No Influence of Low-, Medium-, or High-Dose Tyrosine on Exercise in a Warm Environment

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Revisions read well. Just one minor comment. The revised paragraph (342 to 387) is too long. Can authors break it into two to improve readability? Same comment for discussion 416 to 455.

A new paragraph has been added in line 370 and in line 446.

No influence of low, medium or high dose tyrosine on exercise in a warm environment.

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Abstract

Purpose: Tyrosine administration may counter exercise fatigue in a warm environment, but the typical dose is inconclusive, with little known about higher doses. We explored how three tyrosine doses influenced the circulating ratio of tyrosine: amino acids competing for brain uptake, and hypothesised a medium and high dose would enhance exercise performance in a warm environment.

Methods: Eight recreationally trained, non-heat acclimated males [mean (± SD) age, 23 ± 4 years; stature, 181 ± 7 cm; body mass, 76.1 ± 5.9 kg; peak oxygen uptake ($\dot{V}O_2$peak), 4.1 ± 0.5 $L \cdot min^{-1}$] performed a $\dot{V}O_2$peak test, two familiarisation trials, then four experimental trials in a randomised order separated by 7 d. Prior to exercise, subjects drank 2 x 300 mL sugar-free drinks delivering zero (PLA), 150 (LOW), 300 (MED) or 400 (HIGH) mg·kg body $mass^{-1}$ tyrosine in a double-blind fashion. Subjects performed 60 min constant intensity cycling then a simulated time trial in 30°C and 60% relative humidity.

Results: Time trial performance (P = 0.579) was not influenced by tyrosine ingestion. The plasma ratio of tyrosine: ∑(free-tryptophan, leucine, isoleucine, valine, phenylalanine, methionine), a key determinant of brain tyrosine influx, increased relative to PLA (P < 0.001). The increase was similar (P > 0.05) in MED (7.7-fold) and HIGH (8.2-fold), and greater than LOW (5.3-fold; P < 0.05). No differences existed between trials in core and skin temperature, heart rate, RPE or thermal sensation (P > 0.05).

Conclusion: Exercise performance in a warm environment was not influenced by tyrosine availability in recreationally trained males. The results provide novel data informing future studies, on the tyrosine dose maximising the circulating ratio of tyrosine: amino acids competing for brain uptake.

Keywords: amino acids, catecholamines, fatigue, warm environment.

Introduction

The ability to perform prolonged single-limb and whole-body exercise in a hot environment is impaired compared to the same exercise in cooler conditions (1). In addition to peripheral mechanisms such as altered circulatory and fluid balance factors, changes within the central nervous system (CNS) strongly influence fatigue during prolonged exercise in the heat. Altered brainwave activity associated with reduced arousal has been reported, thermal sensation and subjective effort increase during exercise with hyperthermia, voluntary muscle recruitment declines, which ultimately impair prolonged exercise performance (1). Reduced brain catecholamine function is a possible mediator of this central fatigue as dopamine and noradrenaline are involved in motor initiation and control (2), reward mechanisms and high motivation and arousal states (3), and heat loss mechanisms during exercise (4). Reductions in brain dopamine activity in several brain areas have also been reported at the point of exhaustion in exercised rats (5). There may be a similar functional link between CNS catecholamine impairment and exercise fatigue in humans.

Brain catecholamine synthesis is largely dependent upon impulse flow through neuronal populations and adequate precursor availability. The availability of brain tyrosine, the amino acid precursor to CNS catecholamine synthesis, is generally adequate under basal conditions, as the rate-limiting enzyme tyrosine hydroxylase is thought to be near saturation (6). Dopamine and noradrenaline neuronal activity in the CNS is strongly upregulated in response to stress (7) and if the exposure is prolonged, tissue availability of tyrosine can become depleted in neurons which are firing rapidly, impairing catecholamine synthesis (8). Under these stressful

48 conditions, experimentally increasing brain tyrosine content in rats maintains catecholamine
49 synthesis (8). Orally administered tyrosine shows promise in reducing decrements in mood
50 and cognitive function in humans during stress exposure, using doses up to 300 mg·kg body
51 mass^{-1} (9, 10) but an optimal tyrosine dose is yet to be established (9). Several studies have
52 attempted to influence exercise capacity and performance with 150 mg·kg body mass^{-1} tyrosine
53 in healthy subjects (11-15), with a separate study administering 20 g (16). Only one of these
54 studies reported increased exercise capacity in a warm environment (13) with the remainder
55 unable to confirm an influence of tyrosine on exercise in temperate (11, 12, 16) or warm
56 environment (14, 15). This is surprising, considering the demands which prolonged exercise
57 with hyperthermia places on the CNS (1) and the body of work suggesting a positive influence
58 of tyrosine on cognitive and psychomotor function during stress exposure. A key determinant
59 of brain influx of tyrosine is the circulating concentration ratio to large neutral amino acids
60 which compete for a common L-carrier system at the blood-brain barrier (17). Acute
61 administration of 150 mg·kg body mass^{-1} increases this ratio several-fold (13, 14, 18), but there
62 is a lack of data on the effect of higher tyrosine doses in humans. Considering that tyrosine
63 administration up to 300 mg·kg body mass^{-1} is effective in reducing cognitive impairments
64 during exposure to demanding environmental conditions, it is reasonable to propose that this
65 might also counter exercise fatigue in warm ambient temperatures.

66

67 The primary aim of this study was to examine the effect of three different tyrosine doses on the
68 ability to perform prolonged endurance exercise in a warm environment. We hypothesised that
69 a tyrosine dose similar to that used in the majority of previous exercise studies (defined as a
70 low dose for the purpose of this study) (11-15) would not influence, but that a medium or high
71 dose would enhance cycling performance in in a warm environment, compared to a placebo

containing no tyrosine. A secondary aim was to describe the effects of several acute tyrosine doses on the circulating ratio of tyrosine to amino acids competing for brain influx.

Methods

Subjects and ethics procedures

Nine healthy recreationally trained male volunteers, who were unacclimated to exercise in a warm environment, were recruited for the study. All testing took place in the Autumn and Winter months in Wales within the UK when air temperature is typically 15°C or lower. All subjects were permanently resident in the UK for at least one month before commencing the study. One volunteer withdrew from the study before completing all testing due to time constraints outside the study, and the remaining eight completed all testing [mean (± SD) age, 23 ± 4 years; stature, 181 ± 8 cm; body mass, 76.1 ± 5.9 kg; absolute peak oxygen uptake ($\dot{V}O_2$peak) from ramp incremental test, 4.2 ± 0.5 $L \cdot min^{-1}$; relative $\dot{V}O_2$peak, 55.1 ± 7.1 $mL \cdot kg \cdot min^{-1}$; absolute peak power achieved during ramp test, 327 ± 38 W; relative peak power from ramp test, 4.3 ± 0.5 $W \cdot kg^{-1}$; maximal heart rate during ramp test, 185 ± 8 $beats \cdot min^{-1}$]. Subjects were not all specifically trained endurance cyclists but had a minimum of three years training history, and participated in sports training at least 3 days per week, for a minimum of 3.5 hours total per week (classified as performance level 2) (19). Verbal and written information was given on the protocol and subjects were allowed to ask questions before providing written informed consent to proceed with the study. Subjects were free to withdraw from the study without prior notice, and without any penalty. The study was approved by the Research Ethics Committee at Aberystwyth University (reference number 14611).

