

## Aberystwyth University

### *Failure of oral tyrosine supplementation to improve exercise performance in the heat*

Tumilty, Les; Davison, Glen; Beckmann, Manfred; Thatcher, Rhys

*Published in:*

Medicine and Science in Sports and Exercise

*DOI:*

[10.1249/MSS.0000000000000243](https://doi.org/10.1249/MSS.0000000000000243)

*Publication date:*

2014

*Citation for published version (APA):*

Tumilty, L., Davison, G., Beckmann, M., & Thatcher, R. (2014). Failure of oral tyrosine supplementation to improve exercise performance in the heat. *Medicine and Science in Sports and Exercise*, 46(7), 1417-25. <https://doi.org/10.1249/MSS.0000000000000243>

#### **General rights**

Copyright and moral rights for the publications made accessible in the Aberystwyth Research Portal (the Institutional Repository) are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the Aberystwyth Research Portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the Aberystwyth Research Portal

#### **Take down policy**

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

tel: +44 1970 62 2400

email: [is@aber.ac.uk](mailto:is@aber.ac.uk)

# Medicine & Science IN Sports & Exercise

The Official Journal of the American College of Sports Medicine  
[www.acsm-msse.org](http://www.acsm-msse.org)

***... Published ahead of Print***

## **Failure of Oral Tyrosine Supplementation to Improve Exercise Performance in the Heat**

Les Tumilty<sup>1</sup>, Glen Davison<sup>2</sup>, Manfred Beckmann<sup>3</sup>, and Rhys Thatcher<sup>1</sup>

<sup>1</sup>Department of Sport and Exercise Science, Aberystwyth University, Wales, United Kingdom; <sup>2</sup>Endurance Research Group, Centre for Sports Studies, University of Kent at Medway, Chatham, United Kingdom; <sup>3</sup>Institute of Biological, Environmental and Rural Sciences, Aberystwyth University, Wales, United Kingdom

Accepted for Publication: 4 December 2013

***Medicine & Science in Sports & Exercise® Published ahead of Print*** contains articles in unedited manuscript form that have been peer reviewed and accepted for publication. This manuscript will undergo copyediting, page composition, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered that could affect the content.

## Failure of Oral Tyrosine Supplementation to Improve Exercise Performance in the Heat

Les Tumilty<sup>1</sup>, Glen Davison<sup>2</sup>, Manfred Beckmann<sup>3</sup>, and Rhys Thatcher<sup>1</sup>

**Institutional affiliation:** <sup>1</sup>Department of Sport and Exercise Science, Aberystwyth University, Wales, United Kingdom.

<sup>2</sup>Endurance Research Group, Centre for Sports Studies, University of Kent at Medway, Chatham, United Kingdom.

<sup>3</sup>Institute of Biological, Environmental and Rural Sciences, Aberystwyth University, Wales, United Kingdom.

**Corresponding author:** Les Tumilty, Department of Sport and Exercise Science, Carwyn James Building, Penglais Campus, Aberystwyth University, Aberystwyth, Ceredigion, Wales, UK, SY23 3FD.

**Telephone:** (+44) 1970 628425

**Facsimile:** (+44) 1970 628557

**E-mail:** llt07@aber.ac.uk.

**Running title:** Tyrosine and endurance exercise in the heat.

**Funding disclosure:** The authors did not receive funding for this work.

**Conflict of Interest:** The authors report no conflict of interests.

## Abstract

**Purpose:** Acute oral tyrosine administration has been associated with increased constant-load, submaximal exercise capacity in the heat. This study sought to determine whether self-paced exercise performance in the heat is enhanced with the same tyrosine dosage.

**Methods:** Following familiarisation, seven male endurance-trained volunteers, unacclimated to exercise in the heat, performed two experimental trials in 30°C (60% relative humidity) in a crossover fashion separated by at least 7 days. Subjects ingested 150 mg·kg body mass<sup>-1</sup> tyrosine (TYR) or an isocaloric quantity of whey powder (PLA) in 500 mL of sugar-free flavoured water in a randomised, double-blind fashion. Sixty minutes following drink ingestion subjects cycled for 60 min at  $57 \pm 4\%$  peak oxygen uptake ( $O_{2peak}$ ), then performed a simulated cycling time-trial requiring completion of an individualised target work quantity ( $393.1 \pm 39.8$  kJ).

**Results:** The ratio of plasma tyrosine plus phenylalanine (tyrosine precursor): amino acids competing for brain uptake (free-tryptophan, leucine, isoleucine, valine, methionine, threonine, lysine) increased 2.5-fold from rest in TYR, and remained elevated throughout exercise ( $P < 0.001$ ), whereas it declined in PLA from rest to pre-exercise ( $P = 0.004$ ). Time-trial power output ( $P = 0.869$ ) and performance ( $34.8 \pm 6.5$  min and  $35.2 \pm 8.3$  min in TYR and PLA, respectively;  $P = 0.4167$ ) were similar between trials. Thermal sensation ( $P > 0.05$ ), RPE ( $P > 0.05$ ), core temperature ( $P = 0.860$ ), skin temperature ( $P = 0.822$ ) and heart rate ( $P = 0.314$ ) did not differ between trials.

**Conclusion:** These data indicate that acute tyrosine administration did not influence self-paced endurance exercise performance in the heat. Plasma tyrosine availability is apparently not a key determinant of fatigue processes under these conditions.

**Keywords:** amino acids; catecholamines; mild hyperthermia; central fatigue; prolonged exercise

## Introduction

Exercise performance is clearly impaired in high ambient temperature compared to cooler conditions (33). This impairment has been primarily attributed to fatigue caused by central nervous system changes, secondary to increased brain temperature (21), although peripheral factors including high skin temperature, dehydration and alterations in circulatory and thermoregulatory factors likely also contribute to this (28). A definitive neurobiological cause has yet to be established for this central fatigue but one, or several, neurochemical systems are likely to be involved. There is a well-defined role for the brain catecholamines dopamine and noradrenaline in increased motivation, arousal and reward (4), acute stress responses (26), motor initiation and control (14) and thermoregulation (9). Therefore, it is plausible these neurotransmitters modulate the central fatigue associated with prolonged exercise in the heat.

The amino acid tyrosine is a nutritional substrate precursor for dopamine and noradrenaline. Brain tyrosine concentrations are above the  $K_m$  for tyrosine hydroxylase, the rate-limiting enzyme in catecholamine synthesis (8). Catecholamine synthesis and release is also limited by neuronal firing rate so that during periods of low impulse flow tyrosine hydroxylase is highly susceptible to catecholamine end-product inhibition (8). Microdialysis measurements in the rat confirm that augmentation of cerebral catecholamine release following systemic tyrosine injection, while under basal conditions, is generally limited in magnitude and duration (12). In animals exposed to extreme environmental stress which substantially increases impulse flow through catecholamine neurons, such as nigrostriatal lesioning, electrical stimulation of cerebral catecholamine pathways or tail-shock, neurotransmitter turnover is elevated and tyrosine may be depleted in some neuronal populations (5, 12, 17). Tyrosine hydroxylase then exhibits increased affinity for tyrosine coupled with a decreased sensitivity to catecholamine end-product inhibition (27). Under these conditions, tyrosine administered to rats by injection or dietary means

maintains cerebral tyrosine levels and augments central nervous system catecholamine turnover in activated neuronal populations (17, 32). Impairments in locomotion, exploratory behaviour, memory and coping behaviour in rats exposed to acute, experimentally-induced stress such as tail-shock, heat or cold exposure are also countered by tyrosine administration (17, 18, 39). There is also a large body of evidence that cognitive, psychomotor and mood impairments and symptom intensity in humans induced by demanding environmental conditions such as cold, hypoxia or prolonged wakefulness, are countered by increasing plasma tyrosine availability via acute oral administration prior to exposure to the environmental conditions (1, 19, 20). Evidence supporting an enhancement of exercise in humans following acute tyrosine supplementation is less clear. Studies have generally failed to show benefits of acute tyrosine administration on exercise capacity, exercise performance, muscle strength and anaerobic power in temperate ambient conditions (7, 30, 31). There are mixed reports of the effectiveness of tyrosine supplementation prior to prolonged exercise in the heat. One recent study reported improved capacity to perform constant-load, submaximal intensity exercise in 30°C heat (60% relative humidity) following acute administration of 150 mg·kg body mass<sup>-1</sup> tyrosine (34) but a separate study using a similar protocol and tyrosine dosage failed to show an effect (35).

