

Angiotensin-(1-7) oral formulation improves physical performance in mountain bike athletes: a double-blinded crossover study

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Research article

Keywords: exercise, performance, nutrition, cardiovascular/cardiorespiratory

Posted Date: August 18th, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-58840/v1>

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Abstract

Objective

This study examined the effects of HPβ-CD-Ang-(1–7) oral supplement on performance of mountain bike (MTB) athletes.

Methods

Fourteen recreational athletes, involved in training programs for at least one year, participated in this crossover design study. Subjects underwent two days of testing with a seven-day interval. HPβ-CD-Ang-(1–7) (1.75 mg) and HPβCD-Placebo were provided in capsules three hours prior to tests. To determine the safety of the HPβ-CD-Ang-(1–7) formulation associated with physical effort, cardiovascular parameters heart rate (HR) and blood pressure (BP) were analyzed. Physical performance was measured using maximal oxygen uptake (VO_2), total exercise time (TET), mechanical work (MW), mechanical efficiency (ME), and rating of perceived exertion (RPE). Respiratory exchange coefficient (REC), lactate and non-esterified fatty acids (NEFAs) were measured. Maximal incremental tests were performed on a progressively loaded leg cycle ergometer.

Results

There were no significant differences in terms of HR or BP at rest and maximum effort between the Ang-(1–7) and placebo groups. The VO_{2max} showed significant differences ($p = 0.04$). It was higher in the Ang-(1–7) condition (66.15 ml/kg/min) compared to the placebo (60.72 ml/kg/min). This was also observed for TET (Ang-(1–7) 39.10 min vs. placebo 38.14 min; $p = 0.04$), MW (Ang-(1–7) 156.7 vs. placebo 148.2; $p = 0.04$), and at the lowest RPE (Ang-(1–7) vs. placebo; $p = 0.009$). No significant differences were observed for REC, NEFAs, or Lactate.

Conclusion

These results suggest that HPβ-CD-Ang-(1–7) improves the physical performance of MTB recreational athletes and could be a promising supplement.

Introduction

It was recently discovered that the renin-angiotensin system (RAS), traditionally recognized as a system involved in cardiovascular control and modulator of electrolyte balance ¹, plays a key role various physiological responses in skeletal muscle ^{2,3}.

The studies have reinforced the role of this system in skeletal muscle, including improving insulin sensitivity ⁴ and inhibiting muscle atrophy ⁵. The Ang-(1–7) through *Mas* receptor, can influence in performing physical exercises. Recent data showed that the ACE2 / Ang (1–7) / *Mas* axis can modulate physical performance, since the Knockout mice for the angiotensin II converting enzyme (ACE2), an enzyme responsible for the production of angiotensin-(1–7) from Ang II, presented less physical performance and less cardiac adaptation to exercise ⁶.

Furthermore Ang-(1–7) augments the bioavailability of nitric oxide (NO), promoting increased expression and activation of endothelial nitric oxide synthase (eNOS) via the Akt (PKB) protein-dependent signaling pathway, inducing vasodilation ^{7,8}. Seeing that NO is an important mediator of several physiological processes, regulating tissue blood flow, muscle contraction, and mitochondrial biogenesis, Ang-(1–7) may act through this molecule, affecting positively physical performance ⁹.

Latest data from our laboratory have shown, that treatment with the oral formulation of Ang-(1–7) included in HPβ-CD, a cyclodextrin used in the formation of drug inclusion compounds that improves stability, solubility, bioavailability, uniform activation, absorption and gastric protection ¹⁰, prevents exercise-induced muscle damage in young people undergoing a protocol of injury induced by physical exertion (i.e., eccentric exercise). HPβ-CD-Ang-(1–7) was associated with lower perception of acute muscle pain, as well as improvement in maximum strength levels and lower levels of proinflammatory cytokines at 48 and 72 hours after the exercise session ¹¹. This suggests that the formulation HPβ-CD-Ang-(1–7) attenuates muscle damage in addition to maintaining physical performance ¹¹.

Based on the evidence of both animal and human models regarding the physiological effects of Ang-(1–7), the present study aims to evaluate the effects of the HPβ-CD-Ang-(1–7) oral formulation on the physical performance of MTB athletes. Therefore, hypothesis is that the oral treatment with the formulation HPβ-CD-Ang-(1–7) improves the physical performance of MTB athletes and, the HPβ-CD-Ang-(1–7) oral formulation does not change cardiovascular parameters.

Materials And Methods

Ethical aspects

This was an experimental study conducted at the Federal University of Ouro Preto, approved by the Human Research Ethics Committee under protocol no. 25402813.2.1001.5150. To participate in the study, the participants were made aware of the study objectives and the possible benefits and risks. All provided informed written consent.

Patient characteristics

A sample of twenty-one cyclists of both sexes volunteered for this study. Average age was 29 ± 5 years; Average body weight was 71 ± 7 kg; Average height was 1.70 ± 0.07 m; Average body mass index was 24 ± 2 kg/m². The inclusion criteria were involvement in training programs for at least 12 months and completion of at least four sessions of MTB training per week.

Participants were excluded if they had used supplements with potential effects on physical performance, had current injuries or in the last six months, were not willing to abstain from intense exercise 24 hours before the test. Participants were tested at the same time of day for each one of the experimental visits.

Figure 1 - Participants recruitment flow diagram.

