UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"
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Programa de Pós-Graduação em Ciências do Movimento

Efeitos agudos da ingestão de L-Arginina na recuperação cardiovascular e na modulação autonômica cardíaca após teste de esforço submáximo em homens saudáveis

Andrey Alves Porto

Maio – 2025

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Epígrafe

"Nada na vida deve ser temido, somente compreendido. Agora é hora de compreender mais, para que possamos temer menos."
— Marie Curie

Resumo

Introdução: A suplementação com L-arginina tem sido amplamente estudada devido ao seu papel como precursora do óxido nítrico (ON), uma molécula fundamental para a regulação da vasodilatação, fluxo sanguíneo e modulação autonômica. Apesar de seu potencial, os efeitos agudos da suplementação de l-arginina na recuperação cardiovascular e autonômica após o exercício ainda não estão completamente esclarecidos. **Objetivo:** Avaliar os efeitos agudos da suplementação de L-arginina sobre a recuperação cardiovascular e da modulação autonômica cardíaca após o exercício em homens saudáveis.. **Métodos:** Homens saudáveis entre 18 e 30 anos de idade foram submetidos a dois protocolos experimentais triplo-cegos e randomizados, com um intervalo de pelo menos 48 horas entre eles. Os participantes ingeriram 3g de Larginina (LARG) ou placebo (amido), em cápsulas idênticas. Após 60 minutos da ingestão, realizaram um teste físico em esteira, começando com 3 minutos de aquecimento (5 km/h) seguidos de incrementos de 1 km/h a cada 2 minutos, até atingir 80% da frequência cardíaca máxima estimada. Os dados de frequência cardíaca (FC), pressão arterial sistólica (PAS) e diastólica (PAD) foram coletados em momentos específicos (30 segundos e minutos: 1, 3, 5, 7, 10 e 20). A análise da variabilidade da frequência cardíaca (VFC) incluiu índices lineares no domínio do tempo, como o desvio padrão da média dos intervalos entre batimentos (SDNN) e raiz quadrada da média dos quadrados das diferenças sucessivas (rMSSD), bem como no domínio da frequência, representados pelas bandas de baixa frequência (LF) e alta frequência (HF). Parâmetros autonômicos e cardiovasculares foram analisados em repouso, exercício e recuperação, fornecendo uma avaliação detalhada da modulação autonômica e respostas cardiovasculares. **Resultados:**. Em relação aos parâmetros cardiovasculares, não foram observados efeitos significativos entre os protocolos: FC ($p = 0,499$), PAS ($p = 0,956$) e PAD ($p = 0,844$). No entanto, observou-se no protocolo placebo uma recuperação mais lenta da modulação autonômica parassimpática (índices rMSSD e SD1) e da variabilidade global (SDNN, SD2 e LF ms2) em comparação com os valores basais (efeito de tempo, $p = 0.001$). Em relação à recuperação imediata após o exercício, não foram identificadas diferenças estatisticamente significativas na frequência cardíaca de recuperação (FCR) ($p = 0,944$), no índice rMSSD30" ($p = 0,562$) e nas respostas da pressão arterial média (MAP) ($p = 0,687$) e da pressão de pulso (PP) ($p = 0,929$) entre os protocolos avaliados. Quando avaliado o período mais longo de recuperação, também não foram encontradas diferenças significativas entre os protocolos para os índices de VFC: SDNN ($p = 0,955$), RMSSD ($p = 0,885$), SD1 ($p = 0,960$), SD2 ($p = 0,966$), $\eta^2P = 0,001$; LF (ms^2) ($p = 0,822$), $\eta^2P = 0,001$ e HF (ms^2) ($p = 0,267$). **Conclusão:** Em síntese, os resultados deste estudo demonstraram que a suplementação aguda de L-arginina não influenciou significativamente as respostas de variabilidade da frequência

cardíaca (VFC) e os parâmetros cardiovasculares entre os protocolos analisados. Contudo, a ingestão de L-arginina promoveu uma recuperação mais rápida da modulação vagal em comparação ao repouso. Esses achados devem ser interpretados com cautela, considerando a ausência de diferenças estatisticamente significativas entre os protocolos e a possibilidade de que pequenas variações observadas não apresentem relevância clínica ou fisiológica.

Palavras-chave: Arginina; Exercício; Sistema Nervoso Autônomo; Óxido Nítrico; Recuperação Pós-Exercício

Abstract

Introduction: L-arginine supplementation has been widely studied due to its role as a precursor of nitric oxide (NO), a key molecule in the regulation of vasodilation, blood flow, and autonomic modulation. Despite its potential, the acute effects of L-arginine supplementation on cardiovascular and autonomic recovery after exercise are not yet fully understood. **Objective:** To evaluate the acute effects of L-arginine supplementation on cardiovascular recovery and cardiac autonomic modulation after exercise in healthy men, considering different physical effort protocols. **Methods:** Healthy men between 18 and 30 years of age underwent two randomized, triple-blind experimental protocols, with an interval of at least 48 hours between them. Participants ingested 3 g of L-arginine (L-ARG) or placebo (starch) in identical capsules. Sixty minutes after ingestion, they performed a physical test on a treadmill, starting with a 3-minute warm-up (5 km/h) followed by 1 km/h increments every 2 minutes, until reaching 80% of the estimated maximum heart rate. Heart rate (HR), systolic blood pressure (SBP) and diastolic blood pressure (DBP) data were collected at specific time points (REC 1, 3, 5, 7, 10 and 20). The analysis of heart rate variability (HRV) included linear indices in the time domain, such as the standard deviation of the mean intervals between beats (SDNN) and the root mean square of successive differences (rMSSD), as well as in the frequency domain, represented by the Low-Frequency (LF) and High-Frequency (HF) bands. Autonomic and cardiovascular param- eters were analyzed at rest, exercise and recovery, providing a detailed assessment of autonomic modulation and cardiovascular responses**. Results:** When assessing the longer recovery period, no significant differences were found between the protocols for HRV indices: SDNN ($p = 0.955$), RMSSD ($p = 0.885$), SD1 ($p = 0.960$), SD2 ($p = 0.966$), $\eta^2P = 0.001$; LF (ms^2) ($p = 0.822$), $\eta^2P = 0.001$, and HF (ms^2) ($p = 0.267$). The same was observed for cardiovascular parameters, with no significant effects between the protocols: HR ($p = 0.499$), SBP ($p = 0.956$), and DBP ($p = 0.844$). However, it was observed that in the placebo protocol, there was a slower recovery of parasympathetic autonomic modulation (rMSSD and SD1 indices) and global variability (SDNN, SD2, and LF ms^2) compared to baseline values (time effect, $p = 0.001$). Regarding immediate recovery after exercise, no statistically significant differences were identified in heart rate recovery (HRR) ($p = 0.944$), the rMSSD30" index ($p = 0.562$), and the responses of mean arterial pressure (MAP) ($p = 0.687$) and pulse pressure (PP) ($p = 0.929$) between the evaluated protocols. **Conclusion:** In summary, the results of this study demonstrated that acute L-arginine supplementation did not significantly influence heart rate variability (HRV) responses and cardiovascular parameters among the protocols analyzed. However, L-

arginine intake promoted a faster recovery of vagal modulation compared to rest. These findings should be interpreted with caution, considering the absence of statistically significant differences between the protocols and the possibility that small variations observed do not present clinical or physiological relevance.

Keywords: Arginine; Exercise; Autonomic Nervous System; Nitric Oxide; Post-Exercise Recovery.

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Lista de Abreviaturas e Siglas

ANS: autonomic nervous system

BMI: body mass index

BP: blood pressure

DBP: diastolic blood pressure

DCV: doenças cardiovasculares

FC: frequência cardíaca

HF: high frequency

HR: heart rate

HR peak: peak heart rate

HRR: heart rate recovery

HRV: heart rate variability

Kg: kilograms

LF: low frequency

L-ARG: l-arginine

mmHg: millimeters of mercury

ms: milliseconds

ON: óxido nítrico

PAD: pressão arterial diastólica

PAS: pressão arterial sistólica

RMSSD: root-mean-square of differences between adjacent normal RR intervals in a timein-terval expressed in milliseconds.

SBP: systolic blood pressure

SD: standard deviation

SDNN: standard deviation of all normal RR intervals expressed in milliseconds

SPSS: Statistical Packages for Social Sciences

VFC: variabilidade da frequência cardíaca

Lista de símbolos

>: Maior que

<: Menor que

=: Igual

(): Parênteses

%: Porcentagem

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Apresentação

Este é um modelo alternativo de tese e contempla a pesquisa intitulada: **Efeitos agudos da ingestão de L-Arginina na recuperação cardiovascular e na modulação autonômica cardíaca após teste de esforço submáximo em homens saudáveis,** realizada no Laboratório de Fisiologia do Estresse da Faculdade de Ciências e Tecnologia – FCT/UNESP.

Em concordância com as normas do modelo alternativo do Programa de Pós-Graduação em Ciências do Movimento da Faculdade de Ciências e Tecnologia da Universidade Estadual Paulista "Júlio de Mesquita Filho", a presente dissertação está dividida da seguinte forma:

- ✓ Introdução Geral, contendo a contextualização do tema pesquisado;
- ✓ **Artigo I:** Andrey Alves Porto; Luana Almeida Gonzaga; Rayana Loch Gomes; Rodrigo Daminello Raimundo; Luiz Carlos Marques Vanderlei; Vitor Engrácia Valenti. Acute effects of L-arginine intake on heart rate variability after a submaximal exercise test in healthy men: randomized clinical trial. Que está em revisão no perió- dico: *Nitric Oxide* (normas para submissão no site:https://www.sciencedirect.com/journal/nitric-oxide/publish/guide-for-authors).
- ✓ **Artigo II:** Andrey Alves Porto; Luana Almeida Gonzaga; Felipe Ribeiro; Camila Marcondes de Oliveira Luiz Carlos Marques Vanderlei; Vitor Engrácia Valentia. L-arginine supplementation did not impact the rapid recovery of cardiovascular and autonomic functions following exercise in physically active healthy males: A triple-blind randomised placebo-controlled crossover trial. v. 16, p. 4067, 2024. https://doi.org/10.3390/nu16234067.
- ✓ Conclusões, obtidas por meio da pesquisa realizada; e referências

Introdução Geral

A L-arginina é um aminoácido semiessencial amplamente comercializado como suplemento dietético devido ao seu papel como precursor na síntese de óxido nítrico (ON). O ON é uma molécula sinalizadora endógena que desempenha funções críticas no organismo, incluindo a regulação da vasodilatação, aumento do fluxo sanguíneo, controle da respiração mitocondrial e modulação da função plaquetária (SUREDA, 2012). Essas propriedades tornam a L-arginina atrativa para diferentes aplicações, especialmente em contextos de saúde cardiovascular e desempenho físico. Além disso, há evidências de que a suplementação de L-arginina pode influenciar os níveis do hormônio do crescimento (GH), o que contribui ainda mais para sua popularidade entre praticantes de atividade física e esportes (KANALEY, 2008).

Apesar do potencial biológico do ON e da relevância da L-arginina como seu precursor, os efeitos agudos da suplementação de L-arginina sobre o desempenho esportivo ainda não são completamente compreendidos, apresentando resultados inconsistentes na literatura. Muitos produtos comercializados alegam que a suplementação de L-arginina melhora o desempenho físico ao atuar como um potente vasodilatador, o que teoricamente aumentaria o aporte de oxigênio e nutrientes aos músculos em atividade, favorecendo assim o desempenho esportivo (STREETER, 2019). No entanto, estudos controlados frequentemente não corroboram essas alegações. Por exemplo, Alveras (2012) não observou diferenças significativas no desempenho físico de indivíduos suplementados, e Fahs (2009) relatou ausência de impacto no fluxo sanguíneo muscular em jovens saudáveis.

Nesse contexto, revisões sistemáticas recentes ajudam a esclarecer o papel da L-arginina em diferentes aspectos fisiológicos. Uma meta-análise conduzida por Porto et al. (2023) avaliou o impacto da suplementação de L-arginina e L-citrulina em parâmetros inflamatórios e estresse oxidativo induzidos pelo exercício. A revisão, que incluiu sete ensaios clínicos randomizados, não encontrou evidências significativas de que a suplementação promove melhorias nesses parâmetros em comparação ao placebo (PORTO, 2023). No entanto, outro estudo conduzido por Porto et al. (2021) destacou o potencial da L-arginina para reduzir a pressão arterial sistólica e diastólica após o exercício, especialmente em indivíduos hipertensos. Esses achados reforçam que os efeitos da suplementação podem variar substancialmente dependendo da condição clínica e do perfil dos participantes (PORTO, 2021).