Experimental procedures

The experimental procedures are similar to a previous study conducted in our lab (14). Subjects completed initial testing to measure $\dot{V}O_2$peak then 48 h later, they performed the first of two familiarisation trials separated by 7 days, and one week later the first of four experimental trials, each separated by 7 days. Randomisation of the experimental trial order was carried out using an open source software package (PEPI for Windows, Brixton Health). All experimental trials started in the morning between 0700 and 0830, and at the same time for each participant at each subsequent visit. A 24 h dietary intake record was completed by each subject before the first familiarisation, to enable diet replication before each experimental trial, but diet was not analysed. Subjects arrived for each experimental trial after an overnight fast of at least 8 h except for drinking 500 mL ordinary tap water 2 h before arrival. Subjects were instructed to sleep at least 8 h the night before to ensure they were well rested and verbal confirmation of adherence to these instructions was obtained at each visit. The consumption of alcohol and participation in strenuous or unaccustomed physical activity was not permitted for 48 h prior to each laboratory visit.

Initial testing

Subject's height (Holtain Ltd, Crymych, UK) and nude body mass (Seca 899, Hamburg, Germany) were recorded and they were fitted with a radiotelemetric heart rate monitor (Polar, FS2C, Kempele, Finland). Seat height and handle bar position was adjusted on an electrically braked cycle ergometer (Lode Excalibur Sport 2, Groningen, Netherlands) and replicated for each subject in subsequent visits. Exercise commenced for 3 min at 0 W then power output increased by 1 W every 2 s until volitional exhaustion, operationally defined as an inability to maintain 60 rpm pedal cadence for 5 s and voluntary withdrawal from exercise. Subjects were instructed to remain seated throughout the exercise test and all subjects adhered to these

instructions. Expired gases were analysed throughout exercise using an online breath-by-breath system (Quark PFT, Cosmed, Rome, Italy) which was calibrated before each test. Peak power was recorded as the highest power output achieved and $\dot{V}O_2$peak was identified as the highest $\dot{V}O_2$ recorded over a consecutive 30 s average during the test. The gas exchange threshold (GET; defined as non-linear increase in $\dot{V}CO_2$ with a linear increase in $\dot{V}O_2$, accompanied by an increase in the ventilatory equivalent for oxygen, $\dot{V}E/\dot{V}O_2$, while the ventilatory equivalent for carbon dioxide, $\dot{V}E/\dot{V}CO_2$, continued to decrease or level off) was identified for each subject and confirmed by a minimum of two separate, experienced investigators. The power output equivalent to 10% Δ (the $\dot{V}O_2$ at the GET plus 10% of the difference between the GET and $\dot{V}O_2$peak) was calculated for each subject.

Familiarisation and experimental trials

The purpose of the familiarisations was to appease any anxiety and to allow the subjects to become accustomed to the protocol and exercising in the warm environment, and to minimise any learning effects which might influence performance time. The familiarisations were identical to the placebo trial except no blood was drawn.

Nude body mass was recorded (Seca 899, Hamburg, Germany). Subjects fully emptied their bladder so urine volume could be measured and approximately 1 mL urine was retained to analyse urine osmolality (Osmostat 030, Gonotec, Berlin). The baseline urine osmolality for each subject was ≤ 700 mosmol·kg^{-1} at each laboratory visit, so all subjects were assumed to be euhydrated (20). A radiotelemetry band was fitted to the chest for continuous heart rate measurement (Polar, FS2C, Kempele, Finland) and skin thermistors (Grant Instruments, Cambridge, England) were attached using breathable adhesive medical tape (Hypafix, Bsn

144 Medical, Hull, UK), to the left calf, left anterior thigh, upper left chest, and left posterior upper
145 arm. Subjects fitted a rectal thermistor (Grant Instruments, Cambridge, England), in private,
146 10 cm beyond the anal sphincter. Rectal temperature (used as in indicator of core temperature)
147 and skin temperature at four sites were continuously logged using an electronic data logger
148 (Squirrel SQ2020, Grant Instruments, Cambridge, England). The skin temperature values were
149 used to calculate mean weighted skin temperature [0.3·(chest temperature + arm temperature)
150 + 0.2·(thigh temperature + calf temperature)] after each trial (21). Subjects were seated quietly
151 in the laboratory for 15 min then a blood sample (Baseline) was drawn from a superficial
152 antecubital vein using a 21-g sterile needle (BD Vacutainer Systems, Plymouth, UK), with
153 minimal stasis, into a heparinised vacutainer (BD Vacutainer Systems, Plymouth, UK). A 300
154 mL drink was administered containing ordinary tap water with 20% sugar-free lemon and lime
155 flavoured cordial (Morrisons, Bradford, UK), and consumed by the subject within 5 minutes.
156 The quantity of tyrosine powder (Nutricia Ltd., Liverpool, UK) added to the drink differed,
157 depending upon on the experimental trial undertaken (see Tyrosine administration below)

158

159 Subjects remained seated quietly in a thermoneutral laboratory (18°C, 50% relative humidity,
160 RH) for 60 min. A second venous blood sample (Pre-exercise) was drawn and the second 300
161 mL drink was administered, as described above. Subjects entered the climate chamber (Design
162 Environmental, Gwent, Wales), which was maintained at 30°C and 60% RH, and started
163 cycling (Lode Excalibur Sport 2) without a warm up, at a fixed intensity equivalent to 10% Δ
164 (129 ± 17 W) for 60 min. This is defined as heavy intensity exercise and was used to induce
165 hyperthermia prior to the main performance time trial, without eliciting exhaustion (14).
166 Subjects were instructed to remain seated throughout all exercise periods and adhered to these
167 instructions in all trials. Subjects were provided drinks (2 mL·kg body mass^{-1} tap water and
168 20% sugar-free, lemon and lime cordial) after every 15 min of exercise elapsed. A third venous

