics. 4th ed. **Stamford: Appleton and Lange**; 1999.

SHEN, J. G. et al. Establishing a relationship between the effect of caffeine and duration of endurance athletic time trial events: A systematic review and meta-analysis. **Journal of Science Medicine in Sport**, v. 22, p. 232–8, 2019.

SHIBA, Y. et al. Evaluation of mastication-induced change in sympatho-vagal balance through spectral analysis of heart-rate. Journal Oral Rehabilitation, v. 29, p. 956–960, 2002.

SHROUT, PE & FLEISS, JL. Intraclass correlations: Uses in assessing rater reliability. **Psychological Bulletin**, v. 86(2), p. 420–428, 1979. https://doi.org/10. 1037/0033-2909.86.2.420

SIMMONDS, M.J., MINAHAN, C.L., SABAPATHY, S. Caffeine improves supramaximal cycling but not the rate of anaerobic energy release. European Journal of Applied Physiology, v. 109(2), p. 287-95, 2010. doi: 10.1007/s00421-009-1351-8.

SMITH, A. Effects of caffeine in chewing gum on mood and attention. **Human Psychopharmacol**, 2009 Apr;24(3):239-47. doi: 10.1002/hup.1020.

SMITH, A. Effects of chewing gum on stress and health: a replication and investigation of dose-response. **Stress Health**, v. 29, n. 2, p. 172-4, 2013. doi: 10.1002/smi.2430. Epub 2012 Apr 11. PMID: 22496105.

SOUTHWARD, K.; RUTHERFURD-MARKWICK, K. J.; ALI, A. Correction to: the effect of acute caffeine ingestion on endurance performance: a systematic review and meta-analysis. **Sports Medicine**, v. 48, p. 2425–41, 2018.

STECKER RA, HARTY PS, JAGIM AR, CANDOW DG, KERKSICK CM. Timing of ergogenic aids and micronutrients on muscle and exercise performance. **Journal of International Society of Sports Nutrition**, v, 2;16(1), p. 37, 2019. doi: 10.1186/s12970-019-0304-9.

SUN R, SUN J, LI J, LI S. Effects of caffeine ingestion on physiological indexes of human neuromuscular fatigue: A systematic review and meta-analysis. **Brain and Behavior**, v. 12(4):e2529, 2022. doi: 10.1002/brb3.2529

SYED, S. A. et al. Multiple dose pharmacokinetics of caffeine administered in chewing gum to normal healthy volunteers. **Biopharmaceutics & Drug Disposition**, v. 26, p. 403–9, 2005.

TALANIAN, J. L.; SPRIET, L. L. Low and moderate doses of caffeine late in exercise improve performance in trained cyclists. **Applied Physiology Nutriton Metabolism**, v. 41(8), p. 850-5, 2016. doi: 10.1139/apnm-2016-0053

TAIPALE-MIKKONEN, R.S., et al. Influence of Menstrual Cycle or Hormonal Contraceptive Phase on Physiological Variables Monitored During Treadmill Testing. **Frontiers in Physiology**, v. 16(12), 761760, 2021. doi: 10.3389/fphys.2021.761760.

TALANIAN, J. L.; SPRIET, L. L. Low and moderate doses of caffeine late in exercise improve performance in trained cyclists. **Applied Physiology Nutrition Metabolism**, v. 41, p. 850–5, 2016.

TALLIS, J. et al. The effect of a physiological concentration of caffeine on the endurance of maximally and submaximally stimulated mouse soleus muscle. **Journal Physiology Science**, v. 63, p. 125–132, 2013.

TALLIS, J. et al. Is the ergogenicity of caffeine affected by increasing age? The direct effect of a physiological concentration of caffeine on the power output of maximally stimulated EDL and diaphragm muscle isolated from the mouse. **Journal Nutrition Health Aging**, v. 21, p. 440–8, 2017.

TARNOPOLSKY, M. A. Caffeine and endurance performance. **Sports Medicine**, v. 18, p. 109–25, 1994.