Investigações recentes também reforçam essa disparidade nos achados em parâmetros cardiovasculares. Streeter et al. (2019) avaliaram os efeitos agudos da suplementação de L-

arginina em 30 indivíduos fisicamente ativos e não encontraram evidências de melhora em parâmetros cardiovasculares, como frequência cardíaca (FC), pressão arterial sistólica (PAS) e variabilidade da frequência cardíaca (VFC), durante exercícios resistidos. Por outro lado, Lima et al. (2018) relataram resultados mais promissores ao investigar os efeitos da L-arginina na pressão arterial pós-exercício em pacientes hipertensos. Nesse estudo, a suplementação de 7 g de L-arginina antes de uma sessão de exercício aeróbico contribuiu para uma redução mais pronunciada da pressão arterial durante o período de recuperação.

Além disso, estudos em modelos animais fornecem uma perspectiva interessante sobre os possíveis benefícios da L-arginina quando combinada ao exercício físico. Darband et al. (2019) demonstraram que a suplementação de L-arginina, associada ao treinamento físico, foi eficaz em reduzir estresse oxidativo, inflamação e apoptose no miocárdio de ratos envelhecidos, protegendo o tecido cardíaco contra os efeitos deletérios do envelhecimento. De forma semelhante, Silva et al. (2017) observaram que a suplementação com L-arginina aumentou o desempenho físico e reduziu o estresse oxidativo em ratos submetidos a exercícios aeróbicos, sugerindo que os efeitos benéficos podem estar associados à melhora da função endotelial e à modulação do equilíbrio redox.

Embora o impacto do exercício físico no combate ao sedentarismo e nas doenças cardiovasculares (DCV) seja amplamente documentado (HA FJ, 2017), as DCV permanecem a principal causa de mortalidade global, inclusive no Brasil, mesmo diante de esforços significativos em educação e prevenção (PRÉCONA, 2019). Nesse cenário, estudos epidemiológicos têm evidenciado que a VFC é uma ferramenta eficaz e amplamente validada para a predição de mortalidade cardiovascular, tanto em populações saudáveis quanto em pacientes com hipertensão, insuficiência cardíaca e outras condições (CAETANO, 2015). A VFC reflete diretamente a modulação autonômica cardíaca, sendo um indicador não invasivo do equilíbrio entre os ramos simpático e parassimpático do sistema nervoso autônomo (SNA) (VANDERLEI, 2009).

A análise da VFC vem sendo cada vez mais utilizada em estudos que avaliam os efeitos do exercício físico, dado que o comportamento da VFC durante e após o exercício fornece informações valiosas sobre a adaptação do SNA. Nesse sentido, a intensidade do exercício é um dos fatores mais relevantes na modulação da VFC, sendo que exercícios de alta intensidade frequentemente resultam em maior supressão da atividade parassimpática e, consequentemente, em uma recuperação mais lenta da VFC (MICHAEL, 2017). Esse fenômeno tem implicações

tanto para a monitorização do desempenho esportivo de atletas (LAMBERT, 2009) quanto para a avaliação da aptidão física em diferentes contextos (D'AGOSTO, 2014). Além disso, a cinética da VFC pós-exercício pode ser utilizada como um indicador da aptidão aeróbica, uma vez que uma recuperação mais rápida está associada a maior capacidade cardiorrespiratória e melhor desempenho físico (STANLEY, 2013).

No entanto, lacunas na literatura ainda persistem, particularmente no que diz respeito aos efeitos da L-arginina sobre o comportamento do SNA durante a recuperação pós-exercício máximo. A falta de padronização em variáveis como sexo, nível de aptidão física e homogeneidade das amostras limita a generalização dos resultados disponíveis.

Por outro lado, é amplamente reconhecido que a recuperação do SNA após o exercício fornece informações importantes não apenas sobre o estado de saúde geral, mas também sobre a vulnerabilidade a complicações cardiovasculares (KINGSLEY, 2016). Dado o papel fundamental do exercício físico na promoção da saúde e na prevenção de DCV, a investigação do comportamento autonômico durante a recuperação torna-se um campo de estudo essencial. Nesse contexto, a presente pesquisa busca contribuir para o entendimento dos efeitos da suplementação de L-arginina sobre a modulação autonômica, com foco no comportamento da VFC em indivíduos saudáveis submetidos a testes de esforço máximo.

Para abordar as lacunas existentes na literatura e contribuir para o avanço do conhecimento científico sobre os efeitos da suplementação de L-arginina na modulação autonômica e cardiovascular, foram desenvolvidos dois manuscritos. O primeiro manuscrito, intitulado **"Acute effects of L-arginine ingestion on cardiovascular recovery and cardiac autonomic modulation after submaximal effort test in healthy men: a triple-blinded, randomised, placebo-controlled trial"**, teve como foco principal investigar os efeitos agudos da suplementação de L-arginina sobre a recuperação da modulação autonômica cardíaca e os parâmetros cardiovasculares em resposta ao exercício físico. Para tanto, foi conduzido um estudo rigoroso, utilizando um desenho experimental triplo-cego, randomizado e controlado por placebo, envolvendo indivíduos saudáveis. A pesquisa buscou avaliar a capacidade da L-arginina de influenciar positivamente a recuperação da VFC e outros marcadores cardiovasculares, fornecendo uma análise detalhada de suas possíveis implicações no desempenho físico e na saúde cardiovascular.

O segundo manuscrito, intitulado **“L-arginine supplementation did not impact the rapid recovery of cardiovascular and autonomic functions following exercise in physically active healthy males: A triple-blind randomised placebo-controlled crossover trial”**, teve como objetivo aprofundar a análise sobre o impacto da suplementação de L-arginina, desta vez voltando-se para a recuperação imediata das funções cardiovasculares e autonômicas após o exercício. Este estudo empregou um desenho experimental cruzado, também conduzido em um ambiente triplo-cego e controlado por placebo, com indivíduos fisicamente ativos e saudáveis. O objetivo foi examinar a eficácia da suplementação de L-arginina na aceleração da recuperação pós-exercício, incluindo os efeitos sobre a VFC e outros parâmetros fisiológicos relevantes. Embora as expectativas iniciais fossem de que a suplementação pudesse favorecer uma recuperação mais rápida e eficiente, os resultados encontrados oferecem uma perspectiva crítica e contribuem para o debate científico sobre os reais benefícios da L-arginina em contextos de exercício físico.

Esses dois manuscritos, portanto, complementam-se ao explorar diferentes aspectos dos efeitos agudos da suplementação de L-arginina, utilizando metodologias robustas para responder às questões-chave relacionadas à sua eficácia. Juntos, eles ampliam o entendimento sobre a relação entre suplementação nutricional, modulação autonômica e recuperação cardiovascular, fornecendo subsídios importantes para futuras pesquisas e aplicações práticas no campo da saúde e do esporte.

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Artigo 1

Acute effects of L-arginine intake on heart rate variability after a submaximal exercise test in healthy men: randomized clinical trial

Abstract

The acute effects of L-arginine intake on cardiovascular recovery and cardiac autonomic modulation following a submaximal stress test remain incompletely understood in healthy individuals. Efficient cardiovascular recovery after exercise is crucial for overall cardiovascular health, with cardiac autonomic modulation playing a key role in this process. This study aimed to evaluate the immediate effects of L-ARG supplementation on heart rate variability after exercise in healthy men. In this triple-blind, randomized, placebo-controlled trial, 37 healthy male participants completed two exercise protocols, with a minimum interval of 48 hours between them. In the first protocol, participants consumed a placebo (3 g of starch) before exercise, while in the second, they ingested 3 g of L-ARG. Both protocols involved treadmill exercise at 80% of the participant's maximum heart rate. Heart rate variability (HRV) and cardiovascular parameters, including systolic and diastolic blood pressure, were measured at various recovery time points. Randomization was conducted using the "Research Randomizer" website. L-ARG supplementation led to a faster recovery of vagal modulation in the early recovery phases compared to the placebo. However, no significant differences were observed between the protocols in overall HRV or cardiovascular responses. While blood pressure recovery was slightly slower in the L-ARG group, this difference was not clinically relevant.

The study concluded that acute L-arginine supplementation did not significantly alter HRV responses or cardiovascular parameters between protocols but did enhance vagal modulation recovery compared to the rest period.

Trial registration: ClinicalTrials.gov NCT06405477. Registered on May 06, 2024.

Keywords: arginine; exercise; autonomic nervous system; nitric oxide; post-exercise recovery

Introduction

An ergogenic aid can help prepare the body for exercise, enhance exercise efficiency, support recovery, and improve tolerance to intense training, potentially lowering the risk of injury and promoting overall health during strenuous workouts. In this context, various nutritional supplements have been introduced to the market, aiming to boost muscle strength and hypertrophy gains through resistance training (1).

Supplements containing L-arginine (L-ARG) have been recommended for promoting acute vasodilation by enhancing nitric oxide (NO) production in the active muscle during exercise (2, 3). L-ARG is a semi-essential amino acid that serves as a substrate for endothelial nitric oxide synthase (4), an enzyme capable of stimulating the production of NO metabolites, thus being the main precursor of NO (5) and arginase, two essential enzymes for the functioning of the human body (6). NO is responsible for several essential activities of the human body, such as helping to regulate vasodilation, blood flow, mitochondrial respiration, platelet function (5) and increasing growth hormone levels (7).

In 2001, Maxwell et al. (8) observed that mice receiving L-ARG supplementation demonstrated elevations in post-exercise urinary nitrate excretion, indicative of NO production, alongside enhancements in aerobic capacity. Subsequently, in 2006, Long et al. (9) reported increases in myotube density, total nuclei number, and nuclear fusion index after L-ARG supplementation. Both investigations concluded that the ameliorated exercise performance and skeletal muscle adaptations could be partially attributed to heightened NO production resulting from L-ARG intake. Similarly, in 2006, McConnell (10) concluded that intravenous and oral administration of L-ARG exhibited a modest improvement in aerobic exercise capacity in healthy humans, a finding corroborated by Álvarez and colleagues in 2011 (11). The authors conducted a review and noted that in three out of five studies, acute L-ARG intake enhanced muscular peak performance and total work output and reduced muscular fatigue (11).

Chowdhary (12) highlighted the importance of strategies for enhancing NO production for the autonomic nervous system (ANS) in healthy volunteers. However, academic literature seems to focus on performance outcomes, and it lacks its influence on this system. The ANS plays a critical role in maintaining homeostasis (13), and pre-exercise supplement consumption may benefit (14) or harm ANS recovery (15).

We are committed to better understanding the influence of taking L-ARG before an exercise session. Previous studies have presented different results regarding post-exercise effects for L-ARG intake and other outcomes. Porto et al. 2022 (14) reported that intake of this substance reduced systolic blood pressure (SBP) and diastolic blood pressure (DBP) in adults

with hypertension, while no effect was found on post-exercise antioxidant, anti-inflammatory indices (16) and at the immediate rapid recovery of HRV after a bout of exercise (17). Nonetheless, to our knowledge, our study is the second to evaluate the effect of L-ARG supplementation on HRV recovery parameters (18).

Therefore, our study aims to provide support for a better understanding of the behaviour of the autonomic systems after moderate-intensity aerobic exercise and explore interventions within this context. The aim of this study was to assess the immediate effects of the intake of an L-ARG supplement on heart rate variability after exercise in healthy men. Furthermore, part of the plan is to guide health professionals concerning the use of L-ARG in healthy people and, in the future, as a pilot for studies with other types of health conditions.

Materials and methods

Participants and Ethical Aspects

The present study followed a methodology previously published by Porto et al. (17), who carried out a triple-blind, randomized, placebo-controlled, crossover clinical trial aiming to investigate the effects of L-arginine supplementation on immediate cardiovascular and autonomic recovery after submaximal exercise in physically active, healthy men. This study was approved by the Research Ethics Committee of the Faculty of Philosophy and Sciences of Universidade Estadual Paulista "Júlio de Mesquita Filho" – FFC/UNESP (Approval Number: 4.761.413) and was conducted per ethical guidelines. All participants provided written informed consent after being thoroughly briefed on the study's objectives and procedures. The trial was also registered in the Clinical Trials Network (clinicaltrials.com) under the identification code NCT06405477.

