

## Aberystwyth University

### *Dietary exposure biomarker-lead discovery based on Metabolomics analysis of urine samples*

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# Medicine & Science in Sports & Exercise

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

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<b>Abstract:</b>	<p><b>Purpose:</b> 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.</p> <p><b>Methods:</b> 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 ± 4% peak oxygen uptake (VO<sub>2</sub>peak), then performed a simulated cycling time-trial requiring completion of an individualised target work quantity (393.1 ± 39.8 kJ).</p> <p><b>Results:</b> 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 &lt; 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 ± 6.5 min and 35.2 ± 8.3 min in TYR and PLA, respectively; P = 0.4167) were similar between trials. Thermal sensation (P &gt; 0.05), RPE (P &gt; 0.05), core temperature (P = 0.860), skin temperature (P = 0.822) and heart rate (P = 0.314) did not differ between trials.</p> <p><b>Conclusion:</b> 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.</p>

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**Title:** Failure of oral tyrosine supplementation to improve exercise performance in the heat.

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## **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 ( $\dot{V}O_{2\text{peak}}$ ), 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**

*Paragraph Number 1* Exercise performance is clearly impaired in high ambient temperature compared to cooler conditions (24, 35). This impairment has been primarily attributed to fatigue caused by central nervous system changes, secondary to increased brain temperature (22), although peripheral factors including high skin temperature, dehydration and alterations in circulatory and thermoregulatory factors likely also contribute to this (30). 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 (28), 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.

*Paragraph Number 2* 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 (11). 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, 11, 17). Tyrosine hydroxylase then exhibits increased affinity for tyrosine coupled with a decreased sensitivity to catecholamine end-product inhibition (29). 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, 34). 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, 40). 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, 23). 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, 32, 33). 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 (36) but a separate study using a similar protocol and tyrosine dosage failed to show an effect (37).

**Paragraph Number 3** Both Tumilty et al. (36) and Watson et al. (37) 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).

***Paragraph Number 4*** 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**

***Paragraph Number 5*** 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 (36). One subject did not complete testing due to injury and the corresponding data was omitted from all statistical analysis. The remaining seven subjects [six cyclists and one 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 ( $\dot{V}O_{2\text{peak}}$ ),  $4.6 \pm 0.6$  L $\cdot$ min $^{-1}$ ; peak power output attained during ramp test on a cycle ergometer to elicit  $\dot{V}O_{2\text{peak}}$ ,  $389 \pm 44$  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**

*Paragraph Number 6* Subjects visited the lab on five separate occasions: an initial ramp test to determine peak power output, maximal heart rate and  $\dot{V}O_{2\text{peak}}$  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  $\dot{V}O_{2\text{peak}}$  was determined as the highest oxygen uptake ( $\dot{V}O_2$ ) 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 ( $\dot{V}CO_2$ ) relative to  $\dot{V}O_2$  and an increase in the ventilatory equivalent for  $\dot{V}O_2$  in relation to a levelling off or continued decrease in the

ventilatory equivalent for  $\dot{V}CO_2$  (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  $-80^{\circ}C$  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 (27). 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<sub>3</sub>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 mg·kg body mass<sup>-1</sup> 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 (36). 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  $0.26 \pm 0.1$  m·s<sup>-1</sup> 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  $\dot{V}O_2$  that is 10% of the difference between the  $\dot{V}O_2$  at the gas exchange threshold and  $\dot{V}O_{2peak}$  (16);  $156 \pm 24$  W or  $57 \pm 4\%$   $\dot{V}O_{2peak}$  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 mL·kg body mass<sup>-1</sup> 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.

***Paragraph Number 7*** The time-trial was based on a validated protocol used in previous research examining fatigue during prolonged exercise in the heat (38), 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  $\dot{V}O_{2peak}$  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**

**Paragraph Number 8** Heart rate, T<sub>core</sub> and T<sub>skin</sub> were recorded every 10 min throughout 60 min rest and 60 min of submaximal exercise. Power output, heart rate, T<sub>core</sub> and T<sub>skin</sub> 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 25), 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 (26). Final measurements of heart rate, T<sub>core</sub>, T<sub>skin</sub>, 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).

***Paragraph Number 9*** 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 (10). 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 (36). 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**

**Paragraph Number 10** 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 ± 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 × 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**

**Paragraph Number 11** 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 ( $P = 0.417$ ; effect size = 0.05;  $34.8 \pm 6.5$  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). 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**

**Paragraph Number 12** 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**

**Paragraph Number 13** 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**

**Paragraph Number 14** 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**

**Paragraph Number 15** 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**

**Paragraph Number 16** 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**

*Paragraph Number 17* 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**

*Paragraph Number 18* Previous studies have examined the effect of tyrosine administration on exercise capacity in the heat in man (36, 37), 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.