TEMESI, J. et al. The relationship between oxygen uptake kinetics and neuromuscular fatigue in high-intensity cycling exercise. European Journal of Applied Physiology, v. 117(5), p. 969-978, 2017. doi: 10.1007/s00421-017-3585-1

THOMAS, K. et al. Central and peripheral fatigue in male cyclists after 4-, 20-, and 40-km time trials. **Medicine Science Sports Exercise**, v. 47(3), p. 537–546, 2015. doi:10.1249/MSS.0000000000000448

UMEMURA T, et al. Effects of acute administration of caffeine on vascular function. The Americam Journal of Cardiology, Dec 1;98(11):1538-41, 2006. doi: 10.1016/j.amjcard.2006.06.058.

VANDEWALLE, H. et al. All out anaerobic capacity tests on cycle ergometers. A comparative study on men and women. **European Journal Applied Physiology Occupational Physiology**. v. 54, n. 2, p. 222-9, 1985. doi: 10.1007/BF02335934.

VON RUDEN, L., & NEHER, E. A Ca-dependent early step in the release of catecholamines from adrenal chromaffin cells. **Science**, v. 262, p. 1061–1065, 1993. doi: 10.1126/science.8235626

WACKWITZ, TA, MINAHAN, CL, KING, T, DU PLESSIS, C, ANDREWS, MH, BELLINGER PM. Quantification of maximal power output in well-trained cyclists. **Journal Sports Science**, v. 39(1), p. 84-90, 2021. doi: 10.1080/02640414.2020.1805251.

WAGER, T. D. et al. Predicting individual differences in placebo analgesia: contributions of brain activity during anticipation and pain experience. **Journal Neuroscience**, v. 31, n. 2, p. 439-452, 2011. doi:10.1523/JNEUROSCI.3420-10.2011

WAGER, T. D. et al. Placebo-induced changes in FMRI in the anticipation and experience of pain. **Science**, v. 303, p. 1162–1167, 2004. doi:10.1126/science.1093065

WARREN, G.L. et al. Effect of caffeine ingestion on muscular strength and endurance: a meta-analysis. **Medicine Science Sports Exercise,** 42:1375–87, 2010.

WICKHAM, K. A.; SPRIET, L. L. Administration of Caffeine in Alternate Forms. **Sports Medicine**, v. 48, p. 79–91, 2018.

Wilk, M. et al. The Effects of High Doses of Caffeine on Maximal Strength and Muscular Endurance in Athletes Habituated to Caffeine. **Nutrients**, v. 15;11(8), p. 1912, 2019. doi: 10.3390/nu11081912.

WINTER, E. M. et al. Optimized and corrected peak power output during friction-braked cycle ergometry. **Journal Sports Science**. v. 14, p. 513–521, 1996. doi:10.1080/02640419608727738

WOODFORD, D. W., LESKO, L. J. 1981. Relative bioavailability of aspirin gum. **Journal of Pharmaceutical Sciences**, v. 70, n. 12, p. 1341–1343, 1981.

APÊNDICE A – TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

UNIVERSIDADE FEDERAL DE SANTA CATARINA

CENTRO DE DESPORTOS

PROGRAMA DE PÓS-GRADUAÇÃO EM EDUCAÇÃO FÍSICA

ÁREA DE BIODINÂMICA DO DESEMPENHO HUMANO

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

De acordo com resolução 466/2012 do Conselho Nacional de Saúde, todas as pesquisas conduzidas com seres humanos necessitam do termo de Consentimento Livre e Esclarecido (TCLE), devendo o participante estar ciente dos objetivos do estudo. Você está sendo convidado a participar do projeto que estamos conduzindo intitulado: "**EFEITOS DA SUPLEMENTAÇÃO DE CAFEÍNA EM GOMA NAS RESPOSTAS NEUROMUSCULARES E CARDIORRESPIRATÓRIAS EM CICLISTAS**", que tem como objetivo analisar os efeitos da ingestão de cafeína em goma nas respostas cardiorrespiratórias, neuromusculares, e no desempenho no ciclismo.

O projeto envolve os professores Dr. Luiz Guilherme Antonacci Guglielmo, Dr. Ricardo Dantas de Lucas e o Doutorando Eduardo Marcel Fernandes Nascimento. A participação no estudo é voluntária não envolve nenhum gasto com exceção daqueles advindos de deslocamentos do participante. Todos os materiais necessários serão providenciados pelos pesquisadores. Todas as avaliações serão realizadas no Laboratório do Esforço Físico (LAEF) do Centro de Desportos (CDS); sendo necessárias cinco visitas ao LAEF. Todo o período do experimento durará em torno de um mês e serão realizados testes laboratoriais como será descrito a seguir: 1) Familiarização e teste incremental; 2) Cargas constantes por volta de 12 min em dias diferentes numa intensidade por volta de 70% do máximo individual.