blood sample was drawn immediately after 60 min cycling (Post 60 min). The ergometer was set in linear mode, so the power output and the work accumulated was directly related to pedal cadence, and subjects were free to choose their preferred cadence. A maximum of 2 min elapsed between the end of the 60 min cycling and the start of the time trial and this time was standardised for each subject at each experimental trial. The time trial is based on a validated protocol used previously (18) and requires completion of an individual work target, equivalent to the total work required to complete 30 min cycling at 60% of the ramp test power output eliciting $\dot{V}O_2$peak (326 ± 37 kJ in this group). Subjects were free to self-pace, but were instructed to complete their work target as quickly as possible. The accumulated work portion of the bike console display was visible to the subjects throughout, with values representing 25%, 50%, 75%, and 100% of their individual work target. No additional feedback or motivational encouragement was provided throughout the time trial. Subjects were permitted to drink ad-lib throughout (ordinary tap water with 20% sugar-free lemon and lime cordial) and the volume of fluid consumed was recorded. Heart rate, core temperature and skin temperature at five sites were recorded every five minutes throughout the 60 min pre-exercise period, during the 60 min constant load cycling and the time trial. Additionally, these were recorded at completion of the time trial. Subjective thermal sensation (from -10, unbearable cold, to +10, unbearable heat) and RPE (22) were recorded every 10 min throughout the 60 min constant load exercise, every 5 min throughout the time trial, and at completion of the time trial. Power output was recorded from the ergometer console, with care taken to mask this from the subject, every 5 min throughout the time trial and in the last few seconds before completion. Immediately after subjects achieved their work target, a final venous blood sample was drawn (Post time trial). Subjects were removed from the chamber to a comfortable environment and monitored for 15 min to ensure core temperature was declining. The heart rate band and thermistors were removed, and subjects fully emptied their bladder once more

for measurement of urine volume and osmolality. Finally, subjects were reweighed nude, after towelling off, to assess changes in body mass due to exercising in the warm environment, and accounting for fluid intake.

Tyrosine Administration

The total tyrosine administered was zero (PLA), 150 (LOW), 300 (MED) or 400 (HIGH) mg·kg body mass^{-1}, suspended in 2 × 300 mL drinks. In LOW 150 mg·kg body mass^{-1} was delivered in the first 300 mL drink and in MED and HIGH the tyrosine dose was distributed equally between the two drinks. The 150 mg·kg body mass^{-1} tyrosine was classified as LOW as this is a common dose used in previous exercise and cognitive function studies but with mixed outcomes (9). The MED dose maintained cognitive function in humans exposed to demanding environmental conditions in previous work (10) and HIGH was employed to examine the effect of a higher dose than that previously examined in the literature. The total tyrosine ingested was 11.5 ± 1.0 g in LOW, 23.0 ± 1.9 g in MED and 30.7 ± 2.7 g in HIGH. All drinks were administered double-blinded and were prepared by a separate drinks supervisor not directly involved in data collection. Drinks were served in an opaque sports drink bottle and were shaken vigorously before administering to subjects. Prior pilot work confirmed that the drinks were indistinguishable in taste and texture.

Blood Handling and Analysis

Six millilitres of blood were drawn into a heparinised vacutainer at each sampling time point. One millilitre of whole blood was removed from each sample to measure haemoglobin (ABX Pentra 120, Horiba ABX Diagnostics, Northampton, UK), glucose and lactate (2300 Stat Plus, Yellow Spring Instrument Co., Ohio, USA), and haematocrit (using microcentrifugation).

Haemoglobin and haematocrit values were used to calculate the percentage change in plasma volume relative to the baseline sample (23), and values for blood measures were adjusted based on these changes. The remaining whole blood in each vacutainer was rapidly centrifuged at 1500 *g* for 10 min at 4°C to yield plasma. The plasma was distributed equally into two eppendorfs and immediately stored at -80°C, for later analysis of plasma amino acid concentrations, as described previously (13). All blood analyses were carried out in duplicate except haematocrit measurement which was completed in triplicate.

Statistical Analysis

All statistical analysis was carried out using a commercial software package (IBM Corp., IBM SPSS Statistics for Windows, Version 24.0. Armonk, NY: IBM Corp.). Differences in exercise time to exhaustion were analysed using one-way, repeated measures ANOVA with a Bonferroni correction for multiple comparisons. Data collected at repeated time points in each trial were analysed using a two-way (trial × time) repeated measures ANOVA. Where significant main effects were identified, follow-up paired t-tests with a Bonferroni correction were used to highlight significant differences in the data. Calculations of statistical power and effect sizes (ES) were carried out with G*Power software (version 3.1, Heinrich Heine University, Dusseldorf, Germany). All data are presented as mean ± SD, unless stated otherwise. Statistical significance for all analyses was accepted at $P < 0.05$. Based on the results of a previous study using a similar exercise protocol in a warm environment (18) we estimated 90% probability of measuring 3.4 min difference in time trial performance with 9 subjects, with a resultant statistical power of 0.80 and ES of 0.74.

Results

242 Time trial performance

243 The coefficient of variation for performance time between the second familiarisation trial and
244 PLA was 9.3%. The results were unaffected by experimental trial order ($P = 0.501$). Figure 1
245 contains the group mean and SD (Figure 1A), individual (Figure 1B) performance times and
246 power output during the time trial (Figure 1C). The time trial performance relative to PLA was
247 unaffected by tyrosine ingestion ($P = 0.579$). Power output up to 25 min of the time trial, which
248 was the last time point which all subjects were still exercising, and at completion was similar
249 in all trials ($P = 0.653$). The statistical power for the time trial performance F-test was 0.68
250 with an ES of 0.31. Using this data, a total sample size of 32 is sufficient to identify a
251 significant difference in the entire F-test at $P < 0.05$ (G*Power version 3.1). To achieve
252 statistical significance in the entire F-test ($P < 0.05$) with statistical power of 0.80, and with 8
253 subjects in each group, would require an effect size of 0.35.

254

255 Plasma amino acids

256 Plasma amino acid concentrations are provided in Table 1. There were no differences between
257 trials in the baseline values for all measured amino acids ($P > 0.05$). Tyrosine administration
258 increased the circulating tyrosine concentration relative to PLA ($P < 0.001$). Relative to HIGH,
259 the tyrosine concentration was similar in MED ($P = 0.99$) but was lower in LOW ($P = 0.036$).
260 The plasma concentration ratio of tyrosine: ∑(tryptophan, leucine, isoleucine, valine,
261 phenylalanine, methionine) (Figure 2) was similar between trials at baseline ($P = 0.657$). The
262 ratio increased with tyrosine ingestion (trial × time interaction, $P < 0.001$), but was unchanged
263 in PLA. The peak increase relative to baseline was similar ($P > 0.05$) in MED (7.7-fold) and
264 HIGH (8.2-fold), which was greater than the peak increase in LOW (5.3-fold; $P < 0.05$).

265

266 Blood glucose and blood lactate

267 There were no differences between trials in the blood glucose concentration (P = 0.639). The
268 baseline blood glucose concentration was 4.5 ± 0.3 mmol·L^{-1} in PLA, 4.4 ± 0.3 mmol·L^{-1} in
269 LOW, 4.5 ± 0.2 mmol·L^{-1} in MED and 4.5 ± 0.3 mmol·L^{-1} in HIGH. There was a decline in
270 blood glucose at pre-exercise (P = 0.003) and post time trial (P = 0.004) compared to baseline,
271 with no difference at post 60 min relative to baseline (P = 0.249). The blood glucose
272 concentration post time trial was 4.0 ± 0.3 mmol·L^{-1} in PLA, 4.0 ± 0.4 mmol·L^{-1} in LOW, 3.9
273 ± 0.1 mmol·L^{-1} in MED and 3.8 ± 0.6 mmol·L^{-1} in HIGH. Blood lactate was unaffected by trial
274 condition (P = 0.865) but increased in response to exercise and was higher at post 60 min (P =
275 0.030) and post time trial (P = 0.004) than at baseline. The peak blood lactate value, recorded
276 at the end of the time trial, was 3.0 ± 1.1 mmol·L^{-1} in PLA, 2.7 ± 1.6 mmol·L^{-1} in LOW, 3.0 ±
277 1.0 mmol·L^{-1} in MED and 2.9 ± 1.3 mmol·L^{-1} in HIGH.