*Paragraph Number 19* 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 (36). 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 (35) 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, 22). Changes in central catecholamine activity are implicated in these processes due to their intrinsic connection with increased arousal and motivation (4), thermoregulation (9) 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) (38), highlighting the importance of cerebral catecholamine activity in the ability to tolerate exercise with heat stress.

***Paragraph Number 20*** 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 (36). 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).

**Paragraph Number 21** 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 (36). But a separate study employing a similar tyrosine dosage and exercise protocol reported no effect (37). 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 (36). 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 (32), simulated time-trial performance (7) or muscle strength and anaerobic endurance (33). 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, 32), this does not adequately explain the discrepant findings between two studies adopting similar prolonged exercise protocols in the heat and tyrosine dosage (36, 37) and the underlying reasons for this require further clarification.

**Paragraph Number 22** 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 (31). 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 (31). 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 similar values for RPE and thermal sensation, throughout 60 min of submaximal exercise and the simulated time-trial, suggests that the subjective interpretation of prolonged exercise in the heat cannot be manipulated by acutely increasing plasma tyrosine availability.

***Paragraph Number 23*** Subjects were specifically administered an isocaloric quantity of whey protein as a placebo to clarify whether any effect of tyrosine administration was due to a peripheral (or central) effect of additional substrate or energy availability. This could explain, in part, the differing results from previous tyrosine studies examining prolonged exercise in the heat (36, 37). Estimated macronutrient oxidation rates were the same in both trials during 50 min of submaximal exercise. Also, subjects were euglycemic before commencing the constant-load exercise, the simulated time-trial and on the completion of the time-trial, suggesting that substrate supply did not limit exercise. Reports of macronutrient oxidation rates in the heat are mixed, with some (12), but not all (21) studies reporting increased glycogen oxidation rates compared to cooler conditions. However, subjects generally reach exhaustion during prolonged exercise in the heat with adequate muscle substrate and little

muscle metabolite accumulation, suggesting that these are not primary limitations under such conditions (21, 24), at least when the heat stress is uncompensable. Blood lactate concentrations in the present study, although elevated following exercise as might be expected, were not suggestive of muscle concentrations which would markedly impair performance.

**Paragraph Number 24** If macronutrient and/or metabolite accumulation were not the primary cause of fatigue in the present study, then a direct fatiguing influence of hyperthermia seems probable. Subjects in the present study were clearly hyperthermic in both trials and the T<sub>core</sub> values attained at the end of the time-trial are consistent with exhaustion values in subjects of similar fitness to the present cohort, following prolonged exercise in the heat (6). As cerebral catecholamines have been implicated in heat loss processes during exercise (15), it might therefore be expected that some difference would be apparent in thermoregulatory responses to exercise between trials in the present study, if central catecholamine activity was augmented by tyrosine administration. The similar data on T<sub>core</sub>, T<sub>skin</sub> and rate of body mass losses, irrespective of drink ingested, implies that thermoregulatory processes were not affected despite a plasma amino acid profile, following ingestion of the experimental mixture, which would favour brain uptake of a direct catecholamine precursor (13).

**Paragraph Number 25** 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.

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## **Conflict of Interest**

The authors report no conflict of interests.

The results of the present study do not constitute endorsement by ACSM.

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**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, ††significantly different versus Rest in TYR, ( $P < 0.01$ ); §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.

	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, ( $P < 0.05$ ;  $n = 7$ ).