Experimental Design

The distribution of the supplement was double-blinded and randomized. Participants received either the formulation of HP β -CD-Placebo or the HP β -CD-Ang-(1-7) (1.75 mg), ensuring that 50% of subjects randomly used HP β -CD-Placebo in the first session and HP β -CD-Ang-(1-7) in the second session or vice versa. The double-blinding and randomization of the experiments was made by a third person who was not involved in data collection. The groups were revealed to other researchers only at the time of data interpretation.

A single dose of the HP β -CD-Placebo or formulation HP β -CD-Ang-(1-7) was given orally, both in capsule form, three hours before the beginning of each test. The capsules were identical in color, size and without flavor, ensuring the blinding of the participants.

During the intervention, we requested that physical training, food intake, and sleep hours be maintained. The volunteers were subjected to two days of tests with a seven-day interval between them, which can be considered a safe washout time. Upon return to the laboratory, we took histories from the subjects to determine if there were adverse reactions and if they had maintained their diet and physical training routines.

Figure 2 - Experimental trial schematic.

Physical exercise protocol using the leg ergometer cycle

The test protocol was based on previous studies with MTB athletes ¹². The tests were performed using a leg ergometer cycle (Biotec 2100, CEFISE Biotechnology), with 10 minutes of warm up and a load of 12.5 W for both genders. After warming up, a continuous progressive load test was performed with an initial load of 25W for women and 37.5W for men. The load was increased by 12.5 W every three minutes for both genders. The test finished when the individual reached voluntary exhaustion or when the rotation could not be kept at 70 rpm. At the end of each stage, heart rate (HR), ratings of perceived exertion (RPE), maximal oxygen uptake (VO_2), and respiratory exchange coefficient (REC) were collected.

Supplementation protocol

The formulation was developed by the Laboratory of Hypertension and Laboratory of Chemical of the University of Federal of Minas Gerais; the details were described previously ¹³. This compound (HP β CD/Ang-(1-7)) was patented (BR 10 2016 0244064).

Oral supplementation with HP β CD-angiotensin-(1-7) (1.75mg) or HP β CD-placebo (1.75mg) was administered 3 hours before the test protocol. The 3h time was pre-established considering the peak action of angiotensin-(1-7) which has a window of action between 2 to 6 hours ¹⁴.

Considering toxicity and adverse responses in humans, the present study used a dose 16 times lower than the dose used in a study conducted in cancer patients (400 μ g/kg)¹⁵ that showed no collateral effects. In addition, a previous study in healthy younger ¹¹ showed protective effects against muscle damage using the same dose without side-effects.

Plasma analysis

Blood samples were collected from the antecubital vein by a skilled phlebotomist using standard technical venipuncture. Approximately 12 ml were collected in vacutainer tubes containing heparin. Immediately after collection, the blood was centrifuged at 3.000

rpm for ten minutes, and the serum transferred to Eppendorf tubes stored at -80 °C. Aliquots were used to measure non-esterified fatty acids (NEFA) and creatine kinase (CK).

Blood lactate levels

Lactate levels were measured in each participant's index finger by placing one drop (5 µl) of blood on a BM-Lactate reagent strip (Roche Diagnostics GmbH, D68298 Mannheim, Germany) and introducing it into the Accutrend® Lactate meter (Roche Diagnostics GmbH, D-68298 Mannheim, Germany). Lactate was measured immediately at the end of each test.

Non-esterified fatty acids (NEFAs)

NEFAs were analyzed according to the specific colorimetric method using the Randox® kit (Randox Laboratories, Oceanside, CA, USA).

Mechanical work and efficiency

To calculate mechanical work and efficiency, the equations above were used ¹⁶. Work is described as follows: $w = time (min) \times load (kg) \times wheel\ circumference (m) \times rotation (rpm)$, in kg.m., while mechanical efficiency was calculated using the equation:

$$mechanical\ efficiency = \frac{useful\ work \left(\frac{mechanical\ work (kg.m)}{perfect\ machine\ constant (kg.m)} \right)}{energy\ expenditure (kcal)} \times 100,$$

where the perfect machine constant is the energy spent by a machine without loss of efficiency to perform work (1 kcal = 426.4 kg.m) in %.

Evaluation of BP and HR

Blood pressure was measured using an aneroid sphygmomanometer (Missouri®, Brasil) and a stethoscope (Missouri®, Brasil) before and soon after completion of the test

protocol. HR was measured using the Polar RS800 (POLAR, Finland) at rest, throughout the stages, and at the maximum effort peak of the physical test.

Evaluation of subjective rating of perceived exertion (RPE)

Participants evaluated their fatigue using the subjective rating of perceived exertion with reference to the Borg Scale ¹⁷. RPE was determined at each stage completed by the volunteer.

Evaluation of maximum oxygen consumption

The aerobic capacity was determined in the HPβ-CD-placebo and HPβ-CD-Ang-(1-7) conditions using open-circuit spirometry on VO2000[®] (VO2000, MedGraphics®, Saint Paul, Minnesota-USA) equipment during the leg ergometer cycle physical test calibrated before each test. The ventilatory equivalent for oxygen (VE/VO₂), ventilatory equivalent for carbon dioxide (VE/VCO₂), and respiratory exchange ratio (RER) were recorded at each stage completed to determine maximal oxygen uptake and respiratory exchange quotient. The average of the final two minutes of the test was used to determine the relative VO₂ ¹⁸.