Both Tumilty et al. (34) and Watson et al. (35) reported a marked increase in the ratio of plasma tyrosine: neutral amino acids which compete for brain uptake following supplementation. This ratio is a key determinant of brain uptake of a single amino acid rather than the plasma concentration of the amino acid *per se*, as there are several neutral amino acids competing for a shared, saturable carrier molecule across the blood-brain barrier (13). Therefore, increasing the plasma concentration of a single amino acid, or the concentration of competing amino acids, will increase or decrease the brain uptake of a given amino acid, respectively (13).

To date, no study has assessed whether exercise performance (e.g. a time-trial) in the heat is augmented by high tyrosine availability despite evidence that tyrosine supplementation counters the adverse effects of challenging environmental conditions. Due to the self-paced nature of a performance time-trial, which will be highly influenced by motivation and arousal, it is plausible that any improvements following tyrosine supplementation would be more apparent during this type of exercise trial compared to a constant-load capacity trial. Therefore the present study was designed to test the hypothesis that acute tyrosine administration would improve exercise performance in the heat.

## Methods

### Subjects

Eight male volunteers, unacclimated to exercise in the heat, and participating in regular endurance training at least four times per week, gave written informed consent to take part in the study. This sample size provided sufficient power in a previous study to highlight an effect of tyrosine supplementation on prolonged exercise capacity in the heat (34). One subject did not complete testing due to injury and the corresponding data was omitted from all statistical analysis. The remaining seven subjects, classified as Performance level 3 (10), [six competitive cyclists and one competitive runner with regular experience of cycling; median age, 20 (range, 26) years; median stature, 1.83 (range, 0.13) m; mean  $\pm$  SD for body mass,  $77.9 \pm 11.7$  kg; peak oxygen uptake ( $O_{2peak}$ ),  $60 \pm 11$  mL $\cdot$ kg $\cdot$ min $^{-1}$ ; peak power output attained during ramp test on a cycle ergometer to elicit  $O_{2peak}$ ,  $389 \pm 44$  W; maximal heart rate attained during ramp test,  $186 \pm 8$  beats $\cdot$ min $^{-1}$ ] completed all trials. Testing was carried out between October and June in Wales within the UK when the average daytime air temperature typically ranges between 3°C and 17

°C. All subjects were resident here for at least one month before the commencement of testing. The study was approved by Aberystwyth University Research Ethics Committee.

## **Experimental procedures**

Subjects visited the lab on five separate occasions: an initial ramp test to determine peak power output, maximal heart rate and  $O_{2peak}$  using an online breath-by-breath system (Jager Oxycon Pro, Hoechberg, Germany); 2 familiarisation visits; and 2 main experimental trials. No strenuous or unaccustomed exercise was permitted for 24 h before each test. Subjects were instructed to sleep for  $\geq 8$  h the night before each laboratory visit to ensure they were rested, and verbal confirmation of adherence to this was given on arrival for each test day. All exercise was performed on an electrically braked cycle ergometer (Lode Excalibur Sport, Groningen, Netherlands). Subjects wore comfortable clothing, which was kept consistent between trials, typically consisting of shorts, t-shirt and sports shoes or cycling shorts, short-sleeved cycling top and cycling shoes. During the ramp test, power output was increased at a rate of  $0.5 \text{ W}\cdot\text{s}^{-1}$  until the subject reached volitional exhaustion and  $O_{2peak}$  was determined as the highest oxygen uptake ( $O_2$ ) averaged over a 30 s period. Gas exchange threshold was identified using the v-slope method, determined as the first disproportionate increase in carbon dioxide output ( $CO_2$ ) relative to  $O_2$  and an increase in the ventilatory equivalent for  $O_2$  in relation to a levelling off or continued decrease in the ventilatory equivalent for  $CO_2$  (2). Heart rate was recorded continuously during all trials using radiotelemetry (Polar RS800CX, Polar Electro Oy, Tampere, Finland) and the peak value measured during the ramp test was recorded as maximal heart rate. At least 48 h elapsed between the ramp test and the first familiarisation visit and at least 7 d separated each of the remaining four trials. The familiarisation visits were designed to appease any anxiety and to



allow subjects to become accustomed to the time-trial protocol in the heat. These visits were identical to the main experimental trials except that blood samples were not taken and the placebo drink (see below) was administered for both familiarisations. All of the familiarisation and main trials commenced between 0630 h and 0930 h with each subject performing all four trials at the same time of day to control for diurnal variation. Subjects recorded their food and drink intake for 48 h, and physical activity for 24 h, prior to the first familiarisation, and this enabled duplication prior to each subsequent visit. After arriving at the laboratory following an overnight fast of at least 8 h, except for 500 mL of ordinary tap water which they drank exactly 2 h before arriving, subjects emptied their bladder into a pyrex beaker. Urine volume was measured to the nearest mL before a 1.0 mL aliquot was frozen at -80°C for later measurement of osmolality, in duplicate, using freezing point depression (Osmostat 030, Gonotec, Berlin). Nude, post-void body mass was measured to the nearest 0.1 kg (Seca 645, Seca GMB and Co., Hamburg, Germany), and stature was recorded (Holtain Ltd., stadiometer, Crymych, UK). A rectal thermistor (Grant Instruments, Cambridge, England) was self-positioned by each subject 10 cm beyond the anal sphincter to enable core temperature measurement ( $T_{core}$ ) and surface skin temperature probes (Grant Instruments, Cambridge, England) were attached to the calf, thigh, chest and tricep using breathable medical tape (Hypafix, Bsn Medical, Hull, UK) so skin temperature ( $T_{skin}$ ) could be measured.  $T_{core}$  and  $T_{skin}$  were recorded from an electronic data logger (Squirrel SQ2020, Grant Instruments, Cambridge, England) and from these data, mean weighted skin temperature was calculated (24). Subjects were seated for 15 min to minimize the effect of plasma volume changes before a 10.5 mL resting blood sample was obtained (Rest), with minimal stasis, from an antecubital vein, comprising 6 mL into a heparinised vacutainer and 4.5 mL into a  $K_3$ EDTA treated vacutainer (BD Vacutainer Systems, Plymouth, UK). The experimental or placebo drink were administered in a randomised, double-blind and counterbalanced manner, before subjects were seated in a quiet, comfortable environment (20.5