Figure 1  
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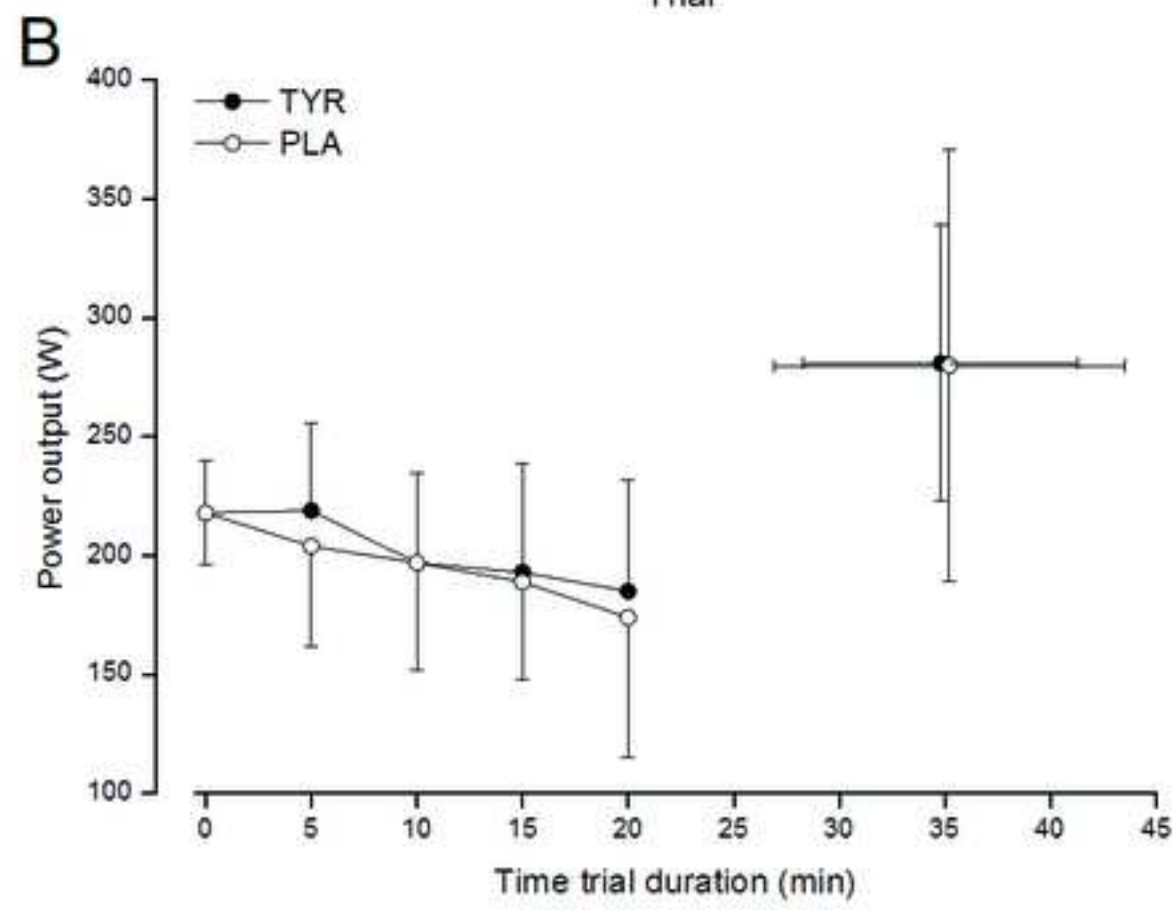
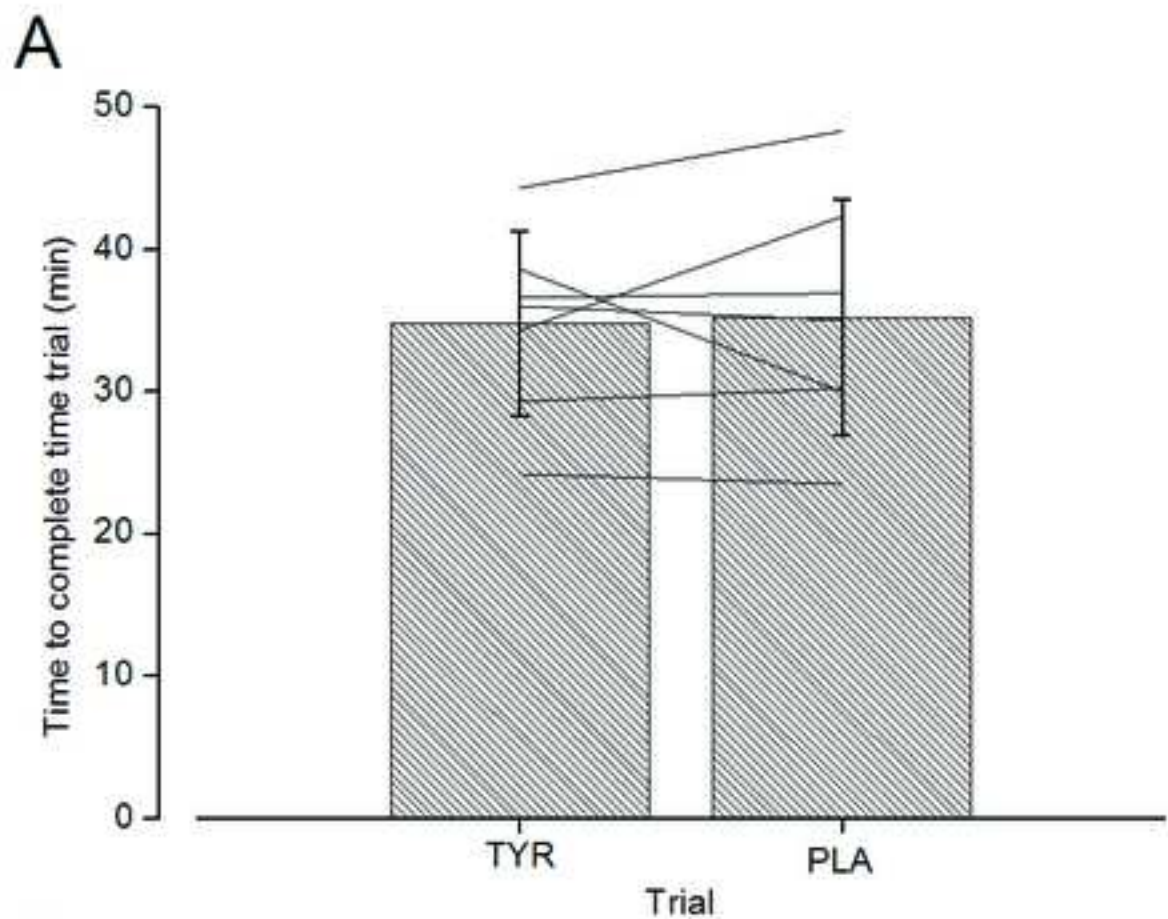