Statistical analysis

Data normality was tested using the D'Agostino & Pearson test. The data that were normally distributed were compared using the paired *t*-test. For non normal data, the Wilcoxon matched pairs signed rank test was used. Information regarding data normality was added to the figures. Data were expressed as mean ± standard deviation (SD) and the significance level was *p* <0.05 for all tests. For the evaluation throughout the stages of the physical test, a regression using equations of straight line were also used. To run the test, we chose the fitting method of least squares regression without weighting. The null hypothesis was that one curve would fit the two conditions (HPβ-CD-Placebo and HPβ-CD-Ang-(1-7)). The curve comparison method was the extra sum-of-squares F-test and the *p*-

value was fixed at 0.05. The measurement of effect size used was Cohen's d (Cohen's $d = (M2 - M1) / SD_{\text{pooled}}$). The effect size was evaluated based on Cohen's guidelines: small (0.2), medium (0.5), and large (0.8). The lower 95% CI of the mean and the upper 95% CI of the mean for both groups were added to the figure legend.

Results

There were no differences between conditions in terms of resting SBP (HP β CD-placebo = 123.30 ± 12.91 mmHg vs. HP β CD-Ang-(1-7) = 123.30 ± 13.89 mmHg; $p = 0.9845$; Paired t-test) or maximum SBP (HP β CD-placebo = 167.70 ± 22.79 mmHg vs. HP β CD-Ang-(1-7) = 168.50 ± 19.94 mmHg; $p = 0.8802$; Paired t-test) (Fig. 3a). There were no differences between conditions in terms of resting DBP (HP β CD-placebo = 80.71 ± 9.16 mmHg vs. HP β CD-Ang-(1-7) = 76.43 ± 9.28 mmHg; $p = 0.1855$; Wilcoxon) or maximum DBP (HP β CD-placebo = 67.86 ± 8.92 mmHg vs. HP β CD-Ang-(1-7) = 69.29 ± 11.41 mmHg; $p = 0.6714$; Paired t-test) (Fig. 3b). There were no differences between groups in terms of resting MAP (HP β CD-placebo = 95.48 ± 9.21 mmHg vs. HP β CD-Ang-(1-7) = 92.60 ± 9.62 mmHg; $p = 0.3246$; Paired t-test) or maximum MAP (HP β CD-placebo = 100.00 ± 11.61 mmHg vs. HP β CD-Ang-(1-7) = 101.90 ± 11.00 mmHg; $p = 0.5071$; Paired t-test) (Fig. 3c).

The heart rates at rest were as follows: HP β CD-placebo = 58.0 ± 9.34 bpm vs HP β CD-Ang-(1-7) = 55.5 ± 9.06 bpm. In the first stage of the test, heart rate responses were as follows: HP β CD-placebo = 88.61 ± 11.49 bpm vs HP β CD-Ang-(1-7) = 92.28 ± 5.73 bpm (Fig. 3d). In the final stage, heart rates were as follows: HP β CD-placebo = 191.50 ± 10.60 bpm vs HP β CD-Ang-(1-7) = 189.50 ± 17.67 bpm (Fig. 3d).

No differences were observed in terms of respiratory exchange coefficient (Fig. 4a). Note that the columns almost overlap and that a single line explains the behavior of the two groups for respiratory exchange coefficient (Fig. 4a). Interestingly, the ratings of perceived exertion were significantly different. In this case, individuals who received HP β -CD-Ang-(1-7) during the performance of physical test reported a lower perception of effort throughout the entire test and were able to complete the test one stage ahead, at 48 minutes (Fig. 4b).

The total exercise time was significantly higher, approximately one minute, in the treated condition (HP β -CD-Ang-(1-7)) (Fig. 5a), accompanied (as expected) by a higher VO₂max and a higher level of mechanical work (Fig. 5b and c, respectively). No differences were observed in terms of Mechanical efficiency (Fig. 5d).

To determine the possible energy pathway used during physical effort, we evaluated as lactate levels and the concentration of non-esterified fatty acids (NEFA) (Fig. 5e and f, respectively). There were no differences between conditions in terms of these parameters.

Discussion

The present study is the first to show that there is an effect of using an oral formulation of HP β -CD-Ang-(1–7) with relatively low doses of the peptide (1.75 mg) on the physical performance of MTB athletes. The results show a significant difference in TET, MW, maximal oxygen uptake, and lower RPE.

The oral formulation HP β -CD-Ang-(1–7) was not associated with side-effects. There were no differences in HR and BP between conditions at rest or after strenuous exercise compared to the placebo, suggesting its benefits without damage to the cardiovascular system. Clinical studies in healthy subjects, show that the Ang-(1–7) has no effect in blood pressure^{19,20}. The dose used in the present study is low compared to the study that evaluated antiangiogenic effect of Ang-(1–7), the maximum dose that produces toxicity was 700 $\mu\text{g}/\text{kg}$ ¹⁵, our study used a dose sixteen time lower.

It is well established that performance in aerobic endurance exercise is related to maximal oxygen uptake (VO_2max), mechanical economy/efficiency during exercise and lactate threshold²¹. In the present study, the athlete under the influence of the oral formulation HP β -CD-Ang-(1–7) increased the total effort time, mechanical work and consumed a average of $6\text{mlO}_2\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ more oxygen at peak exercise effort. The possible mechanisms involved in increased physical performance may involve effects of Ang-(1–7) such as vasodilation, increased blood flow and glucose uptake in skeletal muscles.