1ª etapa: Familiarização e teste incremental (duração aproximada de 90 minutos) – No primeiro momento será realizada a avaliação antropométrica – o que envolve apenas o risco de: constrangimento ao realizar exames antropométricos; e/ou constrangimento ao se expor durante a realização de testes de qualquer natureza. No entanto, o participante poderá **se retirar do**

estudo a qualquer momento. Familiarização com os equipamentos, protocolos e teste de esforço em cicloergômetro com aquecimento inicial de 3 minutos a 30 W e aumentos de 30 W a cada minuto até à exaustão voluntária (~10 min). A orientação básica é que você alcance o limite de esforço o que pode gerar **algum desconforto físico** devido ao esforço mais intenso, mas ressaltamos que o participante poderá interromper o teste a qualquer momento. No teste de esforço, você usará uma máscara no seu rosto para a análise dos gases de oxigênio e gás carbônico do ar expirado. Usará também um medidor da frequência cardíaca (FC). Estes últimos **procedimentos não oferecem riscos**. O participante utilizará um aparelho de infravermelho na coxa para ver a oxigenação muscular, e algumas oclusões do fluxo sanguíneo serão feitas através de um manguito de pressão por alguns segundos em determinados momentos, o que pode gerar desconforto para o participante, mas sem risco físico.

2ª etapa: cargas constantes (duração aproximada de 90 minutos cada dia) – Com intervalos de no mínimo 24 horas serão realizados em cicloergômetro os testes de carga constante, um por dia sempre respeitando o intervalo de recuperação. Serão em torno de 4 visitas nesse formato. Você utilizará a máscara para análise do O_2 expirado e o medidor da FC. Em duas visitas, após um aquecimento e uma pausa passiva você deverá realizar um sprint de 7 segundos, em seguida deverá mascar a goma de cafeína durante 5 minutos e exatamente 5 minutos após, repetir o sprint de 7 segundos, antes e imediatamente após o exercício realizado na bicicleta a 70% da intensidade entre o primeiro Limiar Ventilatório e o VO_{2max}. Em outras duas visitas, após um aquecimento específico no dinamômetro de força (Biodex), você deverá realizar duas repetições de contração voluntária máxima (CVM), em seguida deverá mascar a goma de cafeína durante 5 minutos e exatamente 5 minutos após, repetir as duas CVM, antes e após o exercício realizado na bicicleta a 70% da intensidade entre o primeiro Limiar Ventilatório e o VO_{2max}. Também será utilizado o aparelho de infravermelho e algumas oclusões com o manguito de pressão, já descritos anteriormente.

Dentre os possíveis desconfortos durante a realização dos testes, estão náusea, enjoo e vômito, após o término do teste. **No entanto, menos de 1% da população americana apresenta desconforto durante este tipo de teste (American College of Sports Medicine).**

Todos os dias de testes serão acompanhados por no **mínimo dois profissionais experientes** e formados **da área da saúde** que realizaram cursos de emergências e primeiros socorros **para qualquer eventual risco** que possa ocorrer devido aos esforços físicos.

Os pesquisadores devem proporcionar: assistência imediata – é aquela emergencial e sem ônus de qualquer espécie ao participante da pesquisa, em situações em que este dela necessite; e assistência integral – é aquela prestada para atender complicações e danos decorrentes, direta ou indiretamente, da pesquisa.

Todos os dados coletados neste estudo são estritamente confidenciais e serão utilizados para produção de artigos técnicos científicos. **A sua identidade será preservada durante todas as avaliações, pois cada sujeito da amostra será identificado por número.** Assim, apenas os pesquisadores terão acesso aos dados, que serão codificados e armazenados em banco de dados, de forma que a identificação por outras pessoas não seja possível. Além disso, essas informações poderão ser requisitadas pelo participante. No entanto, é importante ressaltar que há um risco de quebra de sigilo, comum a todas as pesquisas com seres humanos. **Os pesquisadores seguirão de forma estrita os cuidados para manter o sigilo**, contudo, vale dizer que a legislação inclui uma cláusula genérica sobre indenizações a que o participante pode achar-se no direito de receber por compensação de danos materiais ou morais decorrentes da pesquisa, inclusive relacionados à quebra de sigilo.