Participants were recruited via social media announcements and posters placed across the university campus in Presidente Prudente, São Paulo, Brazil. Volunteers who expressed interest were interviewed and asked to complete the International Physical Activity Questionnaire (IPAQ) to determine their eligibility based on the inclusion criteria. The study included healthy males aged 18 to 30 years, with a body mass index (BMI) between 18.5 and 29.9 kg/m², who were classified as physically active according to the IPAQ assessment (19).

To ensure the accuracy and reliability of the study results, we implemented rigorous exclusion criteria. Individuals were not eligible if they were smokers, had a history of excessive alcohol consumption, or presented any of the following conditions: known cardiopulmonary, neurological, or metabolic disorders; abnormal hemodynamic responses during physical activ-

ity; or orthopedic limitations that could impair their ability to complete the experimental procedures. Additionally, individuals who had been diagnosed with COVID-19 within the last 24 weeks, exhibited disease symptoms, or had been in contact with confirmed cases 14 days prior to recruitment were excluded.

These exclusion criteria were essential to maintain the health integrity of the participants and to minimize potential confounding variables that could affect the interpretation and accuracy of the data collected.

Study Design

Before beginning the experimental procedures, participants visited the laboratory for body measurements and screening to verify their eligibility based on the inclusion criteria. Those who met the criteria proceeded to the subsequent study phases. All data collection was conducted in a controlled research laboratory environment within a university setting.

The experimental procedures comprised two distinct protocols, performed with a minimum interval of 48 hours between them to ensure adequate recovery and minimize residual exercise effects (20). Each session took place between 5:00 pm and 9:00 pm to reduce circadian variations, in a controlled room where the temperature was maintained between 23°C and 26°C, with relative humidity ranging from 60% to 70% (21).

To minimize potential confounding factors, participants followed specific pre-experimental guidelines. They were instructed to avoid consuming alcoholic beverages and caffeine-containing foods for at least 12 hours before each session. Additionally, they were advised to have a light meal, drink 500 ml of water two hours before the procedure, and refrain from engaging in strenuous physical activity the day prior.

The randomization process involved the administration of six capsules containing either 3 g of starch (Placebo Protocol) or 3 g of L-arginine (L-ARG Protocol). To preserve the blinding of the study, both the capsules and the flasks were identical in color and size. Neither the volunteers nor the researchers were aware of the contents of the capsules during the experimental protocols. The protocol order was randomized using the "Research Randomizer" website (www.randomizer.org), followed by allocation concealment through sequentially numbered, opaque, and sealed envelopes. A researcher not involved in the experimental procedures carried out the randomization process.

Due to L-arginine's relatively short plasma half-life (approximately 1.5 hours) (22), an interval of at least 48 hours between protocols was deemed sufficient to prevent carryover effects. This careful planning ensured the accuracy and reliability of the data obtained.

Experimental Procedures

During the experimental protocols, heart rate (HR) was continuously recorded using a Polar RS800CX monitor (Finland), which has been previously validated for this purpose (23). After properly fitting the equipment, participants were instructed to remain seated for 10 minutes in a calm environment, breathing spontaneously and in silence. At the 10th minute, measurements of HR, systolic blood pressure (SBP), and diastolic blood pressure (DBP) were taken. Following this initial phase, participants ingested the assigned substance for the day, according to the randomization process, and waited for 60 minutes.

Next, participants began the treadmill exercise protocol. The warm-up consisted of walking at 5 km/h for 3 minutes, followed by progressive load increments of 1 km/h every 2 minutes until reaching 80% of their maximum HR, as predicted by the Tanaka et al. (2001) formula: 208 - (0.7 × age) (24). Upon completing the exercise protocol, volunteers remained on the treadmill for an additional 3 minutes to initiate the recovery phase, after which they sat in a quiet environment for 17 more minutes while being monitored.

Cardiovascular parameters, including HR, SBP, and DBP, were recorded at specific time points: 1st minute (REC 1), 3rd minute (REC 3), 5th minute (REC 5), 7th minute (REC 7), 10th minute (REC 10), and 20th minute (REC 20). For the analysis of autonomic nervous system (ANS) activity, heart rate variability (HRV) indices were determined at distinct time intervals: Baseline (5th to 10th minute), Recovery 1 (REC 1) (0th to 5th minute), Recovery 2 (REC 2) (5th to 10th minute), Recovery 3 (REC 3) (10th to 15th minute), and Recovery 4 (REC 4) (15th to 20th minute).

HR was continuously recorded throughout the protocol, with beat-by-beat data collected using the Polar RS800CX device. The RR interval series obtained were initially processed using a moderate digital filter in the Polar ProTrainer 5 software (Kempele, Finland) to remove artifacts, followed by manual filtering for greater accuracy. Only series containing at least 95% sinus beats were included in the analysis. For each selected moment, 256 consecutive stable RR intervals were chosen.

To assess cardiac autonomic modulation, linear analysis methods were employed in both time and frequency domains. In the time domain, the rMSSD (square root of the mean square differences between adjacent regular RR intervals) and SDNN (standard deviation of the mean of all regular RR intervals) indices were calculated. The Poincaré plot was also analyzed, with SD1 representing the standard deviation of instantaneous variability and SD2 indicating long-term variability.

For frequency domain analysis, spectral components of low frequency (LF = 0.04–0.15 Hz) and high frequency (HF = 0.15–0.40 Hz) were evaluated, expressed in ms^2. These components were obtained using the Fast Fourier transform algorithm (13). The linear indices in both time and frequency domains were calculated using the Kubios HRV analysis software (Biosignal Analysis and Medical Image Group, Department of Physics, University of Kuopio, Finland) (25).

Data analysis

The sample size calculation was based on data obtained in a pilot project (n=13) for the rMSSD index, which, in the end, indicated the highest minimum number. Considering a standard deviation of 5.03 ms, the minimum difference between groups of 4.35 ms, alpha risk of 0.05 and power of 0.80, the sample size resulted in 22 individuals. Still, considering possible losses, 10% were added to this value, totalling a minimum of 24 individuals per group. Version 3.1.9.7 of GPower (Heinrich-Heine-Universität Düsseldorf, Germany) (26) was used.

Regarding the Triple-blinded method, the researcher who performed the analyses was not informed about the identity of each protocol. For data analysis, descriptive statistics were used to characterise the sample and the results were presented with mean, confidence interval, minimum and maximum values. Data normality was defined by the Shapiro-Wilk test. The Cohen's d effect size was calculated for these analyses (small < 0.50; medium between 0.50 and 0.70; large between 0.80 and 1.20; very large ≥ 1.30) (25).

Comparisons of HRV indices and cardiovascular parameters between protocols, interaction (time vs. protocol), and time were performed using the analysis of variance technique for a two-way ANOVA model. The data from the repeated measurement were checked for sphericity violation using the Mauchly test, and the Greenhouse-Geisser correction was used in cases where sphericity was violated. The partial eta-squared effect size ($\eta 2P$) was calculated for the ANOVA results (small≤0.05; medium between 0.06 and 0.13; large≥0.14) (25).

For the analysis of the moments (baseline vs. moments of recovery), the Bonferroni post-test was used for normal distributions and Dunn for non-normal distributions. Statistical

significance was set at 5% for all analyses. The analyses were performed using GraphPad Instat – version 3.01, 1998 (GraphPad Software, Inc., San Diego, California, USA) and IBM SPSS Statistics - version 22.0 (SPSS Inc., Chicago, IL, USA).

Results

The anthropometric characteristics of the 37 participants were evaluated in this study (Table 1). Figure 1 shows the flowchart of losses during the study (CONSORT flow diagram).

Insert Table 1 here

Insert Figure 1 here

Figure 2 illustrates the behaviour of the HRV indices in the time domain during baseline and recovery. We observed the effect of time for: SDNN - p = 0.001, $\eta 2_P$=0,656; rMSSD - p = 0.001, $\eta 2_P$=0,522; SD1 - p = 0.001, $\eta 2_P$=0,510 and SD2 indices: p = 0.001, $\eta 2_P$=0,667, but no effects were observed between protocols: SDNN - p = 0,955, $\eta 2_P$= 0,001; rMSSD - p = 0,885, $\eta 2_P$= 0,001; SD1 - p = 0,960, $\eta 2_P$= 0,001 SD2 p = 0,966, $\eta 2_P$=0,001 and interaction time vs. protocol: SDNN - p = 0,764, $\eta 2_P$=0,005; rMSSD - p = 0,124, $\eta 2_P$=0,027; SD1 - p = 0,203, $\eta 2_P$=0,021; SD2 - p = 0,771, $\eta 2_P$=0,005. For the rMSSD and SD1 indexes, significantly lower values were observed at Rec1, Rec2 and Rec3 compared to the baseline for the L-ARG protocol. In addition, a lower value at Rec4 was also observed in the Placebo protocol. For the SDNN and SD2 indices, significantly higher values were observed about the baseline in Rec1 and Rec2 for the L-ARG and significantly higher values were observed in Rec1, Rec2, Rec3, and Rec4 for the Placebo protocol.

Insert Figure 2 here

The behaviour of HRV indices in the frequency domain in the protocols performed can be seen in Figure 3. Similarly, for the time-domain indices, time effects: LF ms² - p = 0,001, $\eta 2_P$=0,166 and HFms² - p = 0.001, $\eta 2_P$=0,340 were observed, but not between protocols: LF ms² - p = 0,822, $\eta 2_P$= 0,001 and HF ms² - p = 0,267, $\eta 2_P$= 0,017 and interaction time vs. protocols: LF ms² - p = 0,297, $\eta 2_P$= 0,017 and HF ms² - p = 0,024, $\eta 2_P$= 0,047. For the HF ms² index, significantly lower values of the Rec1, Rec2, Rec3 and Rec4 moments compared to the baseline were observed for both protocols. For the LFms², significantly higher values were found at Rec 1 and Rec 2 for the L-ARG protocol and Rec1 Rec 2 and Rec 3 for the placebo protocol compared to Baseline values. The mean, standard deviation, confidence interval and Cohen's d values of the HRV indices are shown in Supplementary file 1.

Insert Figure 3 here

The behaviour of cardiovascular parameters relative to the baseline measurements and during the recovery period is displayed in Figure 4. We observed an effect of time for HR – p= 0.001; $\eta 2_P$= 0,820; SBP - p= 0.001, $\eta 2_P$= 0,729 and DBP - p = 0.001, $\eta 2_P$= 0,550 but there was no effect between protocols: HR - p = 0,499, $\eta 2_P$= 0,006; SBP - p = 0,956, $\eta 2_P$= 0,001; DBP - p = 0,844, $\eta 2_P$= 0,001 and interaction time vs. protocols: HR - p = 0,338, $\eta 2_P$= 0,016; SBP - p = 0,956, $\eta 2_P$=0,001; DBP - p = 0,844, $\eta 2_P$= 0,001 for these variables.

Insert Figure 4 here

Concerning HR, significantly higher values were observed regarding the baseline in both protocols at all recovery moments (1st to 20th min). For SBP, significantly higher values were found concerning baseline and were observed at placebo protocol 1st and 3rd minute of recovery and 1^{st}, 3^{rd} and 5^{th} for L-ARG protocol. DBP presented significantly higher values between recovery and baseline values at the 1st and 3rd minute for the placebo protocol and the 1^{st}, 3^{rd}, 5^{th} and 7^{th} minute for the L-ARG protocol (Figure 4). The mean, standard deviation, confidence interval and Cohen's d values of cardiovascular parameters are shown in Supplementary file 2.

Discussion

The present study aimed to assess the immediate effects of L-ARG intake on autonomic and cardiovascular recovery following a submaximal exercise in healthy adult males. The study's primary findings indicate that the ingestion of this substance did not impact the recovery of heart rate variability, as assessed by time and frequency domain indices of HRV. Furthermore, no significant changes were observed in cardiovascular parameters (HR, SBP, and DBP) responses after exercise between the different protocols. However, it is observed that placebo treatment resulted in a slower recovery of parasympathetic autonomic modulation (rMSSD and SD1 indices) and global variability (SDNN, SD2 and LF ms^2) compared to the baseline. Regarding cardiovascular parameters, the placebo treatment led to faster recovery of SBP and DBP than the L-ARG treatment.