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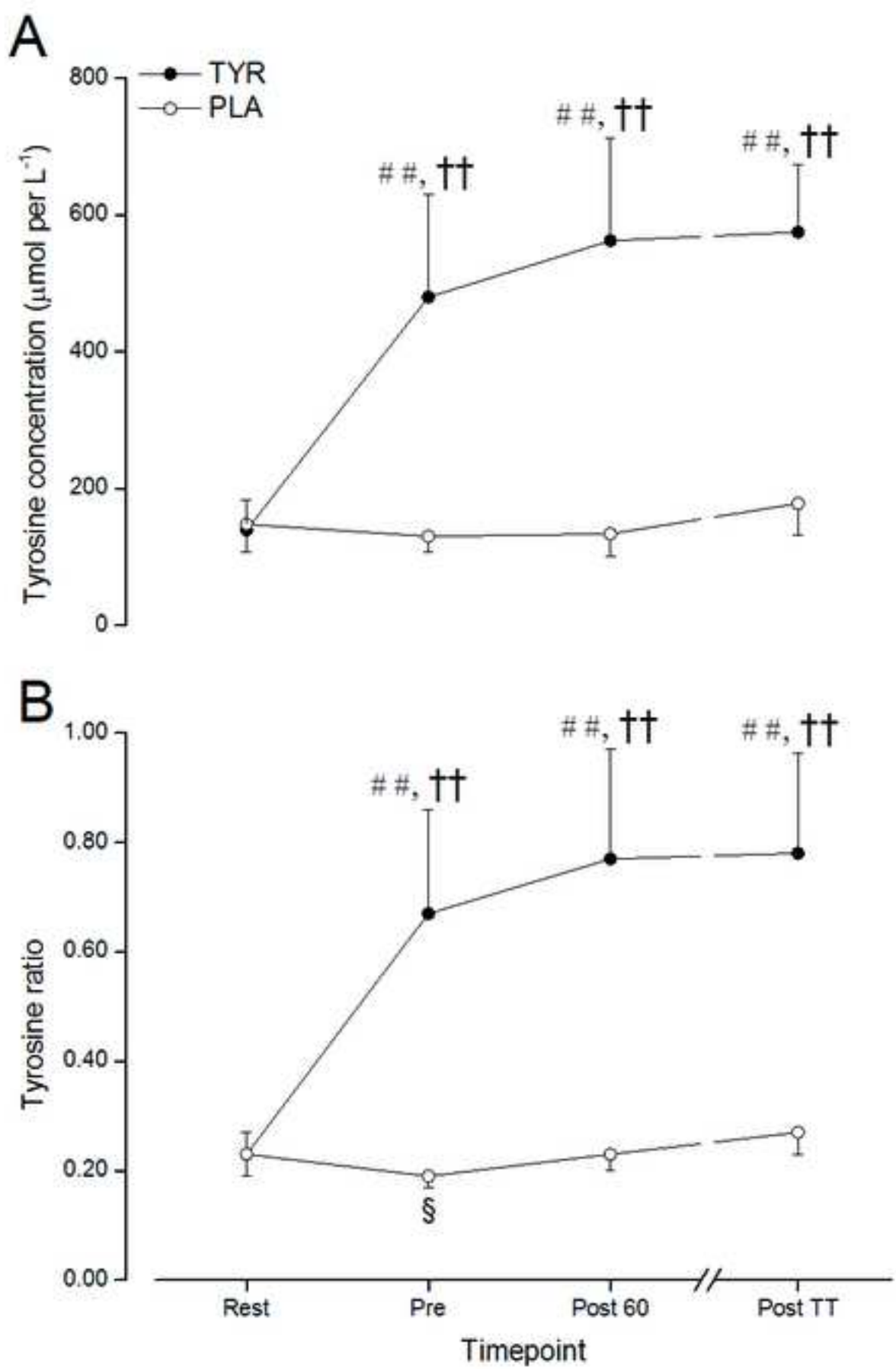