Os benefícios do estudo a curto prazo não existem, a não ser aqueles advindos de se conhecer índices fisiológicos provenientes do consumo da goma de mascar de cafeína, que são frequentemente utilizados por praticantes de esportes que visam o aprimoramento do rendimento esportivo. Este conhecimento poderá ser útil para uma melhor orientação e prescrição de treinamento.

Todos os participantes receberão um relatório detalhado de suas avaliações com orientações para o aprimoramento de exercícios físicos, além disso, os participantes poderão pedir aos pesquisadores a qualquer momento durante ou após a participação orientações de como entender e aplicar os resultados obtidos.

Como citado anteriormente sua participação é voluntária e tem a garantia de plena liberdade de recusar-se a participar ou retirar seu consentimento, em qualquer fase da pesquisa, sem penalização alguma, bastando apenas informar aos pesquisadores. Sua participação no estudo não envolve nenhum outro gasto, a não ser aqueles advindos do deslocamento até o laboratório (ex: gastos com transporte), sendo que os pesquisadores não se responsabilizam pelo ressarcimento.

É importante ressaltar que você tem a garantia de indenização total pelos pesquisadores, diante de eventuais danos decorrentes da pesquisa, conforme item IV.3 (h) da Resolução 466/2012.

Ao perceber qualquer risco ou dano significativos ao participante da pesquisa, previstos, ou não, no Termo de Consentimento Livre e Esclarecido, o pesquisador responsável, deverá comunicar o fato, imediatamente, ao Sistema CEP/CONEP, e avaliar, em caráter emergencial, a necessidade de adequar ou suspender o estudo.

O TCLE é redigido em duas vias e todas as páginas são rubricadas por todos os envolvidos (participante e pesquisador responsável). Você receberá uma via do TCLE para qualquer dúvida possível.

Caso você tenha alguma dúvida, poderá entrar em contato pelo telefone do LAEF: (048) 3721-6248, com: **Prof. Dr. Luiz Guilherme Antonacci Guglielmo;** e-mail: luiz.guilherme@ufsc.br.

Departamento de Educação Física – UFSC

O **Comitê de Ética em Pesquisa com Seres Humanos** (CEPSH-UFSC) é um órgão criado para defender os interesses dos participantes da pesquisa em sua integridade e dignidade e para contribuir no desenvolvimento da pesquisa dentro de padrões éticos. Fica localizado na Rua Desembargador Vitor Lima, nº 222, Prédio Reitoria II, 4º andar, sala 401, Trindade, Florianópolis. Telefone para contato: 3721-6094.

Desde já, agradecemos a sua colaboração.

UNIVERSIDADE FEDERAL DE SANTA CATARINA

CENTRO DE DESPORTOS

DECLARAÇÃO

Declaro para os devidos fins e efeitos legais que, objetivando atender as exigências do Comitê de Ética em Pesquisa com Seres Humanos, e como pesquisador responsável, afirmo que cumprirei os termos da Resolução CNS 466/2012 e suas complementares durante a realização do projeto de pesquisa: **EFEITOS DA SUPLEMENTAÇÃO DE CAFEÍNA EM GOMA NAS RESPOSTAS NEUROMUSCULARES E CARDIORRESPIRATÓRIAS EM CICLISTAS**.

Florianópolis, 10/04/2020.

Prof. Dr. Luiz Guilherme Antonacci Guglielmo

CPF: 134106818-84

UNIVERSIDADE FEDERAL DE SANTA CATARINA

CENTRO DE DESPORTOS

PROGRAMA DE PÓS-GRADUAÇÃO EM EDUCAÇÃO FÍSICA

AREA DE BIODINÂMICA DO DESEMPENHO HUMANO

1. TERMO DE CONSENTIMENTO

Declaro que fui informado, de forma clara e objetiva, sobre todos os procedimentos do projeto de pesquisa intitulado **"Efeitos da suplementação de cafeína em goma nas respostas neuromusculares e cardiorrespiratórias em ciclistas".** Estou ciente que todos os dados a meu respeito serão sigilosos e que posso me retirar do estudo a qualquer momento. Assinando este termo, eu concordo em participar deste estudo.