The vasodilatory effects of Ang-(1–7) were observed in arterioles adipose and atrial of patients²². Intra-brachial infusion of the Ang-(1–7) increased forearm blood flow in healthy and hypertensive subjects²³, reduces Ang II-induced vasoconstriction in mammary arteries of healthy subjects²⁴. Ang-(1–7) stimulated the production of endothelium-derived nitric oxide, prostaglandins, and relaxation factors in endothelial cells in animal models²⁵. Acute infusion of Ang-(1–7) led to significant changes in blood flow distribution and decreased in total peripheral resistance²⁶. Similarly, in another study of transgenic mice²⁷ expressing an Ang-(1–7) producing fusion protein, there was a reduction in total peripheral resistance, suggesting that the acute increase in Ang-(1–7) may lead to important regional and systemic hemodynamic changes.

These data strongly suggest that Ang-(1–7) may recruit muscle microvasculature and increase the area of the microvascular endothelial surface, leading to increased nutrient and oxygen delivery to the skeletal musculature²⁸. Our hypothesis was that the Ang-(1–7) would increase vasodilation and blood flow, thereby improving VO_2max by augmenting the nutrient and oxygen delivery to skeletal muscle. The increased glucose uptake could contribute to ATP replacement, accelerating energy re-synthesis during muscle contraction.

Evidence indicates that, in addition to vasodilatory effects, transgenic rats with high levels of circulating Ang-(1–7) showed better tolerance and insulin-stimulated glucose uptake²⁹. Another study²⁸ demonstrated increased muscle microvascular recruitment following Ang-(1–7) infusion, increasing glucose uptake via the Glut-4 receptor. Recent data from our group suggested that transgenic rats overexpressing circulating Ang-(1–7), when subjected to strenuous exercise, had lower plasma glucose

variations and lower hepatic and muscle glycogen depletion³⁰. These findings suggest that administration of Ang-(1–7) improves glucose metabolism both at rest and during exercise.

Despite that fact that we did not show alterations induced by HPβ-CD-Ang-(1–7) in cardiovascular parameters as arterial pressure and heart rate, this study is the first to measure cardiovascular effects of Ang-(1–7) during exercise in humans. It is possible that systemic responses did not represent the vasodilatory effect in active muscle. Future studies are necessary to investigate its actions in specific tissues.

World MTB competitions are decided by a difference of seconds. Therefore, the present study provides evidence that acute supplementation with the formulation of HPβ-CD-Ang-(1–7) may be potential to achieve decisive results in this modality.

Conclusion

The oral formulation of HPβ-CD-Angiotensin-(1-7) (1.75 mg) improves the physical performance of MTB athletes. There were increases in TET and MW, as well as higher oxygen consumption and lower RPE. There was no difference in cardiovascular parameters between the placebo and the treated condition at rest or at peak physical effort.

Future studies

The mechanisms involved in increase in physical performance describe above as vasodilatory and glucose uptake need to be investigated, local analyses in specific tissues as well skeletal muscle must be analyzed: for example muscle biopsy and regional blood flow by doppler.

Abbreviations

MTB: MTB; HR: heart rate; BP: blood pressure; VO₂: maximum oxygen consumption; ETT: total exercise time; MW: mechanical work; ME: mechanical efficiency; RPE: perceived effort; REC: respiratory exchange coefficient; NEFAs: unesterified fatty acids; CK: creatine kinase; M: mean; SD: standard deviation; SBP: systolic blood pressure; DBP: diastolic blood pressure; MAP: mean arterial pressure.

Declarations

Acknowledgments

The authors would like to sincerely thank the participants for volunteered participating in this study.

Authors' contributions

SSM and ATPM were responsible for data collection, data interpretation, writing and revision of the manuscript, under the guidance and assistance of LKB, DMS and RASS who assisted in each stage and

finalization of the manuscript. NLT and FADMJ assisted in the collection and interpretation of data. ECO assisted in the revision of the manuscript. DBC assisted in the finalization of the manuscript. The authors declare that there is no conflict of interest with the current publication, and all authors have approved the final version of the manuscript.

Funding

Dean of Research, Graduate Studies and Innovation at the Federal University of Ouro Preto (PROPP-UFOP). Foundation of Amparo Research of the State of Minas Gerais (FAPEMIG). Coordination for the Improvement of Higher Education Personnel (CAPES).

Availability of data and materials

The datasets generated and/or analyzed as part of the current study are not publicly available due to confidentiality agreements with subjects. However, they can be made available solely for the purpose of review and not for the purpose of publication from the corresponding author upon reasonable request.

Ethics approval and consent to participate

The research proposal was approved by the Institutional Review Board of Federal University of Ouro Preto, approved by the Human Research Ethics Committee under protocol no. 25402813.2.1001.5150 and all participants gave written informed consent prior to study participation.

Consent for publication

Not applicable, no individual person's data was presented.