$\pm 0.4$  °C,  $44 \pm 6\%$  relative humidity) for 1 h. The experimental drink (TYR) contained  $150 \text{ mg} \cdot \text{kg body mass}^{-1}$  tyrosine (SHS international Ltd., Liverpool, UK) and 7 g vanilla flavouring (Myprotein.co.uk, Cheadle, Cheshire, UK) in 500 mL of fluid [ordinary tap water with 40% sugar-free lemon and lime squash (Morrisons, Bradford, UK)]. This tyrosine dosage was associated with increased exercise capacity in the heat in an earlier study (34). The placebo drink (PLA) contained the same fluid volume and content, with an isocaloric quantity of hydrolysed whey protein (Myprotein.co.uk, Cheadle, Cheshire, UK) instead of tyrosine (equating to  $11.6 \pm 1.6$  g tyrosine or  $6.1 \pm 0.8$  g whey protein;  $22 \pm 3$  kcal) to ensure any performance effects of tyrosine were not due to the additional energy content of the experimental drink. All drinks were coded and prepared by a separate drinks supervisor to ensure they were allocated in a double-blind manner. Prior pilot work with three volunteers, who did not participate in this study, confirmed the drinks were indistinguishable in colour, taste and texture. Care was taken to properly blend the mixtures, which were served in opaque drinking bottles, and were shaken vigorously immediately before ingestion. At the end of the 1 h period, a second 10.5 mL blood sample (Pre) was taken before subjects entered the climate chamber (Design Environmental, Gwent, Wales). Temperature and relative humidity of the chamber were maintained at  $30.1 \pm 0.2$ °C,  $60 \pm 0\%$  respectively, and mean air velocity within the chamber was  $0.26 \pm 0.1 \text{ m} \cdot \text{s}^{-1}$  during all trials. Once inside the chamber, subjects commenced cycling, without a warm up, at a constant power output equivalent to  $10\% \Delta$  [power output requiring a  $\text{O}_2$  that is 10% of the difference between the  $\text{O}_2$  at the gas exchange threshold and  $\text{O}_{2\text{peak}}$  (16);  $156 \pm 24$  W or  $57 \pm 4\%$   $\text{O}_{2\text{peak}}$  in this subject group] for 60 min. The purpose of this exercise period was to induce hyperthermia before commencement of the time trial. No information on time elapsed or motivational encouragement was given to the subjects throughout this exercise period. Drinks ( $2 \text{ mL} \cdot \text{kg body mass}^{-1}$  ordinary tap water with 20% sugar-free lemon and lime squash) were provided at 15, 30, 45 and 59 min. At the end of the 60 min of cycling subjects were quickly

removed to a chair directly adjacent to the cycle ergometer within the climate chamber, where a further 10.5 mL blood sample was obtained (Post 60). Subjects then remounted the cycle ergometer to perform a simulated cycling time-trial. A maximum of 2 min elapsed between subjects dismounting the ergometer and the acquisition of the Post 60 blood sample. The time taken to obtain this sample, and the time between the end of the 60 min of cycling and the commencement of the time-trial, was standardised for each individual subject during subsequent trials.

The time-trial was based on a validated protocol used in previous research examining fatigue during prolonged exercise in the heat (36), and requires subjects to complete a set amount of work as quickly as possible. Individual target work quantities were calculated for each subject as the amount of work which would be completed during 30 min cycling at 60% of the power output eliciting  $O_{2peak}$  during the initial ramp test ( $393.1 \pm 39.8$  kJ in this group). The cycle ergometer was set in linear mode (workload increases as pedalling rate increases) during the time-trial so that the power output, and hence the completed work, was directly related to pedalling cadence. At the start of the time-trial subjects were given their target work quantities and instructed to complete the required amount of work as quickly as possible and no other instructions or motivational encouragement was given throughout. The control console for the ergometer was positioned on the bike frame, and the display was masked with non-transparent adhesive tape so that only the cumulative work portion of the console display was visible. No further feedback on power output, cadence or time elapsed was provided throughout the time-trial. A cumulative work target was also taped to the front of the ergometer handlebars, in full view of the subjects, which detailed the required work to achieve 25%, 50%, 75%, and 100% of their individual target. Subjects were permitted to drink *ad-libitum* throughout (ordinary tap water with 20% sugar-free lemon and lime squash). On completion of the time-trial subjects

dismounted the cycle ergometer, moved quickly to the adjacent chair where a final 10.5 mL blood sample (Post TT) was obtained within a maximum of 2 min, and this time was also standardised for each individual subject during subsequent trials. Subjects emptied their bladder, urine volume was again recorded to the nearest mL before a 1.0 mL aliquot was removed, then frozen at -80°C for later osmolality measurement. Finally, the thermistor and skin probes were removed before subjects showered, towelled dry and then were reweighed nude.

### **Physiological measurements and blood analysis**

Heart rate, Tcore and Tskin were recorded every 10 min throughout 60 min rest and 60 min of submaximal exercise. Power output, heart rate, Tcore and Tskin were recorded at the start of the time-trial, and every 5 min throughout exercise. RPE (3) and thermal sensation, using a 21 point scale ranging from -10 (cold impossible to bear) to +10 (heat impossible to bear) (adapted from 22), were recorded every 10 min throughout 60 min submaximal exercise, after 5 min of the time-trial had elapsed, and then every 5 min throughout the time-trial. One minute expired gas samples were collected in Douglas bags at 30 min and 50 min of the constant-load exercise period. Oxygen and carbon dioxide concentrations were measured using a combined paramagnetic oxygen analyser and infrared carbon dioxide analyser (Series 4100 Xentra, Servomex, Crowborough, UK), which were calibrated before each trial using commercial gases (BOC, Guildford, UK), and expired volume was measured using a dry gas meter (Harvard Apparatus Ltd., Edenbridge, UK). Expired gases were used to estimate fat and carbohydrate utilisation and energy expenditure (23). Final measurements of heart rate, Tcore, Tskin, RPE, thermal sensation, and power output were recorded immediately before subjects completed the time-trial. Wind speed within the chamber was measured every 15 min of exercise as the mean

value of four measurements taken from in front, behind, and to the left and right of each subject at head height while seated on the cycle ergometer, using a hand held anemometer (Kestrel 1000, Richard Paul Russell Ltd, Lymington, UK).

Blood from the K<sub>3</sub>EDTA vacutainer was used to measure haematocrit, haemoglobin, lactate and glucose. Whole blood was drawn into micro-capillary tubes, spun for 5 min at 14000 g using a Hawksley microcentrifuge (Haematospin 1400, Hawksley, Lancing, UK) and the separated red cell volume was measured using a Hawksley haematocrit tube reader, with a coefficient of variation of 0.8% for 10 repeated measurements on the same sample. Haemoglobin was measured using an automated haematology analyser (Pentra 60C +, Horiba ABX Diagnostics, Northampton, UK), with a coefficient of variation of 0.3% for 10 repeated measurements on the same sample standard, containing 14.0 g·L<sup>-1</sup> haemoglobin. Haematocrit and haemoglobin measurements were used to estimate plasma volume changes (11). Blood glucose and blood lactate concentrations were measured using an automated analyser (2300 Stat Plus, Yellow Spring Instrument Co., Ohio, USA), calibrated with standard concentrations for glucose (0.00, and 50.00 mmol·L<sup>-1</sup>) and lactate (0.00 and 30.00 mmol·L<sup>-1</sup>). The coefficient of variation for 10 repeated measurements on a sample standard for blood glucose (6.14 mmol·L<sup>-1</sup>) and blood lactate (5.35 mmol·L<sup>-1</sup>) is 1.7% and 1.5%, respectively. The heparinised blood was immediately centrifuged at 1500 g for 10 minutes at 4°C, the plasma was then separated, and stored at -80°C for later analysis of amino acids using gas chromatography mass spectrometry (34). The coefficient of variation for the measurement of individual plasma amino acids using this technique is: leucine, 8.5%; isoleucine, 9.7%; valine, 5.8%; methionine, 11.4%; threonine, 8.7%; lysine, 5.0%; the free fraction of tryptophan unbound from albumin (free-tryptophan), 5.2%; phenylalanine, 7.5% and tyrosine, 6.5%. Haematocrit was measured in triplicate and all

remaining blood analyses were measured in duplicate. All blood parameters were corrected for plasma volume changes from baseline measurements at Rest.