Nome por extenso_____

Assinatura _____

Florianópolis (SC) _____/_____/_____

_____

Prof. Doutorando Eduardo Marcel Fernandes Nascimento

CPF: 216288868-89

APÊNDICE B – PARECER CONSUBSTANCIADO DO CEP

UNIVERSIDADE FEDERAL DE SANTA CATARINA - UFSC

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Efeitos da suplementação de cafeína em goma nas respostas neuromusculares e cardiorrespiratórias em ciclistas

Pesquisador: Luiz Guilherme Antonacci Guglielmo

Área Temática:

Versão: 3

CAAE: 29934020.5.0000.0121

Instituição Proponente: Universidade Federal de Santa Catarina

Patrocinador Principal: MINISTERIO DA CIENCIA, TECNOLOGIA E INOVACAO

DADOS DO PARECER

Número do Parecer: 4.055.081

Apresentação do Projeto:

Projeto de pesquisa intitulado "Efeitos da suplementação de cafeína em goma nas respostas neuromusculares e cardiorrespiratórias em ciclistas", vinculado ao Centro de Desportos da Universidade Federal de Santa Catarina, coordenado por Luiz Guilherme Antonacci Guglielmo. Este estudo prospectivo parte de uma pesquisa realizada no Laboratório de Esforço Físico (LAEF), localizado no CDS/UFSC.

Trata-se de uma investigação com abordagem experimental que pretende analisar os efeitos da ingestão de cafeína em goma nas respostas cardiorrespiratórias, neuromusculares, e no desempenho no ciclismo. A amostra do estudo será composta por 15 ciclistas moderadamente treinados, do sexo masculino, que realizarão 5 avaliações. Na primeira visita realizarão um protocolo incremental máximo em cicloergômetro para a determinação da intensidade aeróbia máxima, do consumo máximo de O2 (VO2max), e dos limiares de transição fisiológica. Nas próximas 4 visitas, realizarão duas cargas até a exaustão na intensidade do delta 70% e outras duas até 8 min seguida de um sprint de 1 min. Os testes serão realizados de forma randomizada, sendo que dois testes (um de cada modelo) serão realizados com suplementação de goma de cafeína na concentração de 300 mg. Os sujeitos ainda realizarão antes e imediatamente após as cargas até exaustão testes neuromusculares para determinar respostas de fadiga central e periférica. Os dados serão expressos como média ± desvio padrão. A ANOVA two-way com medidas repetidas será utilizada para comparar as mudanças das variáveis fisiológicas e percepção

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Bairro: Trindade **CEP:** 88.040-400
UF: SC **Município:** FLORIANOPOLIS
Telefone: (48)3721-6094 **E-mail:** cep.propesq@contato.ufsc.br

subjetiva de esforço ao longo dos testes e entre os exercícios realizados (placebo e cafeína). A magnitude das diferenças será analisada pelo Effect Size (ES). O nível de significância adotado para todas as análises será p<0,05. A hipótese do presente trabalho é que a ingestão da goma de cafeína irá atenuar a sobrecarga neuromuscular e cardiorrespiratória e aprimorar a performance.

Critério de Inclusão:
Os indivíduos deverão ser saudáveis, do gênero masculino, ter idade entre vinte e quarenta anos, de diferentes níveis de aptidão física (preferencialmente triatletas), preencher fixa de cadastro com dados pessoais e responder os questionários de Fatores de Riscos para Doenças Cardiovasculares (FRDC) e ParQ, conforme recomendação do AMERICAN COLLEGE OF SPORTS MEDICINE, (2003).
Critério de Exclusão:
Os critérios de exclusão serão qualquer resposta afirmativa que for respondida aos questionários de FRDC e/ou ParQ, como também ao não cumprimento dos critérios de inclusão citados anteriormente.