Competing interests

The authors declare that they have no competing interest

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Figures

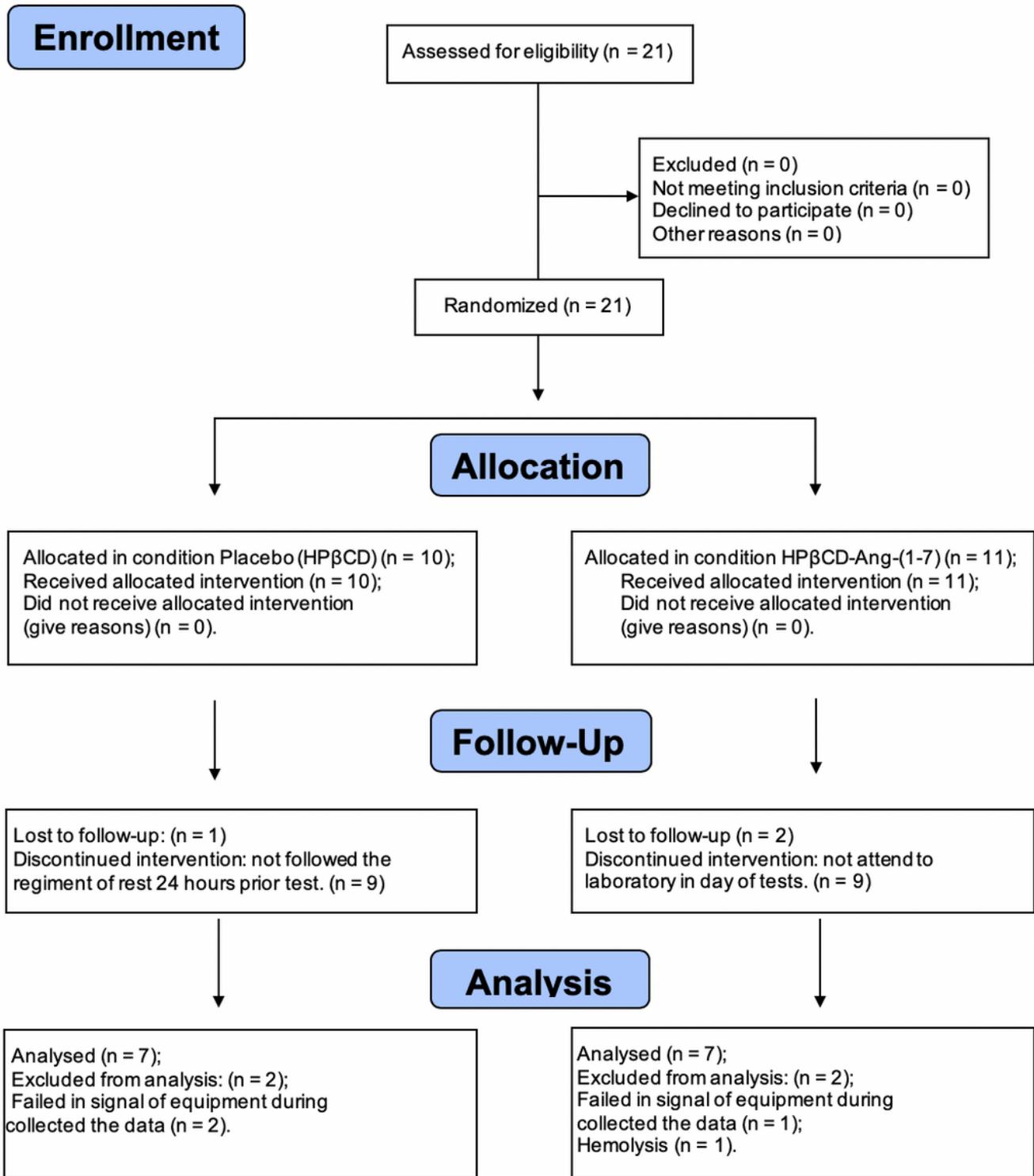


Figure 1

Participants recruitment flow diagram.

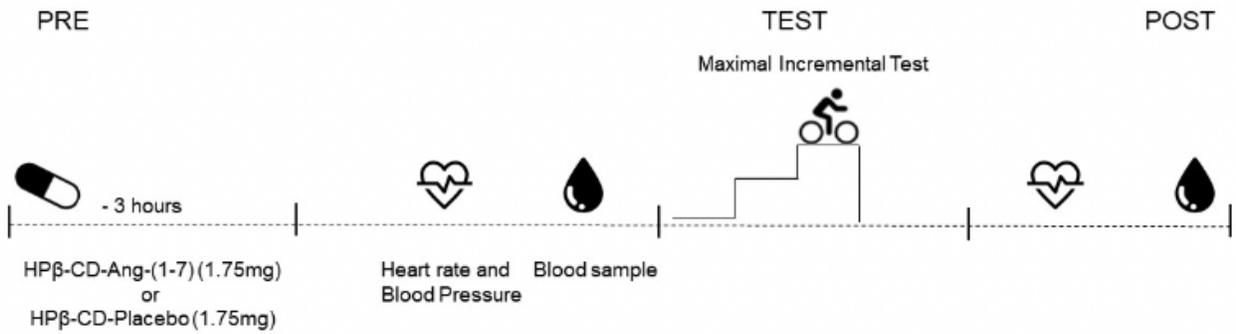


Figure 2

Experimental trial schematic. Participants completed both conditions in a double-blind, randomized, crossover manner, each separated by ≥ 7 days.