## **Statistical analyses**

A computerized statistical package was used to analyse all data (SPSS version 17.0, SPSS inc., Chicago, IL). Normally distributed data are presented as mean  $\pm$  SD. Time trial performance, urine osmolality and rate of body mass losses were examined using Student's paired *t*-test. Cohen's *d* effect size was calculated for the difference in time to complete the time-trial. Differences in data throughout trials were compared using a repeated measures 2 factor (time  $\times$  trial) analysis of variance (ANOVA). Where significant differences were found, *post hoc* analysis was carried out using Student's paired *t*-tests with the Bonferroni correction. End of time-trial values were analysed separately using Student's paired *t*-test to account for the different exercise duration between subjects and trials. Non-normally distributed data are presented as median (range), were analysed using Friedman's tests, and where appropriate, *post hoc* tests were carried out using Wilcoxon matched pairs tests with the Bonferroni correction. Statistical significance was set at  $P < 0.05$ .

## **Results**

### **Time-trial performance**

Following completion of all trials, three subjects reported they felt better or more motivated during the tyrosine trial, but could not distinguish between the TYR and PLA drinks, two subjects incorrectly thought they had received PLA when they had received TYR, and the remaining two subjects were unable to distinguish between trials, indicating successful drinks-

blinding. The coefficient of variation for time to complete the time-trial between the 2<sup>nd</sup> familiarisation trial and the PLA trial was 1.3% and there was no effect of trial order during the experimental trials ( $P = 0.313$ ). TYR did not influence the time to complete the time-trial ( $P = 0.417$ ;  $34.8 \pm 6.5$  min in TYR and  $35.2 \pm 8.3$  min in PLA; Fig. 1A) and power output throughout the time-trial was similar in both trials ( $P = 0.869$ ; Fig. 1B). The effect size for TYR versus PLA is calculated as 0.05 and the resultant statistical power value is 0.11. A SD of 0.6 would be required to detect significant differences in time trial performance between TYR and PLA with 7 subjects per group, yielding an estimated statistical power value of 0.80 ( $P = 0.05$ ; 37). Subjects' performance was even-paced up to 20 min of the time-trial ( $P = 0.061$ ), which represented the last time-point at which all subjects were still exercising.

### **Blood analysis**

Plasma tyrosine concentration at Rest was similar in both trials ( $P = 0.269$ ; Fig. 2A), but was higher in TYR at all remaining sampling times ( $P < 0.01$ ), while the concentration remained unchanged from Rest in PLA ( $P > 0.05$ ). Plasma phenylalanine concentration was unchanged at any time-point in TYR ( $P > 0.05$ ) but was elevated from Rest in PLA on completion of the time-trial ( $P < 0.01$ ). There was no difference between trials in the ratio of plasma tyrosine plus phenylalanine:  $\Sigma$ (free-tryptophan; valine, leucine, isoleucine, threonine, methionine, lysine) at Rest ( $P = 0.838$ ; Fig. 2B) but the ratio was higher in TYR at all other time-points ( $P = 0.001$ ). TYR ingestion increased this ratio over 2.5-fold ( $P < 0.001$ ) from Rest and remained elevated at all other sampling times ( $P < 0.001$ ). In PLA, there was a transient decline in this ratio from Rest to Pre-exercise ( $P = 0.004$ ). The plasma concentrations of the remaining individual amino acids analysed are provided in Table 1. Blood glucose concentration was unaffected by drink

ingested ( $P > 0.05$ ) or exercise ( $P = 0.801$ ) and was  $5.3 \pm 0.9 \text{ mmol}\cdot\text{L}^{-1}$  in both trials at Post TT. At Post 60, blood lactate concentration had increased from Rest in both trials ( $P = 0.018$ ), remaining elevated from Rest at Post TT ( $P = 0.018$ ), but did not differ between trials [ $P > 0.05$ ;  $3.2$  (range,  $3.6$ )  $\text{mmol}\cdot\text{L}^{-1}$  in TYR and  $4.1$  (range,  $4.9$ )  $\text{mmol}\cdot\text{L}^{-1}$  in PLA]. Plasma volume progressively declined, and to a similar extent in both trials, following drink ingestion ( $P < 0.05$ ) reaching  $-8.4 \pm 6.0\%$  in TYR and  $-9.1 \pm 4.5\%$  in PLA at end of time-trial.

### Temperature measurements

Exercise caused a gradual increase in Tcore ( $P = 0.003$ ) at a similar rate in both trials ( $P = 0.131$ ; Fig. 3A). At the end of the time-trial Tcore was  $39.0 \pm 0.7^\circ\text{C}$  in TYR and  $38.9 \pm 0.6$  in PLA ( $P = 0.474$ ). Tskin also increased during exercise ( $P = 0.012$ ), plateauing after 20 min of constant-load exercise and after 15 min of time-trial (Fig. 3B). The drink ingested had no influence on Tskin ( $P = 0.822$ ) and there was no difference between trials in Tskin at end of the time-trial ( $34.8 \pm 0.8^\circ\text{C}$  in TYR and  $35.0 \pm 1.3^\circ\text{C}$  in PLA;  $P = 0.575$ ).

### Heart rate

There was a gradual increase in heart rate throughout exercise (Fig. 4;  $P < 0.001$ ) which was unaffected by drink ingested ( $P = 0.314$ ). At end of the time-trial heart rate reached  $100 \pm 4\%$  and  $99 \pm 4\%$  of maximum in TYR and PLA, respectively ( $P = 0.729$ ).



### **Urine analysis, fluid intake and body mass losses**

Pre-exercise urine osmolality suggested that subjects were similarly hydrated before both trials ( $P = 0.571$ ;  $329 \pm 136$  mosmol $\cdot$ kg $^{-1}$  in TYR and  $369 \pm 148$  mosmol $\cdot$ kg $^{-1}$  in PLA). After the time-trial urine osmolality was similar in each trial ( $198 \pm 56$  mosmol $\cdot$ kg $^{-1}$  in TYR and  $325 \pm 296$  mosmol $\cdot$ kg $^{-1}$  in PLA;  $P = 0.218$ ) and unchanged from pre-exercise ( $P = 0.202$ ). Subjects drank similar volumes of fluid during the time-trial in both trials ( $311.1 \pm 114.4$  mL in TYR and  $363.0 \pm 126.1$  mL in PLA;  $P = 0.415$ ). By the end of the time-trial body mass losses, calculated from the difference between pre- and post-exercise body mass adjusted for fluid intake and urine output following initial weighing ( $2.9 \pm 0.7\%$  and  $3.1 \pm 1.1\%$  of pre-exercise body mass in TYR and PLA, respectively;  $P = 0.528$ ), and the rate of body mass loss ( $P = 0.436$ ;  $4.0 \pm 1.0$  kg $\cdot$ h $^{-1}$  in TYR and  $4.3 \pm 1.6$  kg $\cdot$ h $^{-1}$  in PLA) were similar in both trials.