The published study that evaluated the effects of L-ARG on autonomic modulation after exercise also did not show any influence of this substance on those parameters (18). In the study conducted by Streeter et al. (2019) (17), 30 physically active participants (15 men and 15 women) were evaluated in a randomized, crossover, double-blind, placebo-controlled clinical

trial. After consuming either 3 g of L-ARG or 3 g of placebo, the participants completed five sets of elbow extension-flexion exercises. Significant time effects were observed for autonomic modulation, as measured by rMSSD and pNN50 indices; however, no discernible treatment or interaction effects were noted. As a result, the trial concluded that the presence of L-ARG did not influence the behaviour of the ANS.

Despite similar results, it's important to note some significant methodological differences between studies. Streeter and colleagues only evaluated a 10-minute recovery period and did not divide the recovery period into segments to better observe and compare the effects over time. They also did not present some important indexes corresponding to global HRV modulation, such as SDNN, SD2, and LFms². The main difference between studies is that the exercise protocol involved five sets of 10 maximal isokinetic elbow extensions at 90 degrees per second, with 30-second rests.

Similarly, our study also demonstrated that acute supplementation with L-arginine did not significantly influence autonomic modulation during the recovery period following exercise. These findings are consistent with those of Porto et al.(17), who employed a triple-blind, randomized, placebo-controlled crossover design to investigate cardiovascular and autonomic recovery after treadmill exercise in physically active young men. In that study, L-ARG supplementation did not alter the rapid recovery of heart rate and HRV or blood pressure responses. Although the methodological design was similar, our study employed resistance exercises and evaluated recovery over a different period, while Porto et al. (17) focused on treadmill exercise at 80% of maximum heart rate and monitored recovery for a longer duration. Additionally, Porto et al. (17) presented a more comprehensive evaluation of autonomic modulation, including indices such as RMSSD and pNN50, which were not included in the study by Streeter et al. (18). These differences highlight the complexity of assessing L-arginine's effects on cardiovascular recovery and underscore the importance of further research to elucidate its potential clinical applications.

According to the literature, when we exercise, our cardiovascular system must adjust to support the metabolic needs of our muscles and the thermoregulatory requirements of our skin, all while keeping blood pressure (BP) stable. A prominent model suggests that signals from the brain at the onset of exercise prompt the baroreflex to elevate, swiftly increasing HR by diminishing parasympathetic activity. This initial response is influenced by muscle mechanoreceptors and heightened venous return. As exercise intensity rises, parasympathetic activity decreases, and sympathetic activity increases, causing a shift from parasympathetic control at rest to sympathetic control at high intensity, further heightened by sympathoadrenal activation (27).

Our findings indicated that the L-ARG protocol led to a more rapid recovery of the parasympathetic system and global HRV indices compared to the placebo protocol. Nevertheless, it is essential to exercise caution when interpreting this outcome. rMSSD, SD1 (REC 4 vs. did not recover), SDNN, SD2 (REC 3 vs. did not recover) and LFms² (REC3 vs. REC4) were recovered faster in the L-ARG protocol. However, there were no differences between the protocols, and upon considering the discrepancy in effect sizes as per Cohen's d, it is evident that only the rMSSD index demonstrated a noteworthy disparity between the protocols at REC4 (Δ=0.89). In contrast, the remaining indices exhibited differences of less than 0.5 (refer to Additional file 2). Carefully considering the data, it can be inferred that using L-ARG resulted in a slight improvement in post-exercise HRV recovery.

The faster recovery observed in the L-ARG protocol can be partially attributed to the role of NO as a mediator in the cardiovascular system, facilitating vasodilation by promoting the relaxation of vascular smooth muscle (28). Through this vasodilatory effect, NO counteracts the vasoconstrictive influence of the sympathetic nervous system, thereby helping to maintain a better balance between the sympathetic and parasympathetic components of the ANS (29).

During physical exercise, the regulation of HR and BP changes. This is because the baroreflex function in the brainstem decreases due to activating the metaboreflex. When cellular metabolism increases, metabolic by-products accumulate, activating metaboreceptors. This activation stimulates nerve fibres, leading to increased sympathetic activity, which raises the HR, cardiac output, and BP and causes vasoconstriction in non-active muscles. After exercise, these effects are expected to rapidly decrease due to the reactivation of parasympathetic activity (30). Although parasympathetic control indexes, such as rMSSD and SD1, recovery was quicker in the L-ARG protocol, this wasn't enough to recover HR more quickly. Similar behavior of HRV and HR was presented in the study by Gonzaga et al. (15)

Regarding BP, the placebo group recovered more quickly than the L-ARG group. Both groups presented virtually the same effect size in the moment of statistically significant difference for SBP (REC5: L-ARG = 0.73; Placebo = 0.76) and a slight difference in effect size for DBP (REC7: L-ARG = 0.63 and Placebo = 0.37). Nonetheless, based on effect size and the mean difference between groups at this moment, which is ~1 mmHg, we believe that these differences were not clinically insignificant.

A previous systematic review assessed the impact of L-ARG intake on BP during exercise recovery (14). This meta-analysis of randomized, double-blind, placebo-controlled clinical trials provides evidence supporting the potential of oral L-ARG supplementation to decrease post-exercise SBP by 5.04 mmHg and DBP by 2.96 mmHg compared to the control group in

patients with hypertension over 50 years old. The findings suggest that in patients with hypertension, the reduced NO production is due to endothelial dysfunction. Ageing directly contributes to decreased endothelial activity, increasing the risk of cardiovascular conditions such as hypertension. The reduced availability of nitric oxide diminishes the ability of the body to maintain endothelial homeostasis. In theory, as the main substrate of NO production, L-ARG could address this deficiency (6).

On the other hand, a study conducted by Puga et al. (31) presented a similar result to ours. The authors concluded that a single 9g dose of L-ARG combined with aerobic exercise effectively reduced office diastolic BP during a 90-minute period in normotensive postmenopausal women compared to a control group and a day with no exercise. However, no significant difference was observed between the exercise-only group and the L-ARG plus exercise group.

It is crucial to acknowledge the release of vasodilatory substances such as NO and histamine (32, 33) as well as the reduction in sympathetic activity (34), are just a few of the numerous mechanisms that can impact BP. The absence of a clinically significant reduction in BP following a submaximal effort can also be attributed to factors such as hyperemia resulting from exercise performance (33, 35) baroreceptor activity (36), and reduced cardiac output due to decreased stroke volume (37), all of which play crucial roles in BP regulation.

Finally, it is imperative to emphasise some crucial points of our methodology. We could not analyse nitrate and nitrite plasma concentrations, so we cannot conclusively attribute the results to the effects of L-ARG intake and NO production. However, a study conducted by Puga et al. (31) found lower DBP values after a bout of exercise in normotensive postmenopausal women who consumed L-ARG before exercise and showed no differences in NO bioavailability as assessed by blood test. Additionally, we did not use a standardised ergonomic assessment to measure cardiopulmonary capacity, specifically Vo2 max, which limits the precise characterisation of our volunteers' physical capacity levels. This is a significant limitation of our study. However, our sample size calculation indicates that the number of volunteers in this study is appropriate, and the selection process followed rigorous exclusion criteria, increasing the confidence levels of our data.

Although L-ARG has demonstrated limited effects in modulating HRV and cardiovascular responses, these findings are valuable as they contribute to a more precise understanding of the impact of this substance on healthy individuals. These results play a crucial role in preventing exaggerations regarding the effectiveness of interventions and in aligning expectations for future studies, particularly when they either corroborate or diverge from prior findings. We

advise against generalizing our conclusions to different groups and encourage future studies with varying populations, doses, and treatment durations.

Our research group published a study last year evaluating the effects of L-ARG using the same volunteers participating in the present study. Both works have similar methodologies, as they used experimental protocols with L-ARG supplementation and monitoring of cardiovascular and autonomic variables in physically active individuals, with data extracted from the same clinical trial. However, the main methodological difference between the studies lies in the timing of VFC index assessments during the recovery period.

While the previous study (17) focused on collecting indices within a maximum period of three minutes after exercise, the present study proposed a more comprehensive evaluation, segmenting recovery intervals up to 20 minutes post-exercise. This approach allowed for a more detailed analysis of the temporal dynamics of time and frequency domain indices, providing a deeper understanding of autonomic recovery and parasympathetic reactivation after physical effort.

Furthermore, the difference in the number of participants between the studies is due to the instability in capturing R-R intervals in the short period immediately after exercise, which may have compromised the inclusion of some records in the previous study.

In summary, the findings of this study indicate that acute L-ARG supplementation did not impact on HRV responses and cardiovascular parameters between the protocols. However, L-ARG intake resulted in quicker recovery of vagal modulation than resting. It's important to interpret these results cautiously due to the lack of significant differences between the protocols and the minor variations that may have clinically or physiologically insignificant effects.

Disclosure of interest

The authors report there are no competing interests to declare.

Abbreviations

Blood Pressure (BP); Diastolic Blood Pressure (DBP); Heart Rate (HR); High Frequency (HF); Low Frequency (LF); Nitric Oxide (NO); rMSSD (square root of the mean square of the differences between adjacent regular RR intervals); SD1 (standard deviation of the instantaneous variability of HR); SD2 (long-term standard deviation of the indicators of the RR intervals); SDNN (standard deviation of the mean of all regular RR intervals); Systolic Blood Pressure (SBP); Diastolic Blood Pressure (DBP) L-arginine (L-ARG), Autonomic Nervous System (ANS).

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Table 1. Anthropometric and Physiological Characteristics of Study Participants (n=37).

Variables	Mean ± SD	Minimum – Maximum
Age (years)	22.30± 2.74	[18 – 29]
Height (m)	176.39± 7.28	[1.65 – 1.93]
Weight (kg)	76.32± 11.54	[57.40 – 106.60]
BMI (kg / m²)	24.34 ± 3.02	[19.30 – 29.70]
HR peak (bpm)	156,27 ± 2,32	[152 – 162]

Standard Deviation; Kg = Kilogram; m = meters; Kg/m2 = kilogram/meter2; mmHg = millimeters of mercury; bpm = beats per minute; BMI = body mass index; SBP = systolic blood pressure; DBP = diastolic blood pressure; HR = heart rate.

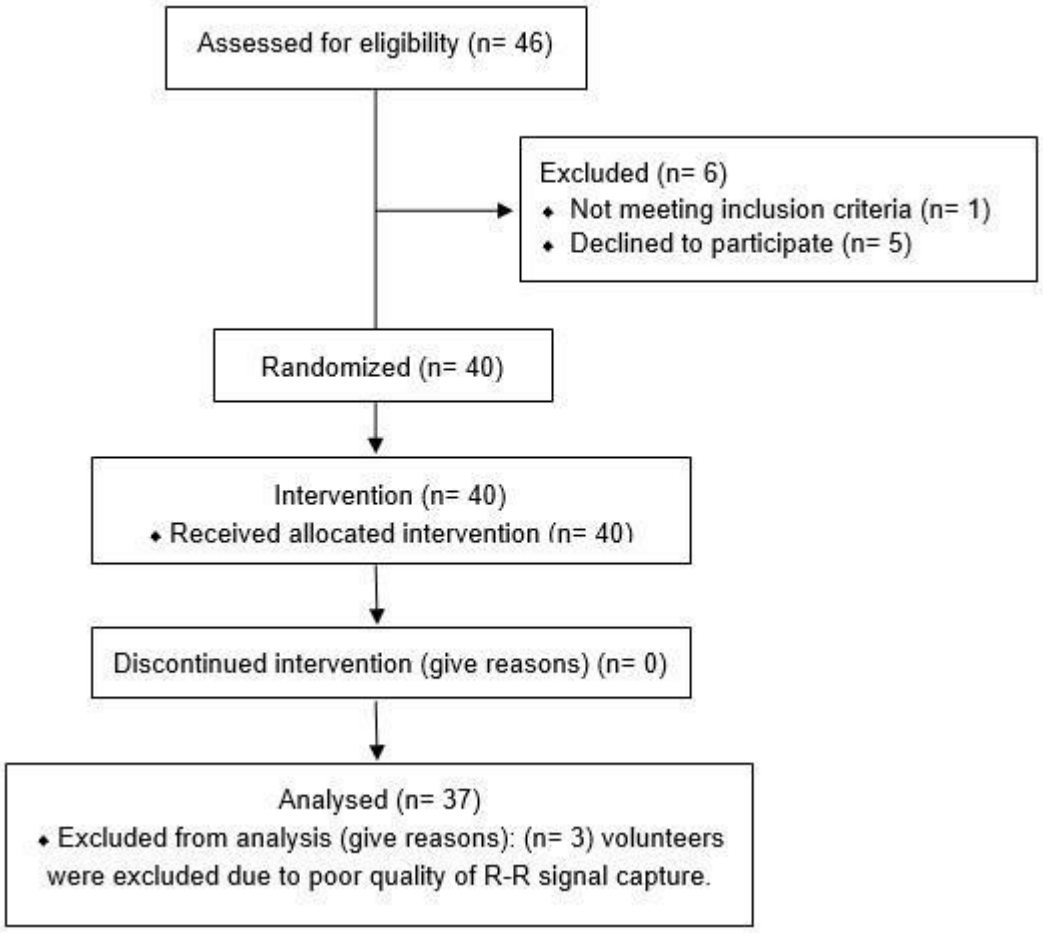

Figure 1. Flow chart of sample loss during the study.