278

279 Core and skin temperatures

280 Core temperature increased relative to pre-exercise after 10 min of exercise, throughout the 60
281 min constant load exercise (P < 0.05) and the time trial (P < 0.001), but no differences were
282 apparent between trials (P > 0.05; Figure 3). Due to technical issues with the skin temperature
283 thermistors, full data sets for five subjects only, were included in the analysis of mean weighted
284 skin temperature. Skin temperature increased during the constant load exercise (P = 0.003)
285 and to a similar extent in each trial (P = 0.347). Skin temperature remained elevated during
286 the time trial, relative to the pre-exercise period, but no differences were apparent between
287 trials (P = 0.704).

288

289 Heart rate

There was a marked increase in heart rate in response to the constant load exercise ($P < 0.001$) and the increase was similar between trials ($P = 0.718$) (Figure 4). Heart rate continued to increase during the time trial ($P < 0.001$) with no differences apparent between trials ($P = 0.477$).

RPE and thermal sensation

There were no differences between trials in RPE during 60 min constant load exercise ($P = 0.942$) or the time trial ($P = 0.538$) (Table 2). Expressed RPE increased after 20 min of exercise, relative to the 10 min value, and remained elevated throughout 60 min of exercise ($P < 0.01$). Perceived exertion was higher at 20 min into the time trial, relative to the 5 min value, and remained elevated until the end of the time trial ($P < 0.05$ for all comparisons). There were no differences between trials in thermal sensation during 60 min of constant load exercise ($P = 0.824$) or the time trial ($P = 0.426$) (Table 2). Thermal sensation increased after 5 min of exercise and remained elevated until the end of 60 min cycling ($P < 0.01$ for all comparisons). Thermal sensation continued to rise during the time trial, relative to the value at the start of the time trial ($P < 0.01$ for all comparisons), peaking at a subjective rating between "Very hot, uncomfortable" and "Extremely hot, close to limit".

Urine measures and body mass changes

The pre-exercise urine osmolality was 327 ± 225 mosmol·kg^{-1} in PLA, 405 ± 284 mosmol·kg^{-1} in LOW, 423 ± 145 mosmol·kg^{-1} in MED and 471 ± 205 mosmol·kg^{-1} in HIGH. Urine osmolality did not change with time ($P = 0.627$) and no differences were apparent between trials ($P = 0.645$). Similarly, no differences were apparent between trials in urine volume ($P = 0.99$). There was an increase in urine volume ($P = 0.026$) from pre-exercise (329 ± 324 mL in

PLA, 129 ± 107 in LOW, 212 ± 162 mL in MED and 156 ± 226 mL in HIGH) to post-time trial (670 ± 389 mL in PLA, 605 ± 474 in LOW, 512 ± 423 mL in MED and 597 ± 439 mL in HIGH). The total volume of fluid consumed ad-libitum during the time trial was similar between trials (P = 0.654; 221 ± 210 mL in PLA; 242 ± 154 mL in LOW; 216 ± 174 mL in MED and 301 ± 238 mL in HIGH). There was a reduction in body mass by the end of the time trial, relative to the pre-exercise value (P = 0.001), which was similar in all trials (P = 0.936), and represented 1.1 ± 0.7 %, 1.4 ± 0.7 %, 1.1 ± 0.7 % and 1.0 ± 0.7 % of the pre-exercise body mass in PLA, LOW, MED and HIGH, respectively.

Plasma volume

Plasma volume declined, relative to baseline, at pre-exercise (P = 0.018), post 60 min (P = 0.010) and post time trial (P = 0.001) with no difference between trials (P = 0.404). By the end of the time trial, the decline in plasma volume relative to baseline was 9.4 ± 5.5 % in PLA, 7.5 ± 3.7 % in LOW, 9.8 ± 2.9 % in MED and 9.3 ± 5.4 % in HIGH.

Discussion

The results from the study demonstrate no influence on simulated time trial performance in recreationally trained males exposed to a warm environment despite large increases in the circulating availability of tyrosine. The amino acid data suggest that 300 mg·kg^{-1} tyrosine maximises the circulating ratio of tyrosine to amino acids competing for brain influx, in young recreationally trained males, with no additional influence seen with 400 mg·kg^{-1}. This has important implications for future work examining tyrosine, given that the acute administration timescale used in our study is common to research in this area. The high dose used in the study is considerably higher than that used in previous studies examining tyrosine administration (10-

16, 24, 25) and was well tolerated by all subjects. This dose was justified given the mixed outcomes from previous studies on exercise tolerance (12-16, 25) and cognitive functions (9, 25) using lower doses.

Previous studies in humans typically administering 150 mg·kg body mass^{-1} tyrosine have generally failed to show an effect of tyrosine on exercise in temperate (11, 12) or high ambient temperatures (14, 15) with only one exception (13). In the Tumilty et al. study (13), exercise was maintained for 15% longer in 30 °C (60% RH) compared to a placebo. However, a separate study using a similar exercise and tyrosine dose protocol could not confirm this finding (15). Additionally, exercise performance was not enhanced with 150 mg·kg^{-1} tyrosine in a separate study employing the same exercise protocol as the present study (14). The rationale for the efficacy of tyrosine to counter exercise impairment is dependent upon stress-induced depletion of CNS tyrosine availability due to the exercise and environmental demands. The core and skin temperatures, heart rate and subjective responses to the exercise protocol and ambient conditions attest to the demanding nature of the exercise protocol used in the present study, and in previous work (13-15). The extent of hyperthermia induced in the present study, evidenced by the core and skin temperature data, is moderate but reflects the fitness of the subjects, and the results are similar to other studies in moderately fit subjects at the point of exhaustion in warm conditions (26). It is likely that CNS dysfunction would be apparent at the core temperatures reached in the present study. Previous work has reported a progressive decline in motor activation areas in the brain with increasing core temperature, and a reduction in maximal voluntary contraction force at 38.5°C (27). These signals most probably integrate with feedback from working muscles and cardiovascular changes as core temperature progressively increases, determining exercise fatigue during prolonged exercise in warm conditions (1). The progressively increasing RPE and thermal sensation data in the present

study provide some support for this. Collectively, the present results seem to suggest that prolonged exercise, with or without exposure to warm ambient temperatures, is insufficiently stressful to lower CNS neuronal tyrosine availability to the extent that catecholamine function is markedly impaired. This is unexpected, considering the specific demands placed on the CNS when exercise is performed in the heat compared to cooler conditions (1), and the role of brain catecholamine function to exercise tolerance in the heat (28).