### **Subjective ratings**

Expressed RPE gradually increased throughout exercise (Fig. 5A). There was no difference in RPE between trials at any time-point ( $P > 0.05$ ). Median RPE at end of the time-trial was 19 (range, 3) arbitrary units in both trials ( $P = 0.689$ ). Thermal sensation gradually increased throughout exercise (Fig. 5B) and was the same in TYR and PLA throughout exercise ( $P > 0.05$ ). Median ratings at end of the time-trial were 7 (range, 5) arbitrary units in TYR, representing a rating between “Very hot, uncomfortable” and “Extremely hot, close to limit”. Median thermal sensation at end of the time-trial in PLA was similar to TYR ( $P = 0.276$ ), and was 8 (range, 6) arbitrary units representing a rating of “Extremely hot, close to limit”.

### **Estimated substrate usage**

Estimated fat ( $P = 0.253$ ) and carbohydrate ( $P = 0.290$ ) oxidation rates and estimated energy expenditure ( $P = 0.200$ ) were unchanged from 30 to 50 min of constant-load submaximal exercise, and were similar in both trials ( $P > 0.05$ ).

### **Discussion**

Previous studies have examined the effect of tyrosine administration on exercise capacity in the heat in man (34, 35), but this is the first study to examine self-paced exercise performance in the heat following acute oral tyrosine administration. The present results demonstrate that, contrary to the hypothesis, acute administration of tyrosine did not improve self-paced exercise performance in the heat compared to a placebo containing an isocaloric quantity of hydrolysed whey protein.

It was hypothesized that additional availability of tyrosine, a nutritional catecholamine precursor, would enhance exercise tolerance in the heat, as reported in an earlier study adopting a constant-load submaximal intensity exercise protocol (34). Additionally, it was expected that any performance effect of tyrosine administration would be more pronounced during a variable intensity time-trial, where subjects could self-pace the exercise and thus the subjective effort, compared to a constant-load trial. The similar power output profiles in each time-trial in the present study, and therefore similar time to complete a set amount of work irrespective of drink ingested, suggests that tyrosine has no performance enhancing effect under the conditions of the present study. This occurred despite a sound neurochemical basis for a benefit of tyrosine administration prior to performance of a time-trial in the heat. The clear impairment in exercise

performance carried out in warm compared to cooler ambient temperature (33) seems to be caused, in part, by alterations in cerebral function, caused by high brain temperature, resulting in reduced voluntary activation of muscle, increased subjective effort and a reduced will or “drive” to exercise (21). Changes in central catecholamine activity are implicated in these processes due to their intrinsic connection with increased arousal and motivation (4), thermoregulation (9, 15) and motor initiation and control (14). Additionally, pharmacological augmentation of central catecholamine activity, via acute bupropion administration, improves simulated time-trial performance in the heat (30°C) but not in cooler conditions (18°C) (36), highlighting the importance of cerebral catecholamine activity in the ability to tolerate exercise with heat stress. As tyrosine is a general catecholamine precursor, the experimental drink may have augmented central noradrenaline activity and this may offer partial explanation for the present results. Recent work has reported an impairment in time trial performance in the heat following acute administration of a noradrenaline reuptake inhibitor, reboxetine, compared to a lactose placebo (25). Enhanced noradrenaline activity in the present study following tyrosine administration may have constrained any performance effect during the simulated time trial.

The current protocol was employed on the basis that the combination of exercise and heat stress would represent a suitably demanding environment such that catecholamine neuronal sensitivity would be up-regulated to precursor availability. The absence of effect on exercise performance in the present study occurred despite striking differences between the plasma amino acid profiles between trials following drink ingestion. The marked increase in the ratio of plasma tyrosine plus phenylalanine: amino acids competing for brain uptake following tyrosine administration was similar in magnitude to one study in which exercise capacity in the heat was prolonged by 15%, 1 hour following administration of 150 mg·kg<sup>-1</sup> tyrosine (34). This would favour the transport of tyrosine into the brain at the expense of other neutral amino acids which compete for

uptake at the blood-brain barrier (13). This plasma ratio transiently declined following ingestion of the placebo mixture in the present study which would reduce brain entry of tyrosine (13).

At least one other study examining tyrosine administration prior to prolonged exercise in the heat provides support for the idea that exercise tolerance in the heat is enhanced following prior oral tyrosine administration (34). But a separate study employing a similar tyrosine dosage and exercise protocol reported no effect (35). It is not entirely clear why there are discrepant findings between the present study and previous work reporting benefit of tyrosine administration prior to exercise in the heat (34). This could be related to the degree of arousal and stress induced by the different protocols adopted, and therefore the degree of activation of the central catecholaminergic system, and the factors involved in the regulation of tyrosine hydroxylase and catecholamine synthesis described above. This may also explain why studies examining acute tyrosine administration in man prior to exercise in temperate conditions, have largely failed to report beneficial effects on exercise capacity (30), simulated time-trial performance (7) or muscle strength and anaerobic endurance (31). While it seems apparent that, in man, exercise alone in the absence of heat stress is insufficiently demanding to up-regulate catecholamine precursor demand (7, 30), this does not adequately explain the discrepant findings between two studies adopting similar prolonged exercise protocols in the heat and tyrosine dosage (34, 35) and the underlying reasons for this require further clarification. Perhaps the adoption of a range of tyrosine doses in future work, particularly higher doses than administered in the present study, may identify whether there is an optimal dose which enhances prolonged exercise in the heat.

As already mentioned, high brain catecholaminergic activity is associated with increased arousal and motivation (4) therefore an augmentation of central catecholamine following tyrosine administration might be expected to highlight differences in the power output profiles during the

time-trial or the subjective response to exercise. By definition, a self-paced time-trial allows the power output throughout the time-trial and thus the relative metabolic demand to be controlled from moment-to-moment. The time-trial power output data in the present study suggest that subjects adopted an even-paced strategy throughout the first 20 min of the time-trial, representing the last common time-point at which all subjects were still exercising, and that plasma tyrosine availability did not affect this pacing strategy. Some investigators suggest that the adoption of a pacing strategy is necessary for the avoidance of catastrophic failure of the organism (29). This is hypothesized to involve feedback and feed-forward control mechanisms in which the brain processes efferent neural commands based on previous experience of similar situations, in order to elicit the most appropriate power output and metabolic rate (29). The present power output data demonstrate that even if a subconscious anticipation of the exercise power output was adopted, tyrosine had no additional effect on this compared to an isocaloric quantity of whey protein. Furthermore, the present data suggests that the subjective interpretation of the exercise cannot be manipulated by a plasma amino acid profile, following ingestion of the experimental mixture, which would favour increased brain uptake of a direct catecholamine precursor (13).

In summary, there was no effect of acute tyrosine administration on simulated time-trial performance in the heat compared to a placebo containing an isocaloric quantity of whey protein. The lack of an association between increased tyrosine availability and exercise performance suggests that, under the conditions of the present study, acutely increasing plasma tyrosine availability does not influence fatigue processes when self-paced endurance exercise is performed with heat stress.

## **Acknowledgements**

The authors wish to thank SHS International for the kind donation of tyrosine powder for use in this study.