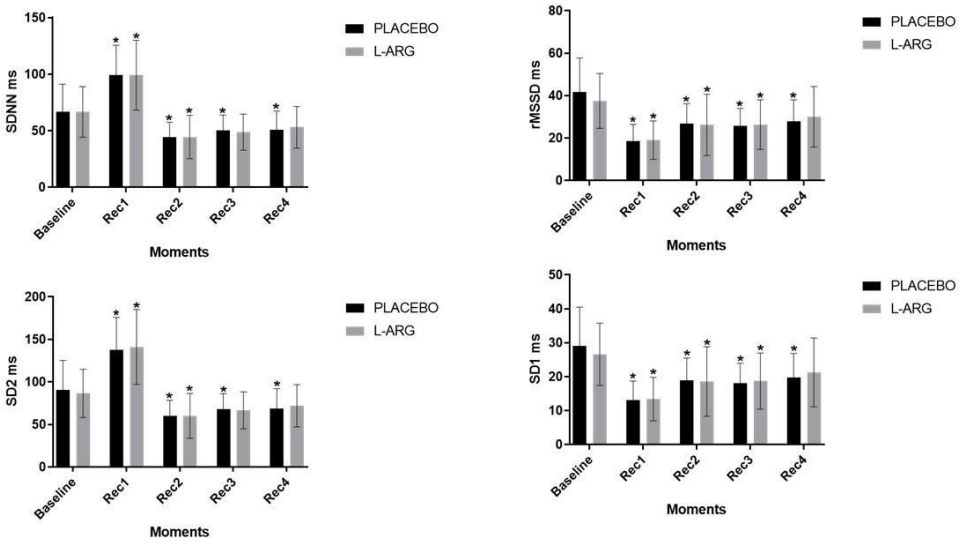

Figure 2. Mean values and their respective standard deviations of indices in the time domain during baseline and recovery (Rec), obtained from the placebo protocol; and L-ARG protocol. **(A) SDNN, (B) rMSSD, (C) SD2 and (D) SD1. Caption:** ms = milliseconds. *Values with significant differences in relation to baseline.

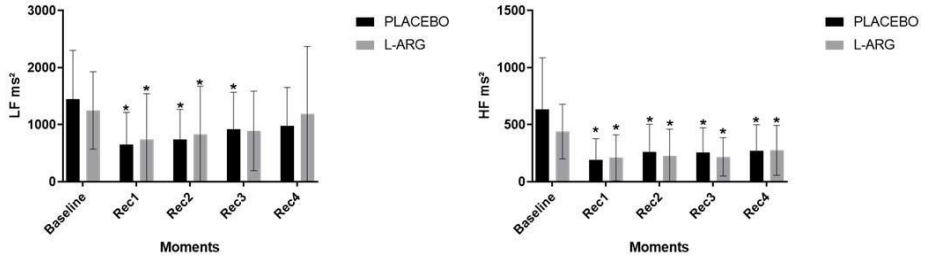

Figure 3. Mean values and their respective standard deviations of indices in the frequency domain during baseline and recovery (Rec), obtained from the placebo protocol; and L-ARG protocol. **(A) LF and (B) HF. Caption:** ms = milliseconds. *Values with significant differences in relation to baseline (P < 0,05).

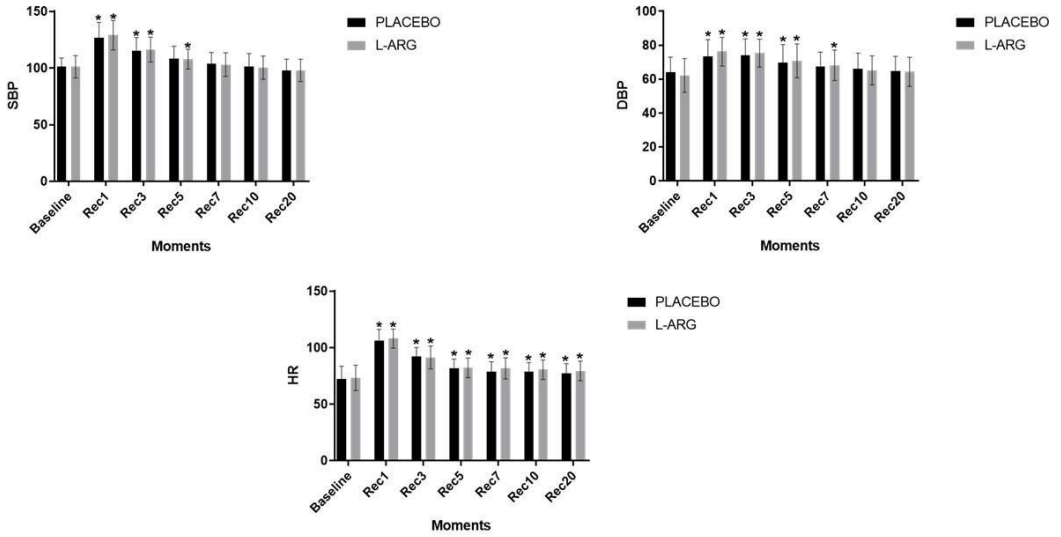

Figure 4. Mean values and their respective standard deviations of heart rate (HR); systolic blood pressure (SBP); diastolic blood pressure (DBP) at baseline and recovery (Rec), obtained from the placebo protocol; and L-ARG protocol. **(A) SBP, (B) DBP and (C) HR.** *Values with significant differences in relation to baseline.

Supplementary material

Supplementary file 1.pdf
Table 1 (Supplementary). Mean, standard deviation, confidence interval and Cohen's d values of HRV indices during the protocols.
Supplementary file 1 shows the mean, standard deviation, confidence interval and Cohen's d values of the HRV indices.

Supplementary file 2.pdf
Table 2 (Supplementary). Mean, standard deviation, confidence interval and Cohen's d values of cardiovascular parameters during the protocols.
Supplementary file 2 shows the mean, standard deviation, confidence interval and Cohen's d values of cardiovascular parameters.

Table 1 (Supplementary). Mean, standard deviation, confidence interval and Cohen's d values of HRV indices during the protocols.

		Moments				
		BASELINE	**REC 1**	**REC 2**	**REC 3**	**REC 4**
SDNN ms	PLACEBO	67.85 ± 24.86 (CI: 59.84-75.86)	**99.28 ± 26.95 (CI: 90.59-107.96) d = 1.27**	**44.33 ± 13.66 (CI: 39.93-48.73) d = 0. 14**	**49.90 ± 13.19 (CI: 45.65-54.15) d = 0.82**	**50.63± 17.16 (CI: 45.10-56.16) d = 0.76**
	L-ARG	64.29 ± 20.28 (CI: 57.75-70.82)	**99.82 ± 31.58 (CI: 89.65-110.00) d= 1.20**	**44.60 ± 19.71 (CI: 38.25-50.96) d= 1.06**	49.05 ± 16.06 (CI: 43.87-54.22) d= 0.92	53.19± 18.59 (CI: 47.20-59.19) d= 0.66
RMSSD ms	PLACEBO	41.83 ± 15.93 (CI: 36.70-46.97)	**18.58 ± 7.86 (CI: 16.05-21.11) d = 1.85**	**26.73 ± 9.43 (CI 23.70-29.77) d = 1.15**	**25.76 ±8.25 (CI: 23.10-28.42) d= 1.27**	**27.90± 10.11 (CI: 24.64-31.16) d= 1.04**
	L-ARG	37.52 ± 12.96 (CI: 33.20-41.84)	**18.99 ± 9.08 (CI: 15.96-22.01) d= 1.66**	**26.23 ± 14.47 (CI: 21.40-31.05) d= 0.82**	**26.36±11.66 (CI: 22.48-30.25) d= 0.91**	30.04± 14.30 (CI: 25.27-34.81) d= 0.15

LF ms²	PLACEBO	1449.85± 855.39 (CI: 1174.23-1725.48)	654.24±560.77 (CI: 473.55-834.93) d = 1.10	743.58±525.66 (CI: 574.20-912.95) d =0.99	923.62±645.36 (CI:715.68-1131.57) d = 0.69	983.13± 670.13 (CI: 767.20-1199.05) d = 0.61
	L-ARG	1252.10±678.11 (CI: 1033.60-244.28)	**743.93± 799.82 (CI: 486.21-1001.64) d= 0.69**	**826.41± 848.13 (CI: 553.13-1099.69) d= 0.55**	891.70±696.60 (CI: 667.25-1116.16) d= 0.52	1190.19±1181.22 (CI: 809.58-1570.80) d= 0.06
HF ms²	PLACEBO	633.29±453.14 (CI:487.28-779.30)	**192.09±185.82 (CI:132.22-251.96) d = 1.27**	**263.24±241.16 (CI:185.53-340.94) d = 1.02**	**253.91±219.48 (CI:183.19-324.63) d = 1.07**	**273.16±226.88 (CI:200.05-346.27) d = 1.01**
	L-ARG	440.33±239.00 (CI:363.32-517.33)	**209.81±201.39 (CI: 144.92-274.70) d= 1.04**	**228.84±232.41 (CI:153.95-303.73) d= 0.90**	**217.80±168.00 (CI:163.66-271.93) d= 1.08**	**275.58±218.93 (CI: 205.03-346.12) d= 0.72**
SD1	PLACEBO	**29.10±11.41 (CI: 25.42-32.77)**	**13.15±5.57 (CI:11.36-14.95) d= 1.78**	**18.85±6.65 (CI:16.71-20.99) d = 1.10**	**18.12±5.85 (CI: 16.24-20.01) d = 1.21**	**19.71±7.17 (CI:17.40-22.02) d = 0.99**

Mean ±	ms	L-ARG	**26.56 ± 9.18** **(CI: 23.61-29.52)**	**13.41±6.40** **(CI: 11.35-15.47)** **d= 1.66**	**18.59 ± 10.25** **(CI: 15.28-21.89)** **d= 0.82**	**18.68 ± 8.27** **(CI: 16.02-21.34)** **d= 0.90**	21.28± 10.13 (CI: 18.02-24.55) d= 0.55
	SD2 ms	PLACEBO	90.56±34.73 (CI: 79.37-101.76)	**137.95±37.91** **(CI: 125.74- 150.17)** **d = 1.30**	**59.85±18.45** **(CI: 53.90-65.79)** **d = 1.10**	**68.10± 18.14** **(CI: 62.25- 73.94)** **d = 0.81**	**68.78±23.42** **(CI: 61.23- 76.32)** **d = 0.74**
		L-ARG	86.52±28.43 (CI: 77.36-95.68)	**141.13 ± 44.08** **(CI: 126.93-155.34)** **d= 1.47**	**60.09± 26.34** **(CI: 51.61-68.58)** **d= 0.96**	66.58±21.80 (CI:59.56-73.61) d= 0.79	71.94±24.78 (CI: 63.96-79.93) d= 0.55

standard deviation; (CI: 95% confidence interval); d= Cohen's d. Values in bold: significant difference between the moments of exercise and rest (P<0.05). [Two-way ANOVA for repeated measures followed by Bonferroni or Dunn post-test]. Caption: REC: recovery; SDNN: Standard Deviation of all normal NN intervals; RMSSD: Root mean square of the successive differences; LF: Low Frequency; HF: Hight Frequency; ms: milliseconds. SD1 and SD2: Standard Deviation

Table 2 (Supplementary). Mean, standard deviation, confidence interval and Cohen's d values of cardiovascular parameters during the protocols.