The present results do not allow us to definitively confirm whether the doses used in the study are optimal to influence exercise tolerance. Additionally, given that the subjects were recreationally trained, a more variable day-to-day cycling performance might be expected compared to experienced, trained cyclists (29). The two familiarisation sessions undertaken prior to the experimental trials in the present study, in subjects involved in regular team sport training, would be sufficient to ensure reproducibility of the simulated time trial performance (29). Several lines of enquiry have reported favourable effects of acute tyrosine supplementation on various cognitive and psychomotor functions while under stress, using doses from 150 up to 300 mg·kg body mass^{-1} (9, 10), providing a rationale for the doses used in the present study. Many of these studies have involved military populations involved in very stressful and prolonged, demanding environments such as sleep deprivation, cold weather and hypoxia exposure (10, 24). Prolonged exposure to these environments presumably requires greater involvement of higher cognitive resources, such as cognitive control and attentional processes, than prolonged exercise in a warm environment. Under these conditions, it might therefore, be expected that brain tyrosine becomes depleted to a greater extent than prolonged exercise in the heat, impairing catecholamine function and dependant processes such as arousal, motivation and cognitive and psychomotor function. This might account for the

greater susceptibility of cognitive function to stress-induced impairment and the reported benefits of tyrosine administration in these studies.

A primary influence on brain influx of tyrosine is the circulating concentration ratio of tyrosine to large neutral amino acids which share a L-transport carrier across the blood-brain barrier. This is the first study to report the effects of several tyrosine doses on this ratio in humans. Previous reports have examined the effect of ingesting mixed meals containing carbohydrate and protein on the circulating ratio of tyrosine: competing amino acids (30), or administered tyrosine in the presence of a mixed meal (25). This is a crucial consideration since the additional presence of ingested protein and carbohydrate would influence the circulating tyrosine ratio independently of tyrosine administration, and directly affect brain uptake and tissue tyrosine levels (17). Additionally, one study examined the effect of two separate tyrosine doses on the circulating tyrosine concentration (150 and 300 mg·kg body mass^{-1}) but did not report the change in the blood ratio of tyrosine to competing amino acids (25). The present data suggest that an acute dose of 300 mg·kg body mass^{-1} represents an upper limit, on balance, between the dose administered and the achievable increase in the circulating tyrosine ratio in recreationally trained male subjects, at least in the time frame adopted in this study. This is likely due to a reduced rate of gastric emptying and/ or reduced intestinal transport of the amino acid into the bloodstream in the presence of the highest ingested tyrosine dose. Plasma amino acid concentrations were measured for approximately 2.5 hours after drink administration. If the measurement period was extended, this may have appreciably influenced the circulating tyrosine ratio, highlighting differences between the medium and high dose trials, but we feel this is unlikely. We have measured the effect of tyrosine administration on the circulating ratio of tyrosine: competing amino acids in several studies (published and unpublished observations), over a time frame of 2.5 to 3 h post-administration. Typically, an initial peak in

412 the ratio of tyrosine: competing amino acids is evident 1 to 2 h post-ingestion, and then a
413 maintenance of this peak, or a modest decline from peak. Therefore, we feel it is unlikely that
414 in the present study, the ratio would have appreciably increased from 3 h post-ingestion, to the
415 extent that differences between the 300 and 400 mg·kg body mass^{-1} doses would be apparent.

416

417 A limitation of this study, and previous tyrosine studies examining exercise in humans, is a
418 lack of direct confirmation of brain uptake of the supplemented tyrosine. The ratio of
419 circulating tyrosine to competing amino acids does correlate well with brain tissue levels in the
420 rat (31). In healthy humans, the brain uptake of tyrosine, measured *in vivo* using positron
421 emission tomography and intravenous ^{11}C-labelled tyrosine, was reduced following oral
422 administration of 175 mg·kg body mass^{-1} (32). This suggests that the L-carrier amino acid
423 transporter operates near saturation in fasted and resting humans. Some caution is warranted
424 in generalising these findings as this may not be representative of brain tyrosine transport
425 kinetics in humans exposed to environmental stress or exercise. Nonetheless, this might
426 account in part for the general lack of influence of tyrosine on exercise tolerance. The accepted
427 thinking for some time is that catecholamine synthesis is tightly controlled within the CNS via
428 receptor-mediated end-product inhibition of the rate-limiting enzyme tyrosine hydroxylase,
429 with around 75% saturation of the enzyme under basal conditions (6). This would limit the
430 extent that CNS catecholamine function was influenced, or even inhibit further catecholamine
431 synthesis (33), in the presence of elevated brain tyrosine availability, unless the stress
432 exposure was excessively demanding and prolonged. This situation is quite different from the
433 central synthesis of serotonin which tends to readily increase in line with brain content of its
434 amino acid precursor tryptophan (34). Recent research using microdialysis in the rat seems to
435 counter the extent to which tyrosine hydroxylase is saturated *in vivo*. Perfusing the striatum
436 and prefrontal cortex with L-tyrosine under awake, resting conditions markedly increased

DOPA in dopamine nerve terminals, with a dose dependant increase in striatum (35). Bypassing the rate-limiting step in catecholamine in humans, for example with Levodopa (L-DOPA) administration, exerts a more pronounced influence on brain catecholamine activity in humans (36). Administering potent stimulators of the catecholaminergic system such as amphetamine (37) or a dopamine/ noradrenaline reuptake inhibitor (18) more robustly enhances exercise performance. Interestingly, selectively inhibiting CNS noradrenaline reuptake impairs prolonged exercise in a warm environment (38) suggesting an important role for CNS dopamine function during prolonged exercise in the heat.

Given that tyrosine is a general catecholamine precursor, some influence on CNS noradrenaline function cannot be ruled out in the present study. Improved blood pressure maintenance and increased auditory event related potential amplitude have been reported in humans during lower body negative pressure exposure, following acute 150 mg·kg body mass^{-1} tyrosine supplementation (39). These effects are consistent with an influence of tyrosine on central noradrenergic function. Recent work reported that 150 mg·kg body mass^{-1} oral tyrosine administration improved skin vasoconstrictive responses to cold exposure in older people, suggesting augmented peripheral noradrenergic effector responses (40). Older people can exhibit lowered endogenous tyrosine availability and blunted peripheral noradrenergic responses (40). This may be unrepresentative of young, healthy males exercising in warm conditions. The absence of an influence of tyrosine administration on skin temperature responses in the present study, and others involving young, healthy subjects exercising in warm conditions (13-15), lends support for this.