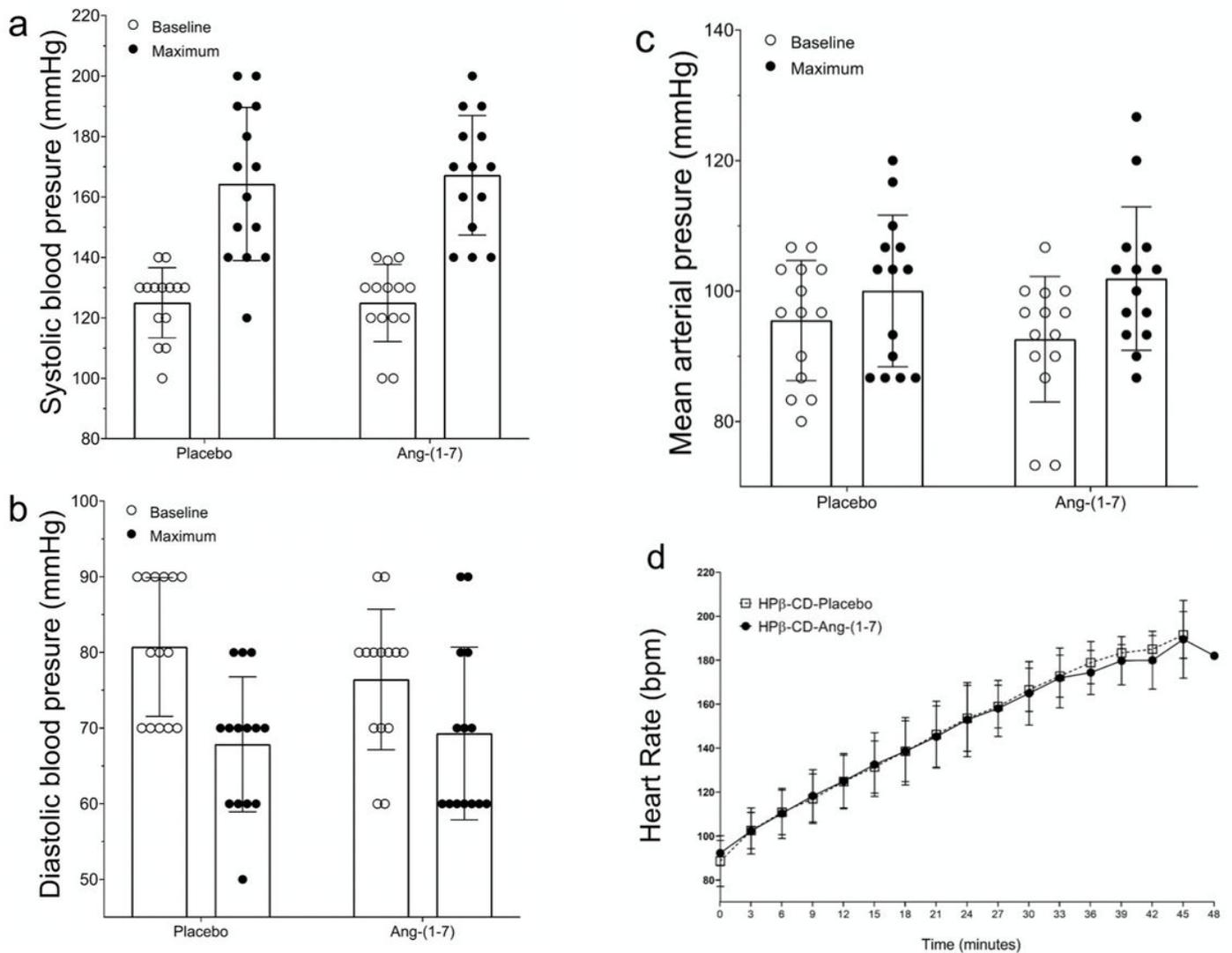


Figure 3

Systolic blood pressure - baseline and maximum (a); Diastolic blood pressure - baseline and maximum (b); mean arterial pressure - baseline and maximum (c); Heart Rate average values throughout the tests (d); in HPβ-CD-Placebo and Hβ-CD-Ang-(1-7). Mean ± SD. No differences for paired t test overall or Wilcoxon. (a) Baseline - $t = 0.01975$; Effect Size = ES (Cohen's $d = 0.00$); Lower 95% CI for Placebo = 116.20 & Ang-(1-7) = 115.60; Upper 95% CI for Placebo = 130.50 & Ang-(1-7) = 131.00 ($n = 14$); (a) Maximum - $t = 0.1540$; ES, $d = 0.03$; Lower 95% CI for Placebo = 153.90 & Ang-(1-7) = 156.40; Upper 95% CI for Placebo = 181.50 & Ang-(1-7) = 180.50 ($n = 14$); (b) Baseline - $w = -28$; ES, $d = 0.46$; 95% CI of median for HPβ-CD-Placebo = 98.71% & Hβ-CD-Ang-(1-7) = 98.71% ($n = 14$); (b) Maximum - $t = 0.4341$; ES $d = 0.14$; Lower 95% CI of mean for HPβ-CD-Placebo = 62.70 & Hβ-CD-Ang-(1-7) = 62.70; Upper 95% CI of mean for HPβ-CD-Placebo = 73.01 & Hβ-CD-Ang-(1-7) = 75.87 ($n = 14$); (c) Baseline - $t = 1.024$; ES, $d = 0.30$; Lower 95% CI for Placebo = 90.16 & Ang-(1-7) = 87.04; Upper 95% CI for Placebo = 100.80 & Ang-(1-7) = 98.16 ($n = 14$); (c) Maximum - $t = 0.6821$; ES, $d = 0.17$; Lower 95% CI for Placebo = 93.30 & Ang-(1-7) = 95.56; Upper 95% CI for Placebo = 106.70 & Ang-(1-7) = 108.30 ($n = 14$); (d) No differences between the two conditions HPβ-CD-Placebo and HPβ-CD-Ang-(1-7) during the performance of physical test were observed for the Heart Rate average ($F = 1.023$; $p = 0.3604$). Mean ± SD in each stage were used to build the graphic ($n = 14$).