		Moments						
		BASELINE	**REC1**	**REC3**	**REC5**	**REC7**	**REC10**	**REC20**
HR bpm	PLACEBO	72.43±11.16 (CI:68.97-75.89)	**106.05±10.05 (CI:102.94-109.17) d:3.17**	**92.41±7.64 (CI: 90.04-94.77) d:2.09**	**81.59±8.25 (CI:79.04-84.15) d:0.93**	**78.57±8.95 (CI:75.79-81.34) d:0.61**	**78.92±7.83 (CI:76.49-81.34) d:0.67**	**77.46±8.29 (CI:74.89-80.03) d:0.51**
	L-ARG	73.19±11.16 (CI:69.62-76.76)	**107.86±8.44 (CI:105.16-110.57) d:3.50**	**91.30±10.08 (CI:88.08-94.52) d:1.70**	**82.08±8.61 (CI:79.33-84.83) d:0.89**	**81.70±9.33 (CI:78.72-84.69) d:0.83**	**80.51±8.64 (CI:77.75-83.28) d: 0.73**	**79.41±8.66 (CI:76.63-82.18) d: 0.62**
SBP mmHg	PLACEBO	101.35±7.41 (CI:99.05-103.65)	**126.49±13.80 (CI:122.2-130.76) d: 2.27**	**115.14±11.77 (CI:111.49-118.78) d:1.40**	108.38±10.78 (CI:105.04-111.72) d: 0.76	103.78±9.96 (CI:100.70-106.87) d: 0.28	101.35±11.43 (CI:97.81-104.89) d: 0.00	97.84±9.90 (CI:94.7-100.91) d: 0.40
	L-ARG	101.08±9.80 (CI:98.04-104.12)	**128.92±13.11 (CI:124.86-132.98) d: 2.41**	**116.22±10.99 (CI:112.81-119.62) d: 1.45**	**107.84±8.74 (CI:105.13-110.55) d: 0.73**	102.97±10.36 (CI:99.76-106.18) d: 0.19	100.27±10.26 (CI:97.09-103.45) d:0.08	97.84±9.90 (CI:94.77-100.91) d:0.33
DBP mmHg	PLACEBO	64.05±8.84 (CI:61.31-66.79)	**73.24±10.15 (CI:70.10-76.39) d:0.97**	**74.05±9.71 (CI:71.04-77.06) d:1.08**	**69.73±10.78 (CI:66.39-73.07) d:0.58**	67.30±8.59 (CI:64.64-69.96) d:0.37	66.22±9.11 (CI:63.39-69.04) d:0.24	64.86±8.58 (CI:62.21-67.52) d:0.09
	L-ARG	62.16±9.90 (CI:59.09-65.23)	**76.22±8.50 (CI:73.58-78.85) d:1.52**	**75.41±8.25 (CI:72.85-77.96) d:1.45**	**70.81±9.97 (CI:67.72-73.90) d:0.87**	**68.11±8.96 (CI:65.33-70.88) d:0.63**	65.14±8.58 (CI:62.48-67.79) d:0.32	64.32±8.56 (CI:61.67-66.98) d:0.23

Mean±Standard deviation; (CI:95% confidence interval of mean); d= Cohen's d. Values in bold: significant difference between the moments of exercise and rest (P<0.05). [Two-way ANOVA for repeated measures followed by Bonferroni or Dunn post-test]. Caption: REC: recovery; HR: heart rate; SBP: systolic blood pressure; DBP: diastolic blo507d pressure; bpm: beats per minute; mmHg: millimetres per mercury.

Artigo 2

L-Arginine Supplementation Did Not Impact the Rapid Recovery of Cardiovascular and Autonomic Function Following Exercise in Physically Active Healthy Males: A Triple-Blind Randomised Placebo-Controlled Crossover Trial

ABSTRACT

Background and Aims: Post-exercise recovery strategies include massage, low-intensity active exercise, thermal contrast, hydration, and nutritional and herbal approaches. These strategies aim to accelerate recovery, enhance performance, and optimise the physical training process. L-arginine (L-ARG) is the physiological precursor of nitric oxide (NO), a crucial mediator of vasodilation and the inhibition of platelet aggregation. A previous study reported that L-ARG supplementation could significantly reduce systolic blood pressure (SBP) and diastolic blood pressure (DBP). This study aimed to investigate the effects of L-ARG on autonomic and cardiovascular recovery immediately following submaximal exercise. Methods and Results: Thirty-two healthy individuals were subjected to two experimental protocols. The first protocol included 60 min of rest, a treadmill warm-up, and load increments until reaching 80% of their maximum HR. Before this protocol, the subjects consumed 3 g of starch (placebo protocol). The second protocol was identical, but the subjects consumed 3 g of L-ARG. Heart rate recovery (HRR), heart rate variability (HRV), and blood pressure (BP) responses were assessed. No significant differences in HRR were found ($p = 0.944$) regarding the root mean square of successive differences in the RR interval (RMSSD30) of HRV ($p = 0.562$) or the BP responses (mean arterial pressure (MAP), $p = 0.687$; pulse pressure (PP), $p = 0.929$) between the protocols. Conclusions: L-ARG supplementation did not significantly alter immediate post-exercise autonomic recovery in healthy males.

Keywords: arginine; exercise; autonomic nervous system; nitric oxide; and post-exercise recovery techniques.

1. Introduction

Studying strategies that optimise post-exercise recovery is essential to prevent injuries, improve workout performance, and reduce the risk of adverse cardiac events. Effective recovery methods contribute to health and well-being by ensuring a quick and efficient recovery, minimizing physical stress, and improving the ability to perform physical activities safely [1].

Exercise acts as a stimulus to the body, eliciting a response from the autonomic nervous system (ANS). During physical exertion, there is a reduction in parasympathetic activity and an augmentation in sympathetic activity to meet the metabolic demands. Following the cessation of exercise, autonomic recovery is characterised by a significant decrease in heart rate (HR) [2]. Thus, heart rate recovery (HRR) can be a non-invasive tool to evaluate the function of the ANS [3] and HRV [4,5].

In this context, and to monitor the subjects' health status, the heart rate (HR) variability (HRV)—a measure of heartbeat oscillation that reflects autonomic nervous system function [6,7]—has been shown to provide valuable clinical insights into the health status [8–11]. HRV has been investigated for many years, with growing interest in understanding its mechanisms and clinical applications in various diseases. The clinical significance of HRV was first recognized when it demonstrated its value in monitoring foetal distress. Later, an association was found between reduced HRV and an elevated risk of mortality following acute myocardial infarction, supporting the use of HRV as a strong and independent predictor of mortality after acute myocardial infarction [12]. Therefore, using HRV to assess quality of life enhances our understanding of patients' health.

The pioneering study by Cole et al. (2000) [5] analysed the HRR after a submaximal exercise test as a predictor of mortality in healthy adults. In a cohort of 5234 participants without cardiovascular disease, abnormal HRR (defined as ≤42 bpm within the first two minutes of recovery) was associated with a higher rate of death. During 12 years of follow-up, abnormal HRR remained a significant predictor of mortality, even after adjusting for various risk factors, with an adjusted relative risk of 1.55 (CI, 1.22 to 1.98), thereby highlighting the clinical importance of HRR in submaximal exercise testing.

Post-exercise recovery strategies include techniques such as massage, low-intensity active exercise, thermal contrast (hot–cold) [1], and hydration [13], as well as nutritional and herbal approaches [14]. These strategies aim to accelerate recovery, enhance performance, and optimise the physical training process [1,4]. Dietary supplements, especially those containing L-ARG, are frequently recommended to help boost energy levels and reduce muscle fatigue

[15,16]. L-ARG is the physiological precursor of NO, a crucial mediator of vasodilation and the inhibition of platelet aggregation through the enhancement of cyclic guanosine monophosphate (GMP) formation [17].

Shiraseb et al. (2022) [18] reported that L-ARG supplementation can significantly reduce the SBP and DBP. Their meta-analysis, which included 22 studies, revealed a weighted mean reduction of 6.40 mmHg in the SBP and 2.64 mmHg in the DBP. Subgroup analyses indicated that L-arginine supplementation consistently lowered the blood pressure, regardless of the baseline blood pressure category (normal, elevated, or hyperten-sion), body mass index (normal, overweight, or obese), or health status.

Furthermore, NO is associated with more efficient autonomic modulation in healthy adults. In the study by Chowdhary et al. (2000) [19], the authors investigated the impact of NO on baroreflex HR control. Their study involved 26 male volunteers and assessed HRV and baroreflex sensitivity during endogenous NO production inhibition with L-NG-monomethylarginine (L-NMMA) and exogenous NO administration with sodium nitroprusside. The results demonstrated that NO enhances cardiac vagal control, as evidenced by the preservation of high-frequency HRV indices during NO administration.

Moreover, there is a body of research examining the effects of L-arginine on cardiovascular and autonomic responses, which has yielded a range of findings. For instance, a study by Streeter et al. in 2019 [29] specifically evaluated L-arginine's influence on recovery following resistance exercise, concluding that it did not significantly improve recovery metrics. This study highlights a particular context in which L-arginine supplementation might not provide the expected benefits. In addition, other studies have explored L-arginine's broader impact on athletic performance, primarily due to its crucial role in the synthesis of nitric oxide. Nitric oxide plays a vital role in vasodilation, potentially enhancing blood flow and oxygen delivery to the muscles, which is critical during and after physical exertion.

Another study [21] examined the effects of citrulline malate (CM) supplementation on CrossFit® performance and the cardiovascular response. Although CM did not significantly improve the workout rounds completed, it increased the time in higher-heart-rate zones, potentially enhancing the aerobic capacity. No significant impact was found on the post-exercise recovery time. Additionally, others [22] found that pre-exercise L-citrulline supplementation in Yili speed-racing horses improved their performance, reduced their post-race lactate levels, accelerated their recovery, and enhanced their antioxidant capacity. Supplementation also increased plasma citrulline, arginine, and other beneficial markers, suggesting positive physiological and biochemical effects during high-intensity exercise.

Although the academic literature frequently focuses on the effects of L-ARG supplementation on performance and exercise outcomes, this study aimed to investigate the im-pact of L-ARG on autonomic and cardiovascular recovery immediately following sub-maximal exercise. This study provides insights into the potential reduction in acute risks post-exercise. It explores interventions that could guide healthcare professionals on the use of L-ARG in healthy individuals and inform future research exploring its effects on various health conditions across different populations.

2. Methods

2.1. Ethical Aspects and Population

The Research Ethics Committee of the Faculty of Philosophy and Sciences at Universidade Estadual Paulista "Júlio de Mesquita Filho" (FFC/UNESP) approved the study procedures (Approval Number: 4.761.413). All volunteers provided informed consent and were thoroughly informed about the study's methods and objectives. Additionally, the study was registered with the Clinical Trials Network (clinicaltrials.com, identification code NCT06405477).

Volunteers were recruited through social media and posters displayed throughout the university in Presidente Prudente, São Paulo, Brazil. Those who expressed interest participated in an interview and were asked to complete the IPAQ [23] questionnaire. Participants who met the inclusion criteria were eligible to proceed to the protocol sessions. In this triple-blinded, randomised, placebo-controlled trial, healthy male participants aged 18 to 30, with a body mass index (BMI) ranging from 18.5 to 29.9 kg/m2 and who were moderate physically active as determined by the International Physical Activity Questionnaire (IPAQ), were enrolled. Based on the IPAQ, we included volunteers who engaged in vigorous activity for at least 20 min per day on three or more days or in moderate-intensity activity or walking for at least 30 min per day on five or more days.

Individuals who smoked or struggled with alcohol dependence, as well as those exhibiting specific characteristics that could compromise the assessment of autonomic modulation and exercise participation, were excluded from the study. This included individuals with cardiopulmonary, neurological, or metabolic disorders; those with abnormal hemodynamic responses during exercise; and individuals with orthopaedic issues that would hinder their ability to complete the experimental procedure. Furthermore, volunteers diagnosed with COVID-19 within the past 24 weeks, those showing symptoms of the disease, or individuals who had been in contact with confirmed COVID-19 cases in the preceding 14 days were also not included.

2.2. Protocols

The participants were instructed to avoid alcoholic beverages and foods containing caffeine for 12 h before each phase of the experimental protocol. They were also advised to have a light meal, drink 500 mL of water two hours before the session, and refrain from in-tense physical activity the day before. The experimental procedures were organised into two protocols, with a minimum interval of 48 h between them. Before both protocols, body measurements were taken. These sessions took place between 5:00 p.m. and 9:00 p.m. to control circadian rhythm effects, in a room maintained at a temperature of 23 °C to 26 °C and with humidity levels between 60% and 70% [21]. The two protocols were randomised to reduce the risk of bias.