Conclusion

Increasing the circulating ratio of tyrosine to amino acids competing for brain uptake with a low, medium or high tyrosine dose had no influence on prolonged exercise performance in a warm environment, in male recreationally trained subjects. The results do not preclude an important role of CNS catecholamines during prolonged exercise in a warm environment, but the weight of evidence suggests this is not appreciably influenced by increasing peripheral precursor availability, in the subject group examined. This probably suggests that the exercise and ambient conditions were insufficiently stressful to lower CNS tyrosine availability in recreationally trained male, and further catecholamine synthesis is feedback-inhibited, or that the additional circulating tyrosine is not fully available to the CNS. The results also report, for the first time, data in humans on the response of several tyrosine doses. The results suggest that 300 mg·kg body mass^{-1} tyrosine is optimal to maximise the circulating ratio of tyrosine: amino acid competing for brain uptake, in recreationally trained males. This provides important insight for future studies examining the acute effect of tyrosine administration.

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Conflict of interest

The authors report no conflict of interest. The results of the present study do not constitute endorsement by ACSM. The authors declare that results of the study are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation.

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587

588 FIGURE 1- Effect of placebo or tyrosine ingestion on the group mean ± SD (A) and individual
589 (B) performance times and power output (C) during a simulated cycling time trial in the heat.
590 PLA 0, LOW 150 mg·kg^{-1}, MED 300 mg·kg^{-1}, HIGH 400 mg·kg^{-1}tyrosine trial.

591

592 FIGURE 2- Effect of placebo or tyrosine ingestion on the plasma concentration ratio of tyrosine
593 to large neutral amino acids competing for brain influx. *$P < 0.05$ denotes significant difference
594 compared to the 150 mg·kg body mass^{-1} tyrosine trial. ***$P < 0.001$ denotes significant
595 difference compared to the placebo trial. Values are mean ± SD. ∑LNAA, ∑(plasma
596 concentration of free-tryptophan, leucine, isoleucine, valine, phenylalanine, methionine). PLA
597 0, LOW 150 mg·kg^{-1}, MED 300 mg·kg^{-1}, HIGH 400 mg·kg^{-1}tyrosine trial.

598

599 FIGURE 3-Core (A) and mean weighted skin (B) temperature responses to exercise in the heat
600 with placebo or tyrosine ingestion. $^{c}P < 0.001$ denotes significant difference to remaining time
601 points in all trials. Values are mean ± SD. PLA 0, LOW 150 mg·kg^{-1}, MED 300 mg·kg^{-1},
602 HIGH 400 mg·kg^{-1} tyrosine trial.

603

FIGURE 4- Heart rate responses to exercise in the heat with placebo or tyrosine ingestion. $^{c}P < 0.001$ denotes significant difference to remaining time points in all trials. Values are mean ± SD. PLA 0, LOW 150 mg·kg^{-1}, MED 300 mg·kg^{-1}, HIGH 400 mg·kg^{-1} tyrosine trial.

Table 1

TABLE 1-Plasma amino acid responses to exercise with placebo or tyrosine ingestion.

	Baseline	Pre-exercise	Post 60 min	Post time trial
Tyrosine ($\mu mol \cdot L^{-1}$)				
PLA	116 ± 17	106 ± 18	112 ± 21	109 ± 16
LOW	113 ± 25	428 ± 154***, a	481 ± 177***, a	404 ± 143***, a
MED	112 ± 17	436 ± 124***, a	635 ± 190***, *, a	618 ± 129***, *, a
HIGH	115 ± 27	448 ± 114***, a	635 ± 162***, *, a	668 ± 141***, *, a
Valine ($\mu mol \cdot L^{-1}$)				
PLA	395 ± 74	356 ± 67	320 ± 65 b	299 ± 77 b
LOW	345 ± 78	377 ± 85	317 ± 59 b	250 ± 66 b
MED	352 ± 44	323 ± 44	296 ± 47 b	271 ± 44 b
HIGH	366 ± 67	340 ± 60	299 ± 70 b	281 ± 51 b
Leucine ($\mu mol \cdot L^{-1}$)				
PLA	211 ± 47	183 ± 37	149 ± 41 a	135 ± 30 a
LOW	175 ± 52	185 ± 62	143 ± 37 a	114 ± 36 a
MED	189 ± 35	162 ± 34	143 ± 31 a	115 ± 20 a
HIGH	207 ± 43	171 ± 32	146 ± 36 a	120 ± 18 a
Isoleucine ($\mu mol \cdot L^{-1}$)				
PLA	93 ± 20	81 ± 16	67 ± 14 a	64 ± 14 a
LOW	81 ± 27	83 ± 25	64 ± 14 a	53 ± 14 a
MED	84 ± 19	73 ± 17	63 ± 12 a	55 ± 10 a
HIGH	84 ± 23	71 ± 17	61 ± 17 a	54 ± 9 a
Methionine ($\mu mol \cdot L^{-1}$)				
PLA	25 ± 2	24 ± 3	25 ± 3	26 ± 3
LOW	26 ± 5	27 ± 4	25 ± 3	24 ± 3
MED	26 ± 4	25 ± 4	25 ± 4	24 ± 4
HIGH	26 ± 5	25 ± 4	24 ± 5	24 ± 5
Phenylalanine ($\mu mol \cdot L^{-1}$)				
PLA	74 ± 8	73 ± 9	80 ± 8	80 ± 6
LOW	69 ± 15	76 ± 11	78 ± 15	75 ± 19
MED	72 ± 11	75 ± 17	76 ± 12	77 ± 16
HIGH	76 ± 7	75 ± 5	79 ± 10	81 ± 15
Tryptophan ($\mu mol \cdot L^{-1}$)				
PLA	122 ± 7	118 ± 14	108 ± 14	87 ± 15 b
LOW	116 ± 23	126 ± 19	114 ± 23	93 ± 23 b

MED	116 ± 16	121 ± 14	106 ± 11	91 ± 10 [b]
HIGH	127 ± 22	126 ± 20	110 ± 25	96 ± 23 [b]

PLA 0, LOW 150 mg·kg^{-1}, MED 300 mg·kg^{-1}, HIGH 400 mg·kg^{-1} tyrosine trial. *P < 0.05 denotes significant difference to the LOW trial. ***P < 0.001 denotes significant difference compared to the PLA trial. [a]P < 0.05 and [b]P < 0.01 denotes significant difference compared to the baseline value in the same trial. Values are mean ± SD.

Table 2

TABLE 2-Subjective responses to exercise in the heat with placebo or tyrosine ingestion.