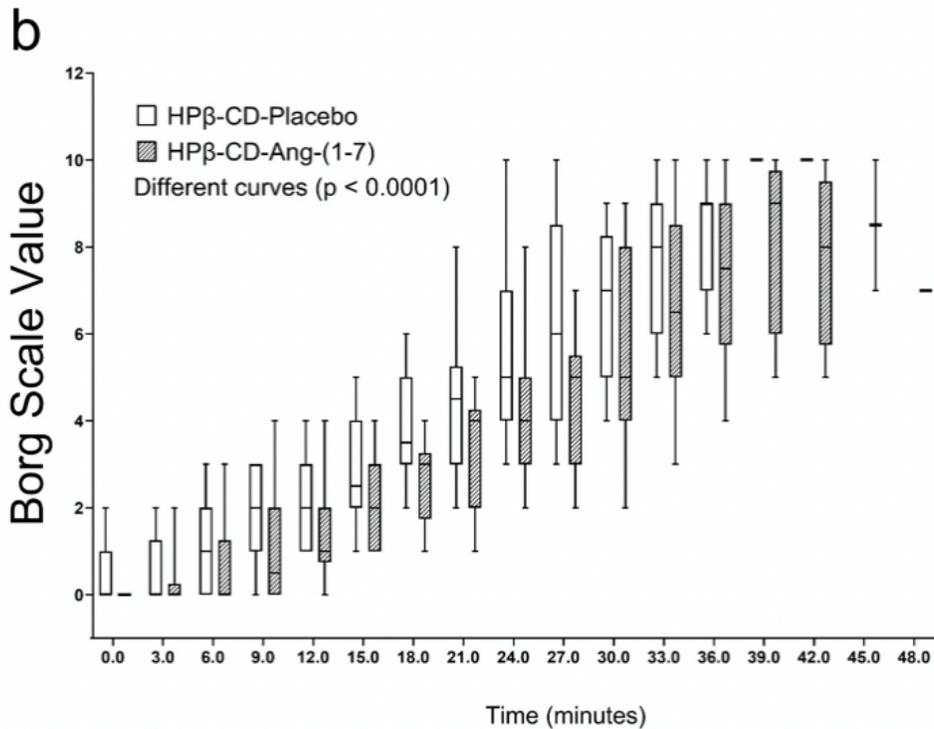
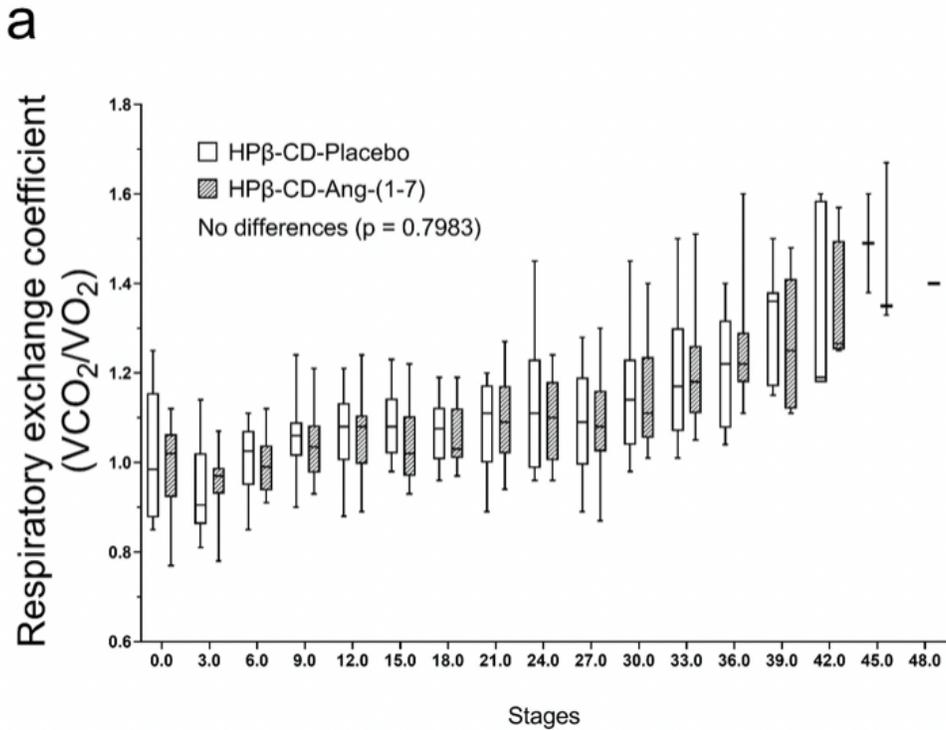


Figure 4

Respiratory exchange coefficient (REC) and Ratings of Perceived Exertion (RPE). (a) There were no differences between the two conditions HPβ-CD-Placebo and HPβ-CD-Ang-(1-7) during the performance of physical test for REC ($F = 0.2255$; $p = 0.7983$) box and whisker (min to max). (b) A significant difference between the two conditions HPβ-CD-Placebo and HPβ-CD-Ang-(1-7) during the performance of physical test was observed for the RPE ($F = 21.77$; $p < 0.0001$) box and whisker (min to max).