Following the randomisation process, the participants took six capsules containing either 3 g of starch (placebo protocol) or 3 g of L-arginine (L-arginine protocol). To preserve the blinding of the study, the bottles and their contents were identical in colour and size. Neither the volunteers nor the researchers were aware of the contents of the capsules during the protocols. The order of the protocols was determined using a randomisation process conducted through the "Research Randomizer" website (www.randomizer.org Accessed February 16th, 2024).

Before commencing the experimental procedures, the participants visited the laboratory for body measurements. Their weight was recorded using a digital scale (Welmy W 200/5, Sao Paulo, Brazil), and their height was measured with a stadiometer (ES 2020-Sanny, Sao Paulo, Brazil). Those who met the specified inclusion criteria for the study proceeded to the next research phase.

During the protocols, the volunteers were equipped with a heart rate monitor (Polar RS800CX,Kempele, Finland) to track their heart rates continuously. After the equipment was correctly set up, they were instructed to remain seated for 10 min. Their systolic blood pressure (SBP) and diastolic blood pressure (DBP) were recorded at the end of this period. Following this, the volunteers consumed the substance assigned to them through randomisation and waited for 60 min.

After adhering to the established procedures, the volunteers began their routine with a warm-up on a treadmill, maintaining a speed of 5 km/h for the first 3 min. Following this, the speed was incrementally increased by 1 km/h every 2 min until they reached 80% of their estimated maximum heart rate, calculated using the formula $208 - (0.7 * \text{age})$. The maximum heart rate projected according to age was determined using the Tanaka formula [24].

Upon completing the exercise, the volunteers remained on the treadmill for 3 min for recovery while being monitored closely.

2.3. Outcomes

Heart rate recovery (HRR) was used as an indirect measure to assess the reactivation of the parasympathetic nervous system. To determine HRR, the heart rate (HR) was recorded at four specific intervals: at the end of the exercise (denoted as HRpeak) and at the first (HR1), second (HR2), and third (HR3) minutes of recovery. The values were calculated by taking the arithmetic mean of five consecutive heartbeats, including two beats before and two beats after the HR measurements at HRpeak, HR1, HR2, and HR3.

After recording these values, the differences between HRpeak and HR1, HR2, and HR3 were calculated as follows: HRpeak − HR1 = HRR1; HRpeak − HR2 = HRR2; HRpeak − HR3 = HRR3.

The validated series of RR intervals recorded by the Polar RS800CX heart rate monitor (Polar Electro,Kempele, Finland) was used to analyse the heart rate variability (HRV). The RR interval series underwent digital filtration using the Polar Pro Trainer software (Ver-sion 5.0, Polar Inc., Kempele, Finland), with only those sets containing more than 95% sinus beats in-cluded in the analysis. Additionally, the RR interval series was visually in-spected to identify any artefacts or arrhythmias that could affect the HRV analysis.

In analysing the RMSSD index, we focused on the first two minutes of the recovery period, divided into four 30s intervals: M1, M2, M3, and M4. This methodology, proposed by Goldberger et al. and utilised in several other studies, is effective in assessing the reactivation of parasympathetic tone following exercise [25].

Systolic blood pressure (SBP) and diastolic blood pressure (DBP) measurements were taken indirectly using a stethoscope (3M Littmann Master Cardiology, Saint Paul, MN, USA) and an aneroid sphygmomanometer (Welch Allyn Tycos, New York, NY, USA) on the partic-ipant's left arm, following a previously described methodology. The pulse pressure (PP) and mean arterial pressure (MAP) were assessed as secondary outcomes.

The pulse pressure (PP) was calculated as the difference between the systolic blood pressure (SBP) and diastolic blood pressure (DBP) using the formula PP = SBP − DBP. The mean arterial pressure (MAP) was then determined by adding one-third of the pulse pressure (PP) to the diastolic blood pressure (DBP) using the formula MAP = (1/3) PP + DBP. For analysis, the baseline values were compared with the measurements taken at the first (1 min) and third (3 min) minutes of recovery.

2.4. Data Analysis

Descriptive statistics characterised the sample for data analysis, presenting the results as means, standard deviations, minimum and maximum values, absolute counts, and percentages. The normality of the data was assessed using the Shapiro–Wilk test. A two-way ANOVA with repeated measures was conducted to compare the HRR, root mean square of successive differences (RMSSD30), mean arterial pressure (MAP), and pulse pressure (PP) across the different protocols and time points, as well as the interaction between these factors. Sphericity violations were evaluated using the Mauchly test, and the Greenhouse–Geisser correction was applied when necessary.

Bonferroni's post hoc test was used for parametric distributions, while Dunn's post hoc test was used for non-parametric distributions, to analyse moments within the same protocol. The partial eta-squared effect size (P) was calculated for the ANOVA results, with the effect sizes categorised as follows: small effect (≤0.05), medium effect (between 0.06 and 0.13), and large effect (≥0.14). Additionally, Cohen's d effect size was calculated for com-parisons between time points, classified as small effects (<0.50), medium effects (between 0.50 and 0.70), large effects (between 0.80 and 1.20), and very large effects (≥1.30). The level of statistical significance was set at 5%. Analyses were performed using IBM SPSS Statis-tics, version 22.0 and GraphPad Instat, version 3.01, 1998 (GraphPad Software, Inc., San Diego, CA, USA).

A pilot project involving 13 participants provided the data necessary for the calculation of the sample size using the RMSSD index, which indicated the highest minimum sample size requirement. With a standard deviation of 5.03 ms and a minimum difference of 4.35 ms between the groups, alongside an alpha risk of 0.05 and power of 0.80, the re-quired sample size was determined to be 22 individuals. An additional 10% was included to account for potential dropouts, resulting in a minimum requirement of 24 individuals per group. This calculation used version 3.1.9.7 of GPower (Heinrich-Heine-Universität, Düsseldorf, Germany). A statis- tical analysis was carried out by a researcher who was unaware of the protocol identities.

3. Results

Table 1 presents the anthropometric characteristics of the 32 participants, while Figure 1 provides a flow diagram illustrating the progress of all participants throughout the study.

Table 1. Characteristics of participants (*n* = 32).

Variable	Mean ± SD	Minimum–Maximum
Age (years)	22.13 ± 2.79	[18–29]
Height (m)	176.45 ± 7.04	[1.65–1.93]
Weight (kg)	77.72 ± 12.47	[57.40–106.60]
BMI (kg/m^2)	24.73 ± 3.26	[19.50–29.80]

SD = standard deviation; Kg = kilogram; m = metres; mmHg = millimetres of mercury; bpm = beats per minute; BMI = body mass index; SBP = systolic blood pressure; DBP = diastolic blood pressure; HR = heart rate.

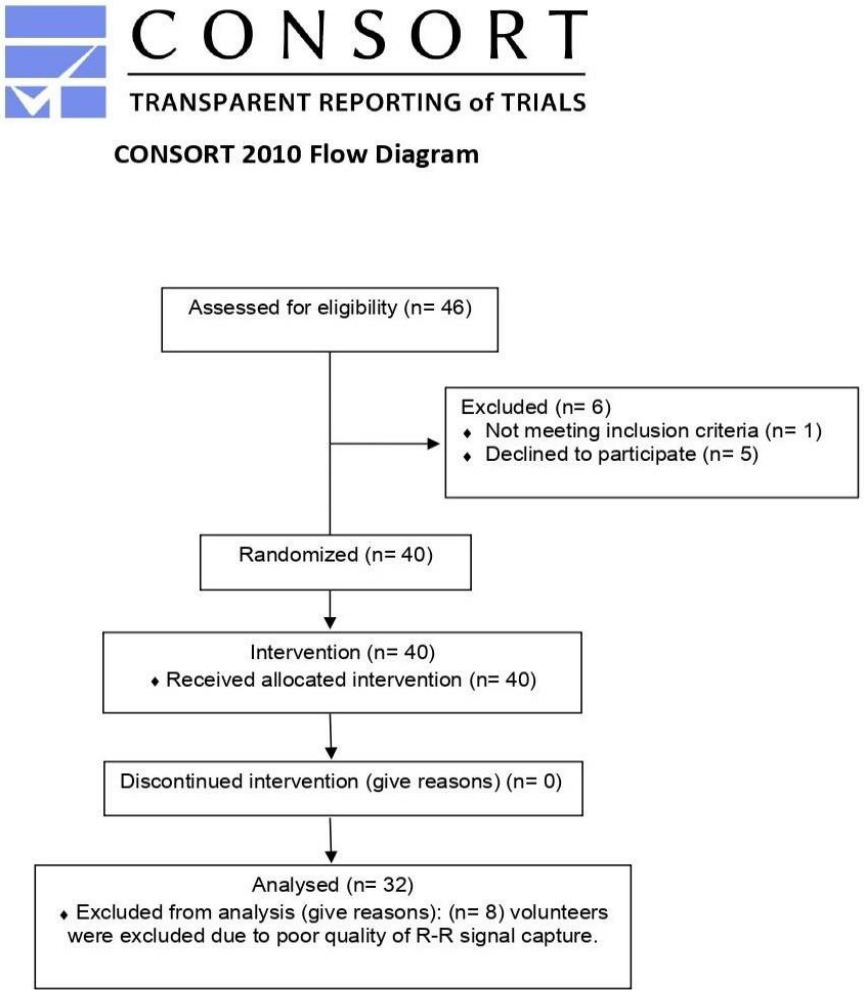

Figure 1. Flowchart diagram.

The HRR values during the recovery period were compared to those recorded during exercise. No significant differences were observed between the protocols (p = 0.944; P = 0.001, power: 0.051) or in the interactions of the moments versus protocols (p = 0.530; P = 0.011, power: 1). However, significant differences were identified between the moments themselves (p = 0.001; P = 0.964, power: 1), as depicted in Figure 2. The mean HRR values, along with their respective standard deviations, during exercise and recovery at the first and second minutes are presented in Table S1 (Supplementary Materials).

For the RMSSD30 index, no significant differences were found between the protocols (p = 0.562; P = 0.005, power: 0.089) or in the interactions of the moments versus protocols (p = 0.766; P = 0.005, power: 1). However, significant differences were observed between the moments (p = 0.001; P = 0.679, power: 1) (Figure 2). The mean values, along with their standard deviations, of the RMSSD30 index obtained during exercise and immediate recovery are available in Table S2 (Supplementary Materials).

Regarding the secondary outcomes of the mean arterial pressure (MAP) and pulse pressure (PP), no significant differences were observed between the protocols (MAP: p = 0.687, P = 0.003, power: 0.068; PP: p = 0.929, P = 0.001, power: 0.051) or in the interactions of the moments versus protocols (MAP: p = 0.390, P = 0.015, power: 1; PP: p = 0.961, P = 0.001, power: 1). However, significant differences were found between the moments for both the MAP (p = 0.001, P = 0.825, power: 1) and PP (p = 0.001, P = 0.497, power: 1) (Figure 3). The values for these outcomes, recorded during exercise and recovery at the first and third minutes, are provided in Table S3 (Supplementary Materials).

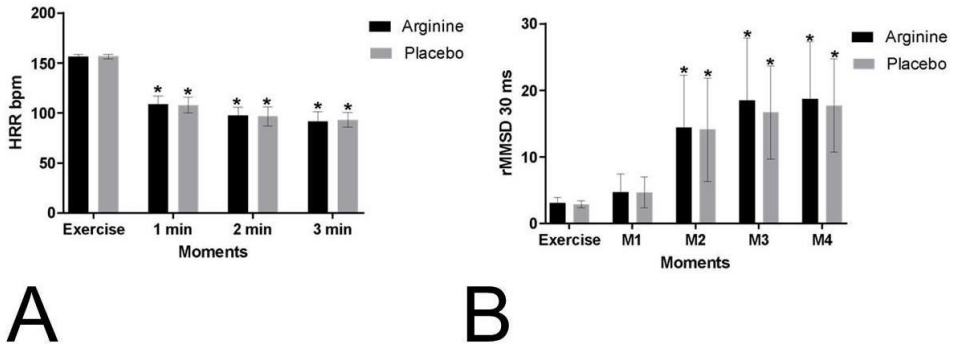

Figure 2. Mean values and their respective standard deviations for heart rate recovery (HRR) (**A**) and root mean square of successive RR interval differences analysed in 30 s

intervals (RMSSD30) (**B**) during exercise and recovery obtained from the placebo protocol (placebo) and the L-arginine protocol (arginine) are presented. Caption: 1 min: first minute of recovery; 2 min: second minute of recovery; bpm = beats per minute; M1 = 0–30 s, M2 = 30–60 s, M3 = 60–90 s, M4 = 90–120 s of recovery; ms = milliseconds. * Values indicate significant differences compared to exercise ($p < 0.05$).