## **Funding disclosure**

The authors did not receive funding for this work.

## **Conflict of Interest**

The authors report no conflict of interests. The results of the present study do not constitute endorsement by ACSM.

## References

1. Banderet LE, Lieberman HR. Treatment with tyrosine, a neurotransmitter precursor, reduces environmental stress in humans. *Brain Res Bull.* 1989;22(4):759-62.
2. Beaver WL, Wasserman K, Whipp BJ. A new method for detecting anaerobic threshold by gas exchange. *J Appl Physiol.* 1986;60(6):2020-7.
3. Borg GA. Psychophysical bases of perceived exertion. *Med Sci Sports Exerc.* 1982;14(5):377-81.
4. Burgess ML, Davis JM, Borg TK, Buggy J. Intracranial self-stimulation motivates treadmill running in rats. *J Appl Physiol.* 1991;71(4):1593-7.
5. Charles Murrin L, Morgenroth III VH, Roth RH. Dopaminergic neurons: effects of electrical stimulation on tyrosine hydroxylase. *Mol Pharmacol.* 1976;12(6):1070-81.
6. Cheung SS, McLellan TM. Heat acclimation, aerobic fitness, and hydration effects on tolerance during uncompensable heat stress. *J Appl Physiol.* 1998;84(5):1731-9.
7. Chinevere TD, Sawyer RD, Creer AR, Conlee RK, Parcell AC. Effects of L-tyrosine and carbohydrate ingestion on endurance exercise performance. *J Appl Physiol.* 2002;93(5):1590-7.
8. Cooper JR, Bloom FE, Roth RH. *The Biochemical Basis of Neuropharmacology.* London: Oxford University Press; 2003., pp. 225-270.
9. Cox B, Lee T. Further evidence for a physiological role for hypothalamic dopamine in thermoregulation in the rat. *J Physiol.* 1980;300:7 - 17.
10. De Pauw K, Cheung SS, de Geus B, Rietjens G, Meeusen R. Guidelines to classify subject groups in sport-science research. *Int J Sports Physiol Perform.* 2013;8(2):111-22.

11. Dill DB, Costill DL. Calculation of percentage changes in volumes of blood, plasma, and red cells in dehydration. *J Appl Physiol.* 1974;37(2):247-8.
12. During MJ, Acworth IN, Wurtman RJ. Dopamine release in rat striatum: physiological coupling to tyrosine supply. *J Neurochem.* 1989;52(5):1449-54.
13. Fernstrom JD, Faller DV. Neutral amino acids in the brain: changes in response to food ingestion. *J Neurochem.* 1978;30(6):1531-8.
14. Freed CR, Yamamoto BK. Regional brain dopamine metabolism: a marker for the speed, direction, and posture of moving animals. *Science.* 1985;229(4708):62-5.
15. Hasegawa H, Meeusen R, Sarre S, Diloer M, Piacentini MF, Michotte Y. Acute dopamine/norepinephrine reuptake inhibition increases brain and core temperature in rats. *J Appl Physiol.* 2005;99(4):1397-401.
16. Jones AM, Poole DC. Introduction to oxygen uptake kinetics and historical development of the discipline. In: AM Jones, DC Poole editors. *Oxygen Uptake Kinetics in Sport, Exercise and Medicine.* London: Routledge; 2005, pp. 3-35.
17. Lehnert H, Reinstein DK, Strowbridge BW, Wurtman RJ. Neurochemical and behavioral consequences of acute, uncontrollable stress: effects of dietary tyrosine. *Brain Res.* 1984;303(2):215-23.
18. Lieberman HR, Georgelis JH, Maher TJ, Yeghiayan SK. Tyrosine prevents effects of hyperthermia on behavior and increases norepinephrine. *Physiol Behav.* 2005;84(1):33-8.
19. Mahoney CR, Castellani J, Kramer FM, Young A, Lieberman HR. Tyrosine supplementation mitigates working memory decrements during cold exposure. *Physiol Behav.* 2007;92(4):575-82.



20. Neri DF, Wiegmann D, Stanny RR, Shappell SA, McCardie A, McKay DL. The effects of tyrosine on cognitive performance during extended wakefulness. *Aviat Space Environ Med.* 1995;66(4):313-9.
21. Nybo L. Brain temperature and exercise performance. *Exp Physiol.* 2012;97(3):333-9.
22. Parsons K. *Human Thermal Environments*. London: Taylor and Francis; 2003. Pp. 81-84.
23. Perronet F, Massicote D. Table of non-protein respiratory quotient: an update. *Can J Sports Sci.* 1991;16:23-9.
24. Ramanathan NL. A New Weighting System for Mean Surface Temperature of the Human Body. *J Appl Physiol.* 1964;19:531-3.
25. Roelands B, Goekint M, Heyman E et al. Acute norepinephrine reuptake inhibition decreases performance in normal and high ambient temperature. *J Appl Physiol.* 2008;105(1):206-12.
26. Roth RH, Tam SY, Ida Y, Yang JX, Deutch AY. Stress and the mesocorticolimbic dopamine systems. *Ann N Y Acad Sci.* 1988;537:138-47.
27. Roth RH, Walters JR, Morgenroth III VH. Effects of Alteration in Impulse Flow on Transmitter Metabolism in Central Dopaminergic Neurons. In: E Usdin editor. *Neuropsychopharmacology of Monamines and Their Regulatory Enzymes*. New York: Raven Press; 1974, pp. 369-84.
28. Sawka MN, Cheuvront SN, Kenefick RW. High skin temperature and hypohydration impair aerobic performance. *Exp Physiol.* 2012;97(3):327-32.
29. St Clair Gibson A, Lambert EV, Rauch LH et al. The role of information processing between the brain and peripheral physiological systems in pacing and perception of effort. *Sports Med.* 2006;36(8):705-22.

30. Strüder HK, Hollmann W, Platen P, Donike M, Gotzmann A, Weber K. Influence of paroxetine, branched-chain amino acids and tyrosine on neuroendocrine system responses and fatigue in humans. *Horm Metab Res.* 1998;30(4):188-94.
31. Sutton EE, Coill MR, Deuster PA. Ingestion of tyrosine: effects on endurance, muscle strength, and anaerobic performance. *Int J Sport Nutr Exerc Metab.* 2005;15(2):173-85.
32. Sved AF, Fernstrom JD, Wurtman RJ. Tyrosine administration reduces blood pressure and enhances brain norepinephrine release in spontaneously hypertensive rats. *Proc Natl Acad Sci U S A.* 1979a;76(7):3511-4.
33. Tatterson AJ, Hahn AG, Martin DT, Febbraio MA. Effects of heat stress on physiological responses and exercise performance in elite cyclists. *J Sci Med Sport.* 2000;3(2):186-93.
34. Tumilty L, Davison G, Beckmann M, Thatcher R. Oral tyrosine supplementation improves exercise capacity in the heat. *Eur J Appl Physiol.* 2011;111(12):2941-50.
35. Watson P, Enever S, Page A, Stockwell J, Maughan RJ. Tyrosine supplementation does not influence the capacity to perform prolonged exercise in a warm environment. *Int J Sp Nutr ExMetab.* 2012;22(5):363-73.
36. Watson P, Hasegawa H, Roelands B, Piacentini MF, Loooverie R, Meeusen R. Acute dopamine/noradrenaline reuptake inhibition enhances human exercise performance in warm, but not temperate conditions. *J Physiol.* 2005;565(Pt 3):873-83.
37. Welkowitz J, Cohen BH, Lea RB. *Introductory Statistics for the Behavioural Sciences.* 7th ed. New Jersey: Wiley; 2012, pp. 281-311.
38. Wurtman RJ, Larin F, Mostafapour S, Fernstrom JD. Brain catechol synthesis: control by brain tyrosine concentration. *Science.* 1974;185(146):183-4.