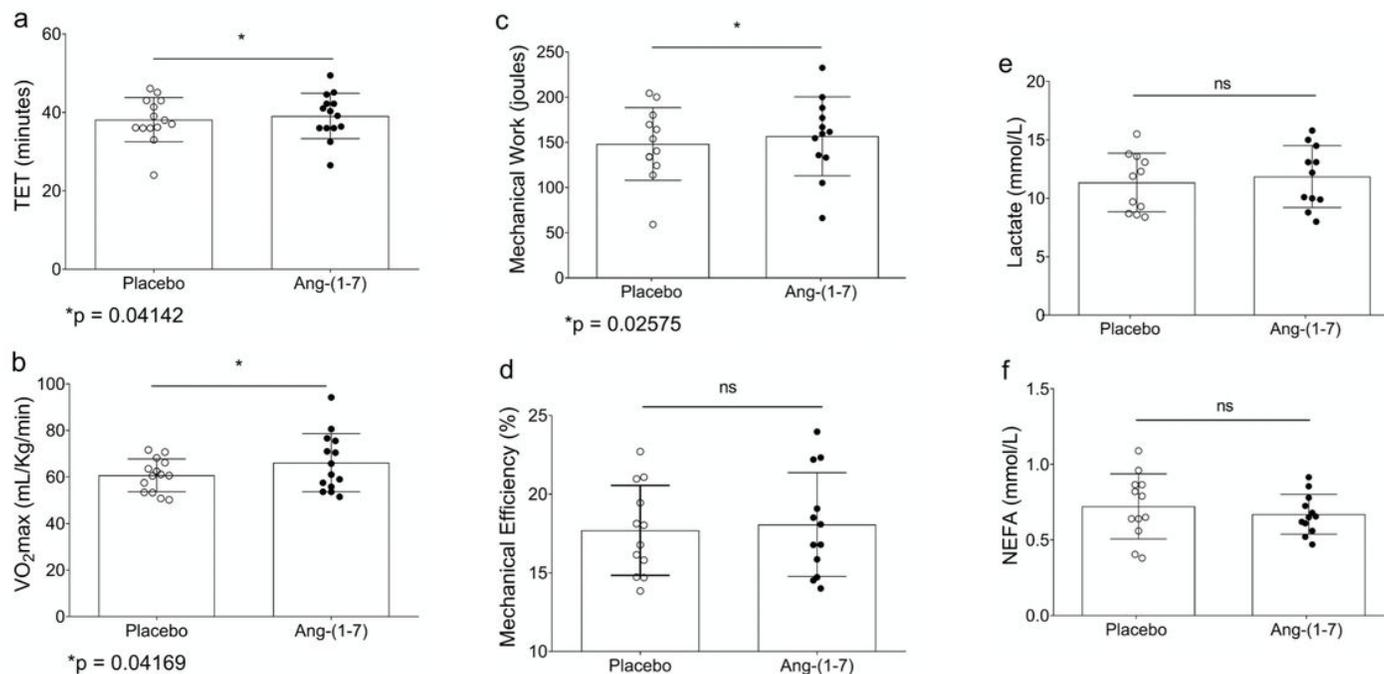


Figure 5

Physical performance and biochemical parameters. (a) Total exercise time (TET); $p = 0.04142$; $t = 2.263$; Effect Size (ES) (Cohen's $d = 0.17$); Lower 95% CI of mean for HP β -CD-Placebo = 34.88 & H β -CD-Ang-(1-7) = 35.76; Upper 95% CI of mean for HP β -CD-Placebo = 41.40 & H β -CD-Ang-(1-7) = 42.44 ($n = 14$); (b) VO₂max; $p = 0.04169$; $t = 2.203$; ES $d = 0.55$; Lower 95% CI for Placebo = 58.07 & Ang-(1-7) = 61.01; Upper 95% CI for Placebo = 64.57 & Ang-(1-7) = 71.60 ($n = 14$); (c) Mechanical work (MW); $p = 0.02575$; $t = 2.577$; ES $d = 0.21$; Lower 95% CI for Placebo = 122.6 & Ang-(1-7) = 128.9; Upper 95% CI for Placebo = 173.7 & Ang-(1-7) = 184.5 ($n = 12$); (d) Mechanical efficiency (ME); $t = 1.385$; ES $d = 0.10$; Lower 95% CI for Placebo = 15.55 & Ang-(1-7) = 15.63; Upper 95% CI for Placebo = 18.95 & Ang-(1-7) = 19.47 ($n = 12$); (e) Lactate; $t = 0.4691$; ES $d = 0.20$; Lower 95% CI for Placebo = 9.671 & Ang-(1-7) = 10.08; Upper 95% CI for Placebo = 13.04 & Ang-(1-7) = 13.64 ($n = 11$); (f) Non-esterified fatty acids (NEFA); $t = 0.7909$; ES $d = 0.29$; Lower 95% CI for Placebo = 0.5853 & Ang-(1-7) = 0.5863; Upper 95% CI for Placebo = 0.8588 & Ang-(1-7) = 0.7537 ($n = 12$); Mean \pm SD (paired t test).