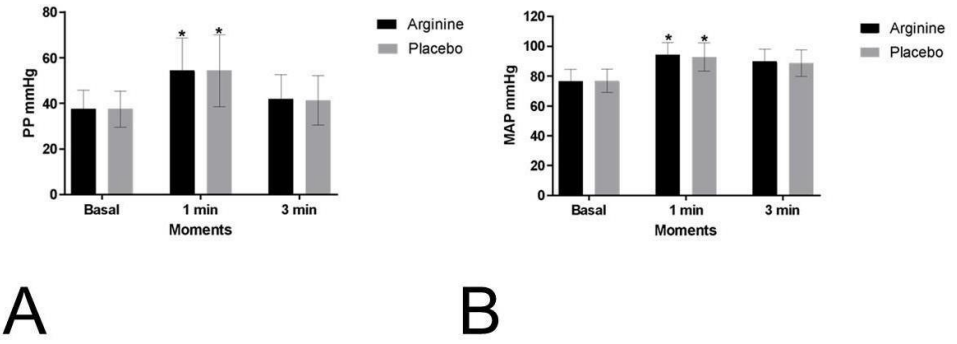

Figure 3. Mean values and their respective standard deviations for pulse pressure (PP) (**A**) and mean arterial pressure (MAP) (**B**) at baseline and during recovery are presented for the placebo protocol (placebo) and the L-arginine protocol (arginine). Caption: 1 min: first minute of recovery; 3 min: third minute of recovery; mmHg = millimetres of mercury. * Values indicate significant differences compared to baseline ($p < 0.05$).

4. Discussion

The current study aimed to assess the effects of L-ARG intake on immediate cardiovascular recovery following a submaximal exercise test in healthy adult males. Our findings suggest that the consumption of this substance did not impact immediate parasympathetic reactivation and post-exercise BP recovery.

The decline in HR following exercise is a crucial indicator of autonomic function. A rapid reduction in the post-exercise HR is linked to a favourable cardiovascular prognosis, indicating a healthy autonomic response [26]. Cole et al. [5] defined an abnormal HRR in the first 1 to 3 min as a decrease of 12 beats per minute or less from the peak HR. HRR in the first minute after exercise is considered a predictor of mortality [24], primarily reflecting parasympathetic reactivation [27].

During exercise, the cardiovascular system must adjust to meet the muscles' metabolic demands and the skin's thermoregulatory needs while keeping the BP stable. According to the literature, a prominent model suggests that signals from the brain at the onset of exercise prompt the baroreflex to elevate, leading to a swift increase in the heart rate by reducing parasympathetic activity. Muscle mechanoreceptors and increased venous return influence this initial response. As the exercise intensity increases, the parasympathetic activity decreases. In contrast,

the sympathetic activity rises, shifting from parasympathetic control at rest to sympathetic control at high intensities, further amplified by sympathoadrenal activation [2].

During physical exercise, HR and BP regulation undergo significant changes. When the cellular metabolism increases, activating the metaboreflex, triggered by the accumulation of metabolic by-products, it reduces the baroreflex function in the brainstem. This activation stimulates nerve fibres, resulting in heightened sympathetic activity and increasing the HR, cardiac output, and BP and vasoconstriction in non-active muscles. Following exercise, these effects are anticipated to diminish rapidly due to the reactivation of parasympathetic activity [28].

Our study examined the physiological behaviour of the heart rate (HR), revealing a significant decrease throughout the recovery period, with a notable reduction occurring in the first minute of passive recovery across all protocols (mean difference in HR during exercise: PLA = 48.85 bpm; L-ARG = 47.41 bpm). In analysing the behaviour of the RMSSD30 index, no significant differences were observed between the protocols, indicating that the ingestion of 3 g of L-ARG did not influence post-exercise parasympathetic reactivation.

Our data are supported by Streeter et al. (2019) [29], who investigated recovery following resistance exercise. The authors [29] conducted the only published study evaluating the effects of L-ARG on autonomic modulation post-exercise and found no influence of this substance on these parameters [29]. This randomised, crossover, double-blind, placebo-controlled clinical trial involved 30 physically active participants (15 men and 15 women). After ingesting either 3 g of L-ARG or 3 g of a placebo, the participants performed five sets of elbow extension–flexion exercises. Significant time effects on autonomic modulation were observed, as indicated by the RMSSD and percentage of successive RR intervals that differed by more than 50 milliseconds (PNN50); however, no treatment or interaction effects were detected. No significant differences were found between the basal values and recovery time for the HR and BP outcomes. Consequently, the study concluded that L-ARG did not affect ANS behaviour.

Despite these similar findings, there are noteworthy methodological differences between the studies. Streeter et al. [29] evaluated a 10 min recovery period without segmenting it to observe and compare the effects over time more effectively, and the recovery values were compared to the basal values. A key difference in the exercise protocol was that it consisted of five sets of 10 maximal isokinetic elbow extensions at 90 degrees per second, with 30 s rests between sets.

We also did not detect significant effects of L-ARG ingestion on post-exercise BP recovery. Across all protocols, this study observed the physiological response of the BP, noting a substantial increase in PP at 1 min of recovery, with the values returning to baseline by 3 min

and the MAP not recovering in any protocol. This suggests that, in healthy individuals, endogenous NO production may sufficiently mitigate the effects of 3 g L-ARG consumption [10]. This finding could explain the discrepancy between the results of our clinical trial and those reported in the systematic review by Porto et al. (2021) [30].

In this context, Porto et al. (2021) [30] conducted a systematic review of the effects of L-arginine supplementation on hypertensive adults post-exercise, involving a sample of 60 individuals. Their results indicated a reduction in post-exercise SBP by 5.04 mmHg and DBP by 2.96 mmHg in the L-ARG group compared to the placebo group. It is important to note key differences between these studies. The systematic review included hypertensive men and women over the age of 50. Ageing is directly associated with reduced endothelial activity, increasing the risk of cardiovascular conditions such as hypertension. The decreased availability of NO impairs the body's ability to maintain endothelial homeostasis [30]. Theoretically, L-arginine, the primary substrate for NO production, could help to address this deficiency [10].

Our study highlights several critical methodological points that warrant attention. Notably, analyses of the plasma nitrate and nitrite concentrations were not conducted, and the low dose of L-ARG may have been insufficient to elicit the expected positive effects of this protocol. A significant limitation of our study was the absence of a standardised ergonomic assessment to measure the cardiopulmonary capacity, specifically VO_2 max, which impacted the precise characterisation of the physical capacity levels of our volunteers. However, our sample size calculation indicated that the number of volunteers included was adequate. The selection process adhered to stringent exclusion criteria, enhancing the reliability of our data. We advise caution in generalising our findings to other groups and recommend that future research involve diverse populations, various doses, and extended treatment durations.

Furthermore, individuals show variability in autonomic recovery post-exercise, particularly in their heart rate recovery (HRR) and blood pressure (BP) responses. While some people return to near-baseline levels within 1–3 min, others may require more time due to intrinsic factors [31]. Recognising this individual variability is crucial in interpreting autonomic recovery results, as quicker recovery generally suggests better cardiovascular and autonomic function. In contrast, slower recovery may require closer monitoring or training adjustments [26].

5. Conclusions

L-ARG supplementation did not significantly change immediate post-exercise autonomic recovery among healthy men.

Supplementary Materials: The following supporting information can be downloaded at: www.mdpi.com/xxx/s1, Table S1. The behaviour of HRR during the protocols; Table S2. The be-haviour of RMSSD30 during the protocols; Table S3. The behaviour of PP and MAP during the protocols.

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Table S1. The behaviour of HRR during the protocols

Variable	Protocol	Exercise	1 min	2 min	3 min	Cohen d'
	L-ARG	156,53±2,27	**109,12±7,90**	**97,62±8,17**	**91,68±9,70**	1 min= 8,16
		(157,34-155,71)	**(111,97-106,27)**	**(100,56-94,67)**	**(95,17-88,18)**	2 min= 9,83
HRR			**[47,41]**	**[58,91]**	**[64,84]**	3 min= 9,21
bpm	PLA	156,74±2,24	**107,89±7,89**	**96,74±9,68**	**93,17±7,28**	1 min= 8,42
		(157,51-155,96)	**(110,63-105,16)**	**(100,09-93,39)**	**(95,69-90,65)**	2 min= 8,54
			[48,85]	**[60]**	**[63,57]**	3 min= 11,80

Mean ± standard deviation; (95% confidence interval); [difference relative to exercise]. Cohen d'= exercise vs. post-exercise recovery (1 min, 2 min and 3 min). Caption= 1 min= first minute of recovery; 2 min= second minute of recovery; HRR= heart rate recovery; bpm= beats per minute; L-ARG= L-arginine protocol; PLA= Placebo protocol. Values in **bold** = significant difference between exercise and rest moments ($P < 0,05$), two-way ANOVA for repeated measures followed by Bonferroni post-test.

Table 2. The behaviour of $RMSSD_{30}$ during the protocols.

Indice	Protocol	Moments					
		Exercise	**M1**	**M2**	**M3**	**M4**	**Cohen d'**
$RMSSD_{30}$ ms	L-ARG	3,09±0,86 (3,39-2,79)	4,71±2,72 (5,66-3,77)	**14,41±7,87 (17,14-11,68)**	**18,51±9,35 (21,75-15,27)**	**18,71±8,59 (21,68-15,73)**	M1= 0,80 M2= 2,02 M3= 2,32 M4= 2,56
	PLA	2,88±0,54 (3,07-2,70)	4,69±2,34 (5,50-3,88)	**14,11±7,77 (16,80-11,42)**	**16,70±7,00 (19,12-14,28)**	**17,74±7,00 (20,17-15,31)**	M1= 1,07 M2= 2,04 M3= 2,78 M4= 2,99

Mean±standard deviation; (95% confidence interval); Cohen d'= exercise vs. post-exercise recovery (M1, M2, M3 and M4). Caption= M1=0–30 seconds (s), M2=30–60 s, M3=60–90 s, M4=90–120 s of recovery; $RMSSD_{30}$= root mean square of successive RR interval differences analyzed in 30-s intervals; ms= milliseconds; L-ARG= L-arginine protocol; PLA= placebo protocol. Values in **bold**= significant difference between the moments of exercise and rest ($P<0,05$), two-way ANOVA for repeated measures followed by Bonferroni or Dunn post-test.

Table S3. The behaviour of PP and MAP during the protocols.

Variables	Protocol	Moments			
		Basal	**1 min**	**3 min**	**Cohen d'**
	L-ARG	37.50±8.29	**54.37±14.34**	41.87±10.73	1 min= 1.44
PP		(59.67-15.32)	**(71.13-37.61)**	(61.70-22.04)	3 min= 0.46
mmHg	PLA	37.50±7.90	**54.37±15.79**	41.25±10.82	1 min= 1.34
		(61.20-13.79)	**(70.56-38.18)**	(61.08-21.41)	3 min= 0.39
	L-ARG	76.56±7.97	**94.37±8.22**	**89.89±8.18**	1 min= 2.20
MAP		(100.26-52.85)	**(116.54-72.20)**	**(100.26-52.85)**	3 min= 1.65
mmHg	PLA	76.87±7.85	**92.81±9.43**	**88.75±8.80**	1 min= 1.84
		(100.58-53.16)	**(113.71-71.90)**	**(110.92-66.57)**	3 min= 1.42

ean±standard deviation; (95% confidence interval); Cohen d'= Basal vs. recovery (1 min and 3 min). Caption: PP: pulse pressure; MAP: mean arterial pressure; L-ARG: L-arginine protocol; PLA: placebo protocol; mmHg: millimeters of mercury; min: minute. Values in **bold**: significant difference between the moments of recovery and exercise ($p < 0.05$). [Two-way ANOVA for repeated measures followed by Bonferroni or Dunn post-test].

Conclusões

I. Os resultados deste estudo demonstraram que a suplementação aguda de L-arginina não influenciou significativamente as respostas da variabilidade da frequência cardíaca nem os parâmetros cardiovasculares entre os protocolos avaliados. Contudo, a ingestão de L-arginina promoveu uma recuperação mais rápida da modulação vagal em comparação ao repouso.

II. A suplementação de L-arginina não influenciou significativamente a recuperação imediata da modulação autonômica e cardiovascular após exercício em homens saudáveis.

III.

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