39. Yeghiayan SK, Luo S, Shukitt-Hale B, Lieberman HR. Tyrosine improves behavioral and neurochemical deficits caused by cold exposure. *Physiol Behav.* 2001;72(3):311-6.

ACCEPTED

**FIGURE 1**—Mean  $\pm$  SD (columns and vertical error bars) and individual (remaining individual lines) times to complete simulated cycling time-trial (A) and power output during time-trial, up to the last time point all subjects were exercising and value recorded at end of time-trial (B), in tyrosine (TYR) and placebo (PLA) trials. Data are expressed as mean  $\pm$  SD, ( $n = 7$ ).

**FIGURE 2**—Changes in plasma tyrosine concentration (A), tyrosine ratio [plasma concentration ratio of tyrosine plus phenylalanine:  $\Sigma$ (f-tryptophan; valine, leucine, isoleucine, threonine, methionine, lysine) (B) in tyrosine (TYR) and placebo (PLA) trials. TT, time-trial. ##Significant difference between trials,  $\dagger\dagger$ significantly different versus Rest in TYR, ( $P < 0.01$ );  $\S$ significantly different versus Rest in PLA, ( $P < 0.05$ ). Data are expressed as mean  $\pm$  SD, ( $n = 7$ ).

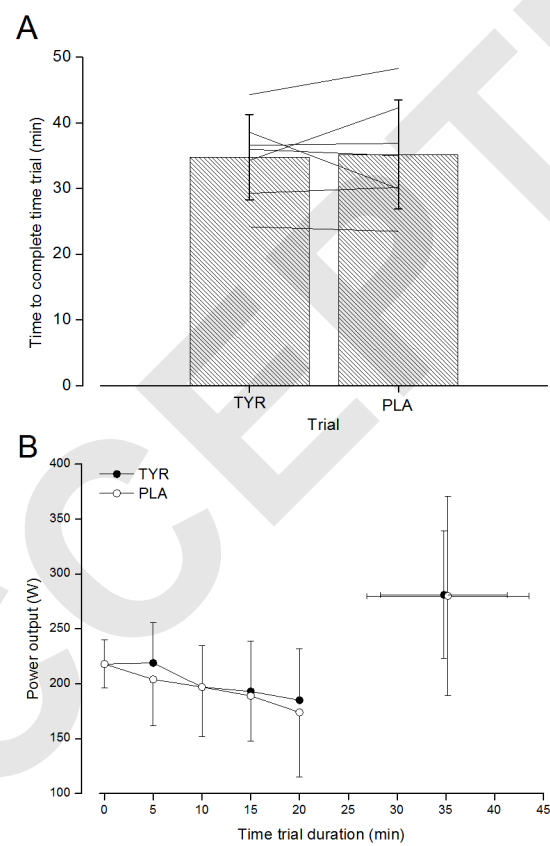
**FIGURE 3**—Core temperature (A) and mean weighted skin temperature (B) responses during 1 h rest, 60 min of constant-load submaximal exercise and simulated cycling time-trial (TT), up to the last time point that all subjects were exercising and value recorded at end of TT, in tyrosine (TYR) and placebo (PLA) trials. \*\*Significantly different versus start value in both trials, ( $P < 0.01$ ). Data are expressed as mean  $\pm$  SD, ( $n = 7$ ).

**FIGURE 4**—Heart rate responses during 1 h rest, 60 min of constant-load submaximal exercise and simulated cycling time-trial (TT), up to the last time point that all subjects were exercising and value recorded at end of TT, in tyrosine (TYR) and placebo (PLA) trials. \*\*Significantly different versus start value in both trials, ( $P < 0.01$ ). Data are expressed as mean  $\pm$  SD, ( $n = 7$ ).

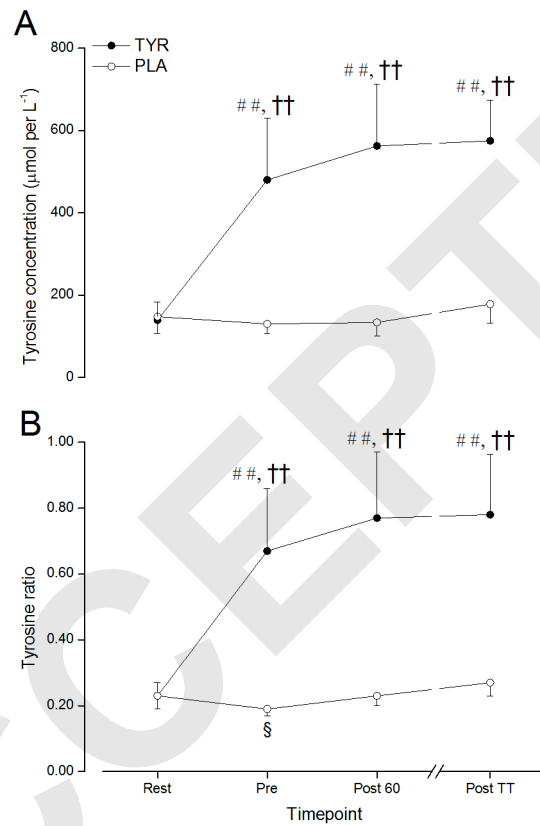
**FIGURE 5**—RPE (A) and thermal sensation ratings (B) during 60 min of constant-load submaximal cycling and simulated cycling time-trial (TT), up to the last time point that all subjects were exercising and value recorded at end of TT, in tyrosine (TYR) and placebo (PLA) trials. \*Significantly different versus 10 min rating in both trials, ( $P < 0.05$ ). Data are expressed as mean  $\pm$  SD, ( $n = 7$ ).