Objetivo da Pesquisa:
Objetivo Primário:
Verificar a influência da cafeína no desempenho de ciclismo e investigar os possíveis mecanismos fisiológicos associados a ela nos parâmetros neuromusculares e respiratórios centrais e periféricos.
Objetivos Secundários:
Determinar as seguintes respostas fisiológicas: consumo máximo de oxigênio - VO2max, frequência cardíaca máxima - FCmax, ventilação máxima - VE, e os limiares de transição fisiológica em protocolo incremental realizado em cicloergômetro; Determinar e comparar as repostas fisiológicas (consumo de oxigênio, frequência cardíaca, volume sistólico, ventilação e lactato sanguíneo) no ciclismo de alta intensidade nos protocolos placebo e suplementação de cafeína. Analisar a influência da ingestão de cafeína nas respostas da cinética de VO2 pulmonar e muscular. Determinar e comparar o desempenho aeróbio de alta intensidade e anaeróbio no ciclismo nos protocolos placebo e suplementação de cafeína; Determinar e comparar a percepção subjetiva de esforço nos diferentes protocolos; Determinar e comparar a resposta da contração voluntária máxima (CVM) antes e após o ciclismo de alta intensidade até a exaustão nos protocolos placebo e suplementação de cafeína; Determinar e comparar o efeito da suplementação de cafeína

na resposta dos parâmetros centrais (ativação voluntária - % AV e Onda - M) e periféricos (peak doublet) dos músculos extensores do joelho após ciclismo de alta intensidade até a exaustão.

Avaliação dos Riscos e Benefícios:

Riscos:

Informação dos riscos está adequada, sendo que o pesquisador informa:

1a etapa: Familiarização e teste incremental (duração aproximada de 90 minutos) – No primeiro momento será realizada a avaliação antropométrica – o que envolve o risco de: constrangimento ao realizar exames antropométricos; e/ou constrangimento ao se expor durante a realização de testes de qualquer natureza. No entanto, o participante poderá se retirar do estudo a qualquer momento. Familiarização com os equipamentos, protocolos e teste de esforço em cicloergômetro com aquecimento inicial de 3 minutos a 30 W e aumentos de 30 W a cada minuto até à exaustão voluntária (~10 min). A orientação básica é que você alcance o limite de esforço o que pode gerar algum desconforto físico devido ao esforço mais intenso, mas ressaltamos que o participante poderá interromper o teste a qualquer momento. No teste de esforço, você usará uma máscara no seu rosto para a análise dos gases de oxigênio e gás carbônico do ar expirado. Usará também um medidor da frequência cardíaca (FC). Estes últimos procedimentos podem gerar o risco de algum desconforto físico ou constrangimento ao se expor durante a realização de testes de qualquer natureza, mas ressaltamos que o participante poderá interromper o teste a qualquer momento. O participante utilizará um aparelho de infravermelho na coxa para ver a oxigenação muscular, e algumas oclusões do fluxo sanguíneo serão feitas através de um manguito de pressão por alguns segundos em determinados momentos, o que pode gerar o risco de algum desconforto físico ou constrangimento ao se expor durante a realização de testes de qualquer natureza, mas ressaltamos que o participante poderá interromper o teste a qualquer momento.

2a etapa: cargas constantes (duração aproximada de 90 minutos cada dia) – Com intervalos de no mínimo 24 horas serão realizados em cicloergômetro os testes de carga constante, um por dia sempre respeitando o intervalo de recuperação.Dentre os possíveis riscos e desconfortos durante a realização dos testes, estão náusea, enjoo e vômito, após o término do teste. No entanto, menos de 1% da população americana apresenta desconforto durante este tipo de teste (American College of Sports Medicine).

Todos os dias de testes serão acompanhados por no mínimo dois profissionais experientes e formados da área da saúde que realizaram cursos de emergências e primeiros socorros para

qualquer eventual risco que possa ocorrer devido aos esforços físicos.Todos os dados coletados neste estudo são estritamente confidenciais e serão utilizados para produção de artigos técnicos científicos. A sua identidade será preservada durante todas as avaliações, pois cada sujeito da amostra será identificado por número. Assim, apenas os pesquisadores terão acesso aos dados, que serão codificados e armazenados em banco de dados, de forma que a identificação por outras pessoas não seja possível. Além disso, essas informações poderão ser requisitadas pelo participante. No entanto, é importante ressaltar que há um risco de quebra de sigilo, comum a todas as pesquisas com seres humanos. Os pesquisadores seguirão de forma estrita os cuidados para manter o sigilo, contudo, vale dizer que a legislação inclui uma cláusula genérica sobre indenizações a que o participante pode achar-se no direito de receber por compensação de danos materiais ou morais decorrentes da pesquisa, inclusive relacionados à quebra de sigilo.
Ao perceber qualquer risco ou dano significativo ao participante da pesquisa, previstos, ou não, no Termo de Consentimento Livre e Esclarecido, o pesquisador responsável, deverá comunicar o fato, imediatamente, ao Sistema CEP/CONEP, e avaliar, em caráter emergencial, a necessidade de adequar ou suspender o estudo.