60 min constant load cycling							
	0	10	20	30	40	50	60
Rating of perceived exertion (RPE; 6 – 20)							
PLA	–	13 ± 1	13 ± 2 [b]	14 ± 2 [b]	14 ± 2 [b]	14 ± 2 [b]	14 ± 2 [b]
LOW	–	12 ± 2	13 ± 2 [b]	14 ± 2 [b]	14 ± 2 [b]	14 ± 2 [b]	15 ± 2 [b]
MED	–	12 ± 1	13 ± 1 [b]	13 ± 2 [b]	14 ± 2 [b]	14 ± 2 [b]	15 ± 2 [b]
HIGH	–	13 ± 1	13 ± 1 [b]	13 ± 1 [b]	14 ± 1 [b]	15 ± 2 [b]	15 ± 1 [b]
Thermal sensation (-10 unbearable cold; 0 neutral; 10 unbearable heat)							
PLA	2 ± 1	4 ± 1 [b]	4 ± 2 [b]	4 ± 2 [b]	5 ± 2 [b]	5 ± 2 [b]	5 ± 2 [b]
LOW	2 ± 1	4 ± 1 [b]	4 ± 2 [b]	5 ± 1 [b]	5 ± 2 [b]	5 ± 2 [b]	6 ± 2 [b]
MED	2 ± 1	4 ± 1 [b]	4 ± 1 [b]	5 ± 1 [b]	5 ± 2 [b]	5 ± 2 [b]	6 ± 2 [b]
HIGH	2 ± 1	4 ± 1 [b]	4 ± 1 [b]	5 ± 2 [b]	5 ± 2 [b]	5 ± 2 [b]	5 ± 2 [b]
Simulated time trial							
	0	5	10	15	20	25	END
Rating of perceived exertion (RPE; 6 – 20)							
PLA	–	15 ± 2	15 ± 2	15 ± 2	16 ± 2 [a]	16 ± 3 [a]	18 ± 2 [a]
LOW	–	15 ± 2	15 ± 2	16 ± 1	16 ± 1 [a]	16 ± 2 [a]	18 ± 2 [a]
MED	–	14 ± 2	15 ± 2	15 ± 2	16 ± 2 [a]	16 ± 2 [a]	18 ± 2 [a]
HIGH	–	14 ± 2	15 ± 2	15 ± 2	16 ± 2 [a]	16 ± 2 [a]	18 ± 2 [a]
Thermal sensation (-10 unbearable cold; 0 neutral; 10 unbearable heat)							
PLA	4 ± 2	5 ± 2 [b]	6 ± 2 [b]	6 ± 3 [b]	6 ± 2 [b]	6 ± 2 [b]	7 ± 2 [b]
LOW	4 ± 2	5 ± 2 [b]	6 ± 2 [b]	6 ± 2 [b]	6 ± 2 [b]	7 ± 2 [b]	7 ± 2 [b]
MED	4 ± 1	5 ± 2 [b]	6 ± 2 [b]	6 ± 2 [b]	6 ± 2 [b]	6 ± 2 [b]	7 ± 2 [b]
HIGH	4 ± 1	5 ± 2 [b]	5 ± 2 [b]	6 ± 2 [b]	6 ± 2 [b]	6 ± 2 [b]	7 ± 2 [b]

PLA 0, LOW 150 mg·kg^{-1}, MED 300 mg·kg^{-1}, HIGH 400 mg·kg^{-1} tyrosine trial. [a]$P < 0.05$ and [b]$P < 0.01$ denotes significant difference compared to the first value recorded in the same trial and exercise period. Values are mean ± SD.