**TABLE 1**—Plasma amino acid concentrations in tyrosine (TYR) and placebo (PLA) trials.

**Figure 1**



**Figure 2**



**Figure 3**

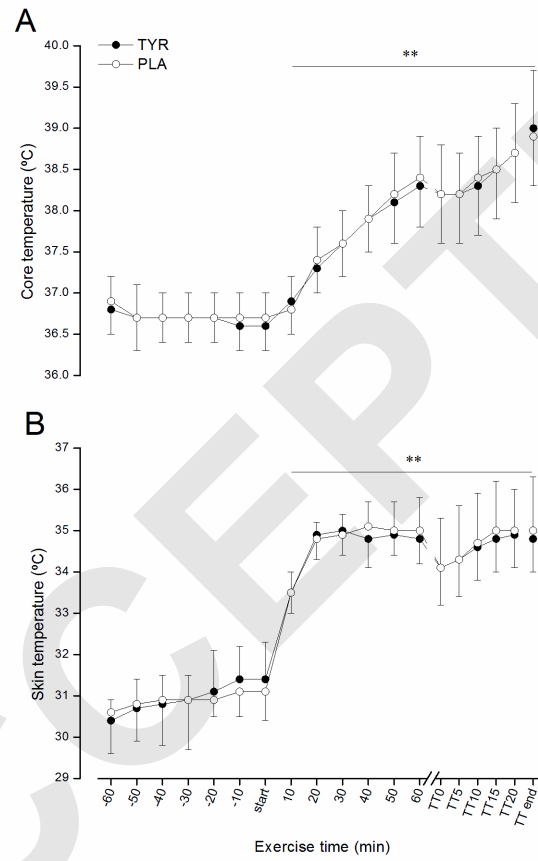
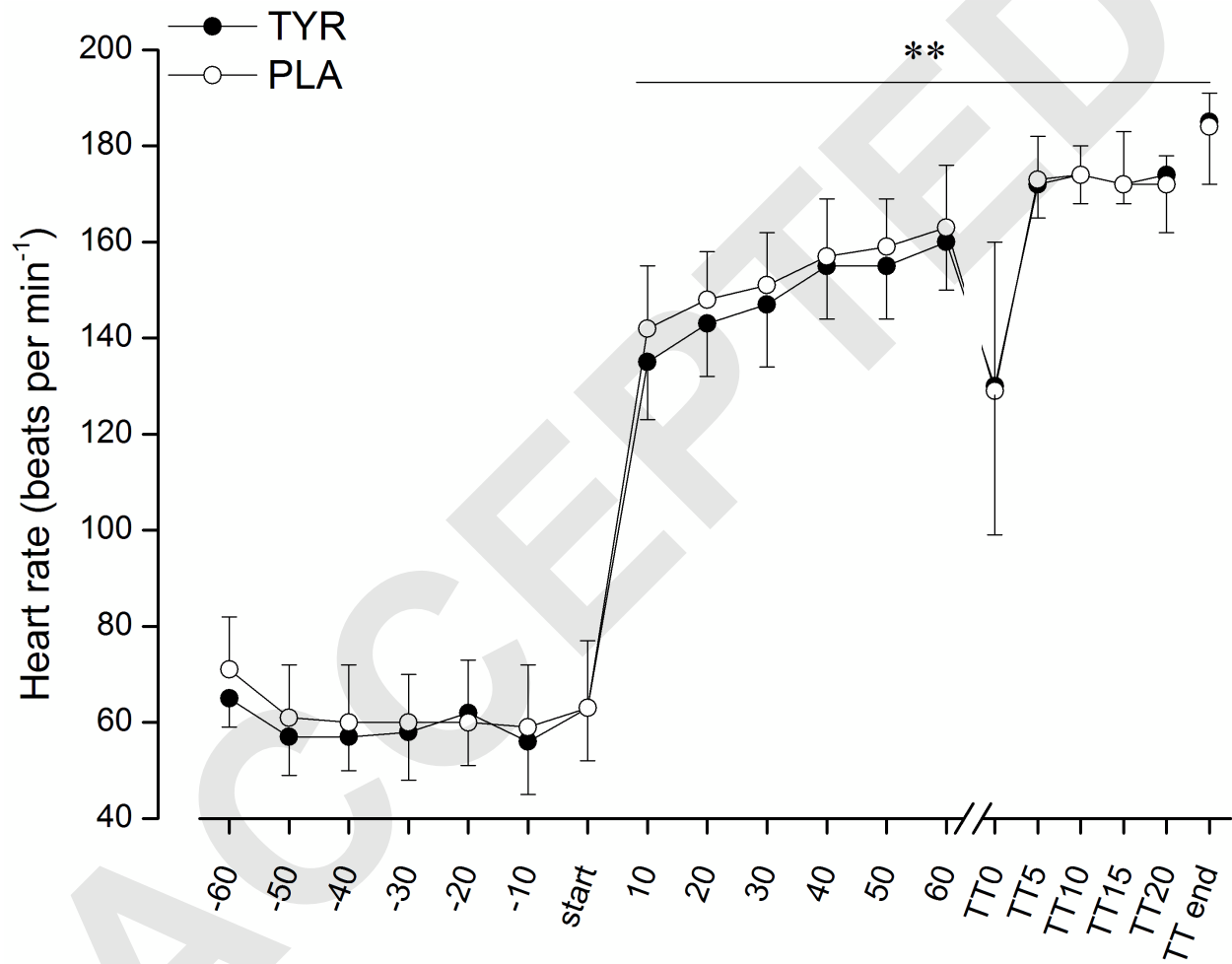
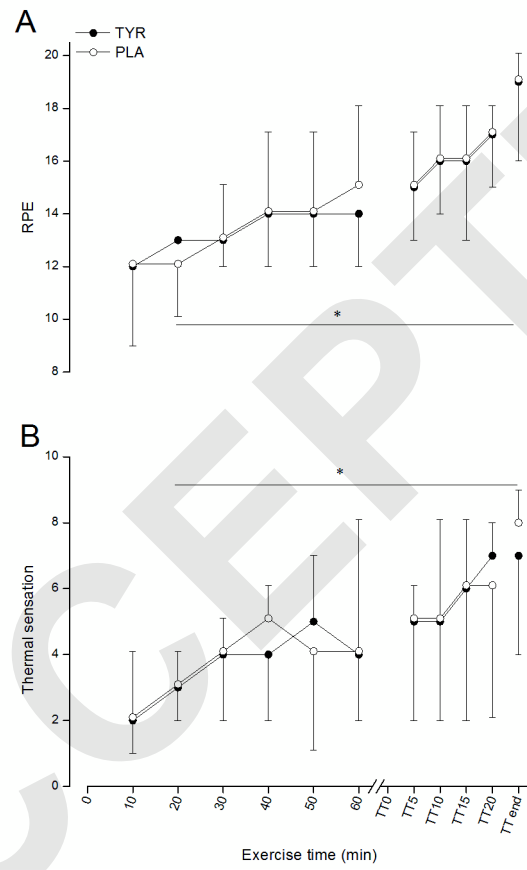




Figure 4



**Figure 5**



**Table 1**

	TYR				PLA			
	Rest	Pre-	Post 60	Post TT	Rest	Pre-	Post 60	Post TT
Amino acids ( $\mu\text{mol}\cdot\text{L}^{-1}$ )								
f-Tryptophan	149 (107)	152 (28)	150 (77)	162 (127)	155 (93)	160 (49)	154 (93)	172 (77)
Valine	158 (69)	135 (51)	159 (81)	143 (73)	165 (85)	166 (53)	144 (102)	161 (108)
Leucine	146 (74)	105 (79)§	130 (89)	131 (117)	154 (128)	181 (83)#	135 (115)	166 (84)
Isoleucine	83 (42)	66 (38)§	62 (35)	73 (43)	77 (75)	97 (42)#	76 (55)	86 (29)#
Threonine	92 (46)	99 (58)	96 (44)	92 (68)	120 (45)	118 (54)	104 (51)	121 (55)
Methionine	30 (9)	27 (9)	28 (9)	34 (22)	30 (9)	30 (7)#	31 (144)	36 (15)§
Lysine	254 (115)	251 (131)	216 (98)	215 (178)	226 (161)	276 (139)	253 (147)	246 (112)
$\Sigma$ ( $\text{mmol}\cdot\text{L}^{-1}$ )	1.0 (0.4)	1.0 (0.3)	0.9 (0.3)	1.0 (0.3)	0.9 (0.3)	0.8 (0.3)#	0.9 (0.3)	0.8 (0.5)

Values are median (range). f-Tryptophan, free tryptophan;  $\Sigma$ ,  $\Sigma$ (valine, leucine, isoleucine, threonine, methionine, lysine). §significantly different from Rest in same trial, ( $P < 0.05$ ;  $n = 7$ ); #Significant difference between trials, ( $P < 0.05$ ;  $n = 7$ ).