Benefícios:
Informação dos benefícios está adequada, sendo que o pesquisador informa: "Os benefícios do estudo a curto prazo não existem, a não ser aqueles advindos de se conhecer índices fisiológicos provenientes do consumo da goma de mascar de cafeína, que são frequentemente utilizados por praticantes de esportes que visam o aprimoramento do rendimento esportivo. Este conhecimento poderá ser útil para uma melhor orientação e prescrição de treinamento.

Comentários e Considerações sobre a Pesquisa:
A pesquisa apresenta pertinência, fundamentação bibliográfica e clareza em seus objetivos.

Considerações sobre os Termos de apresentação obrigatória:
1) A redação dos Riscos e Benefícios não está de acordo com as indicações do documento orientações para evitar pendências do CEPSH/UFSC.
2) Folha de Rosto está adequada, assinada por Luiz Guilherme Antonacci Guglielmo, responsável pela pesquisa, e Michel Angillo Saad, vice-diretor do Centro de Desportos, Campus Florianópolis, UFSC.

UNIVERSIDADE FEDERAL DE SANTA CATARINA - UFSC

3) Carta de anuência: assinada por Michel Angillo Saad, vice-diretor do Centro de Desportos, Campus Florianópolis, UFSC, em 09 de março de 2020. Foi enviada também a declaração de anuência do Laboratório de Exercício Físico (LAEF/CDS/UFSC), assinada por Luiz Guilherme Antonacci Guglielmo, responsável pela pesquisa e coordenador do laboratório, em 06/03/2020.

4) TCLE: apresenta um TCLE para o participante da pesquisa, mas não contempla todas as exigências da resolução 466/2012.

5) Cronograma: O cronograma informa que a coleta de dados para a realização da pesquisa acontecerá a partir de 25/06/2020.

6) Orçamento: informa despesas de R$ R$ 1.673,09 com financiamento Ministério da Ciência, Tecnologia e Inovação.

Recomendações:

Recomenda-se retirar a informação sobre o CPF do participante no Termo de Consentimento Livre e Esclarecido.

Conclusões ou Pendências e Lista de Inadequações:

Sem pendências

Considerações Finais a critério do CEP:

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_1302854.pdf	05/05/2020 12:01:31		Aceito
Outros	Carta_Resposta_Parecer_CEPSH.pdf	05/05/2020 11:58:28	EDUARDO MARCEL FERNANDES NASCIMENTO	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLE.pdf	05/05/2020 11:55:37	EDUARDO MARCEL FERNANDES NASCIMENTO	Aceito
Parecer Anterior	PB_PARECER_CONSUBSTANCIADO_CEP_3999035.pdf	05/05/2020 11:46:42	EDUARDO MARCEL FERNANDES NASCIMENTO	Aceito

UNIVERSIDADE FEDERAL DE SANTA CATARINA - UFSC

Continuação do Parecer: 4.055.081

Projeto Detalhado / Brochura Investigador	Projeto.pdf	05/05/2020 11:42:45	EDUARDO MARCEL FERNANDES NASCIMENTO	Aceito
Folha de Rosto	FolhadeRostoFinal.pdf	09/03/2020 11:17:38	EDUARDO MARCEL FERNANDES NASCIMENTO	Aceito
Declaração de Instituição e Infraestrutura	DeclaracaodoInstituicao.pdf	09/03/2020 11:17:23	EDUARDO MARCEL FERNANDES NASCIMENTO	Aceito
Orçamento	Orcamento.pdf	08/03/2020 22:01:38	EDUARDO MARCEL FERNANDES NASCIMENTO	Aceito
Declaração de Pesquisadores	DeclaracaodoPesquisador.pdf	08/03/2020 21:17:36	EDUARDO MARCEL FERNANDES NASCIMENTO	Aceito

Situação do Parecer:
Aprovado

Necessita Apreciação da CONEP:
Não

FLORIANOPOLIS, 28 de Maio de 2020

Assinado por:
Maria Luiza Bazzo
(Coordenador(a))

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