Figure 1

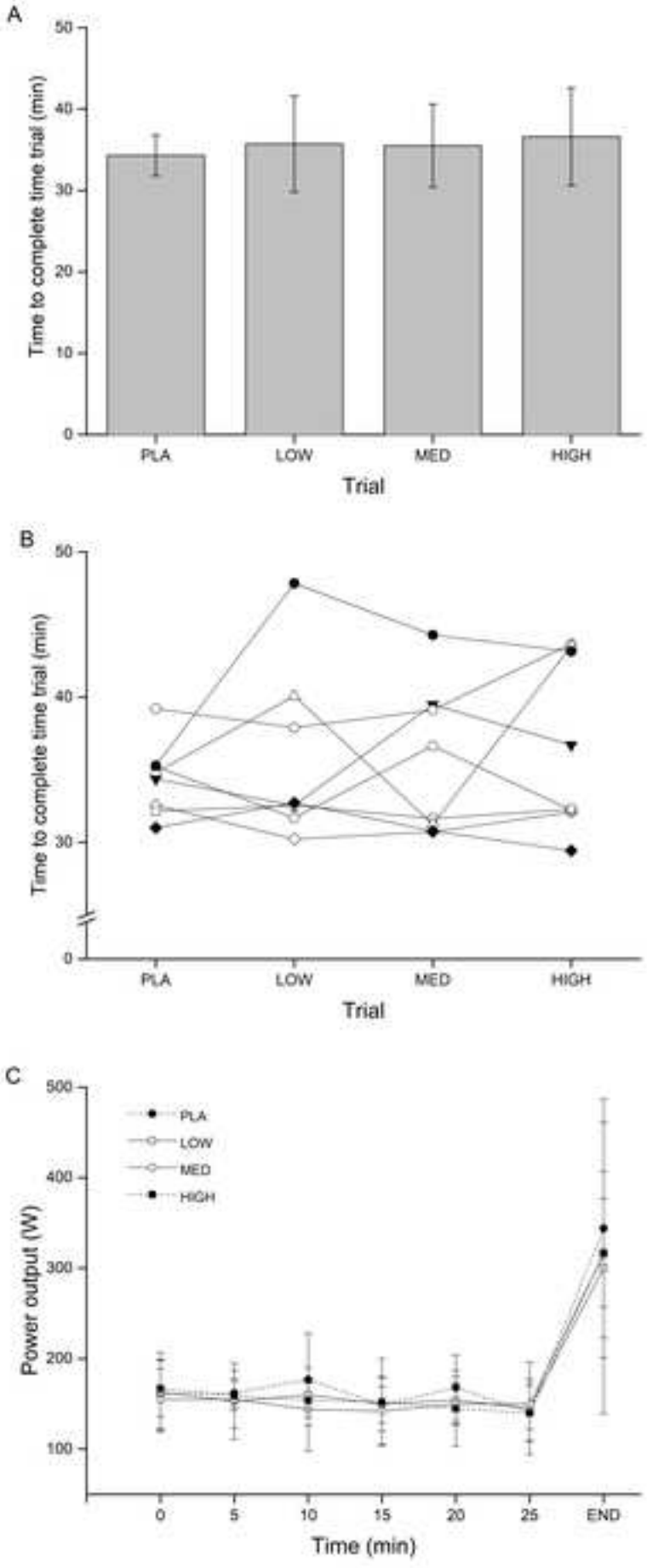

Figure 2

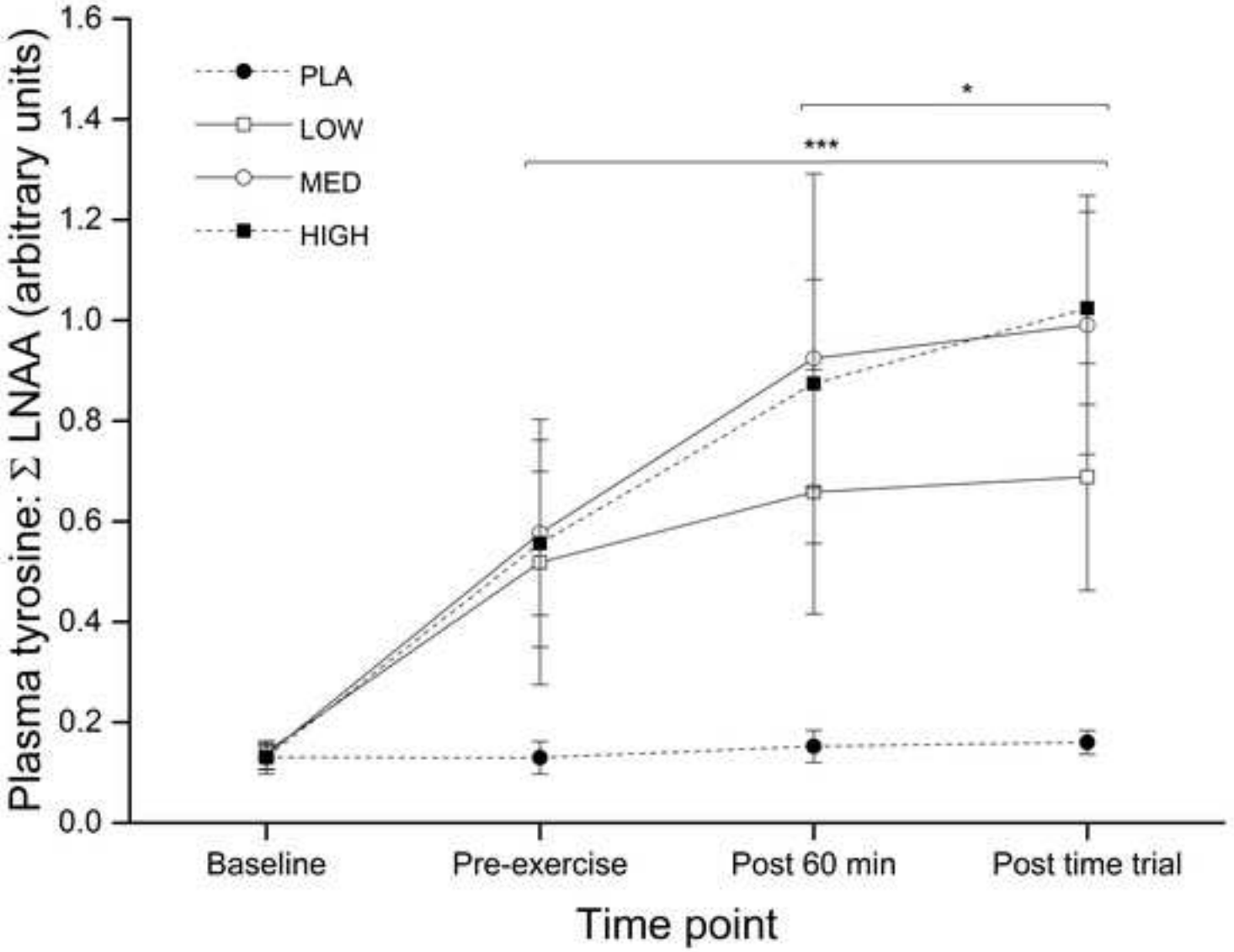

Figure 3

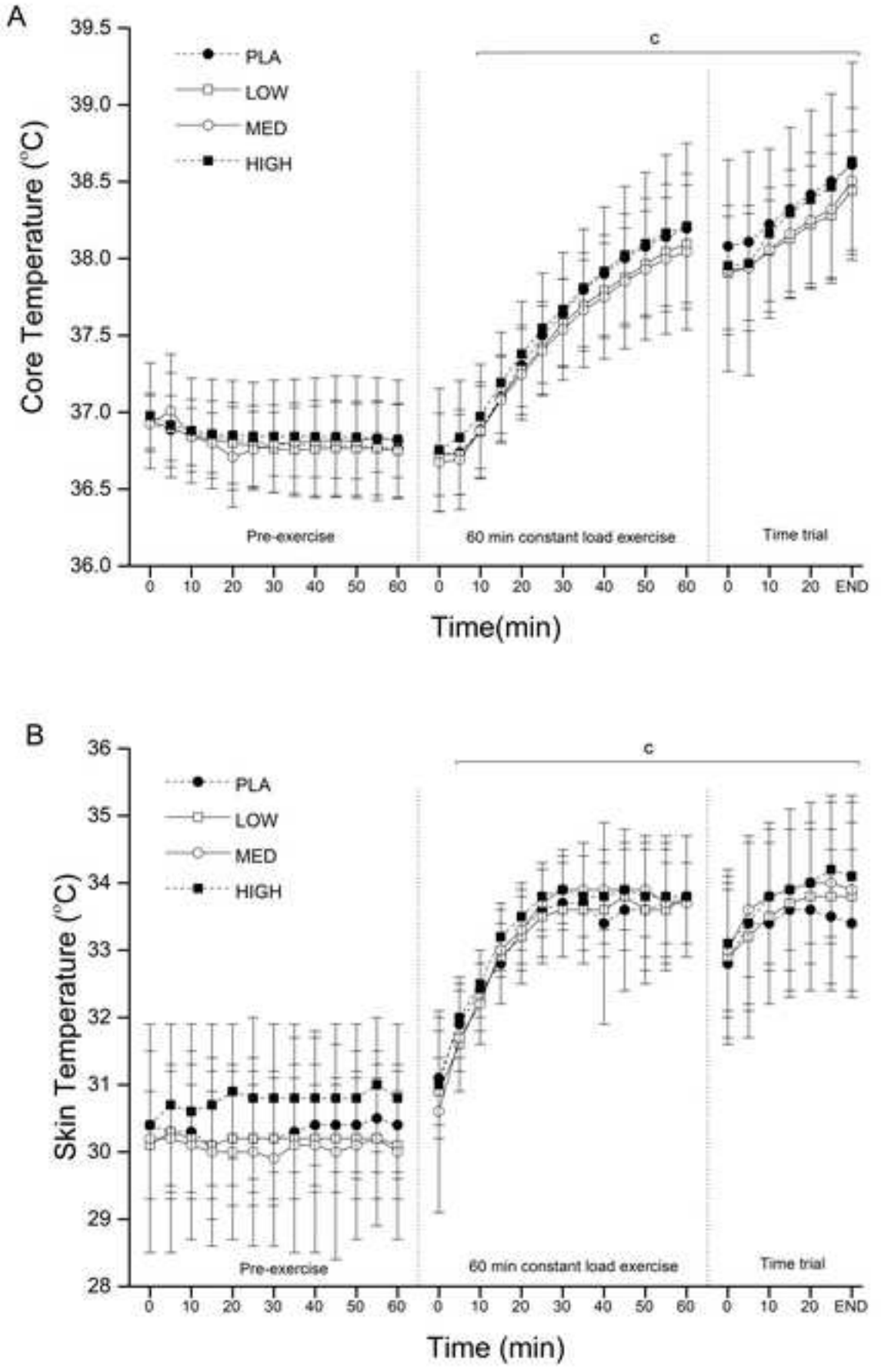

Figure 4

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