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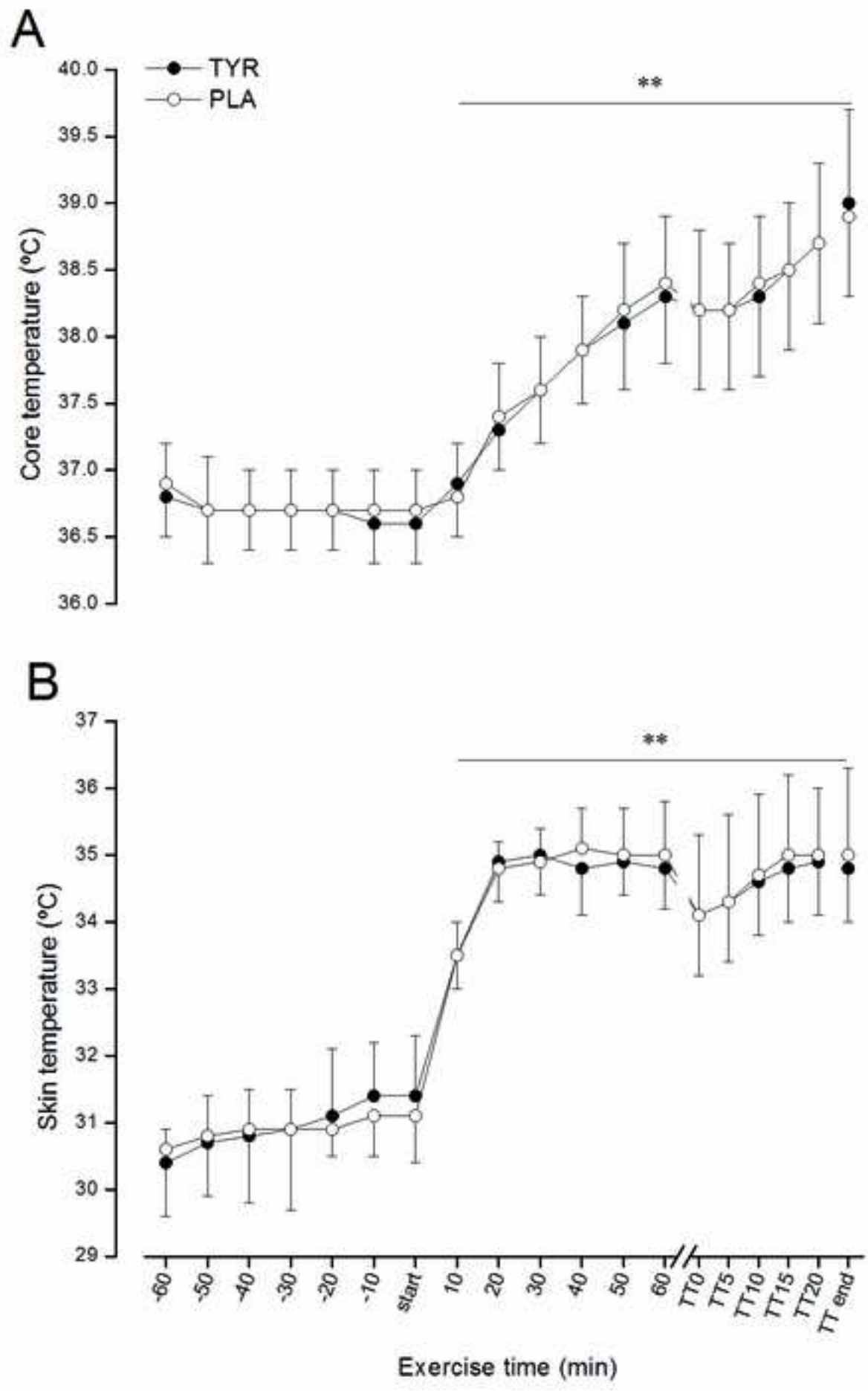


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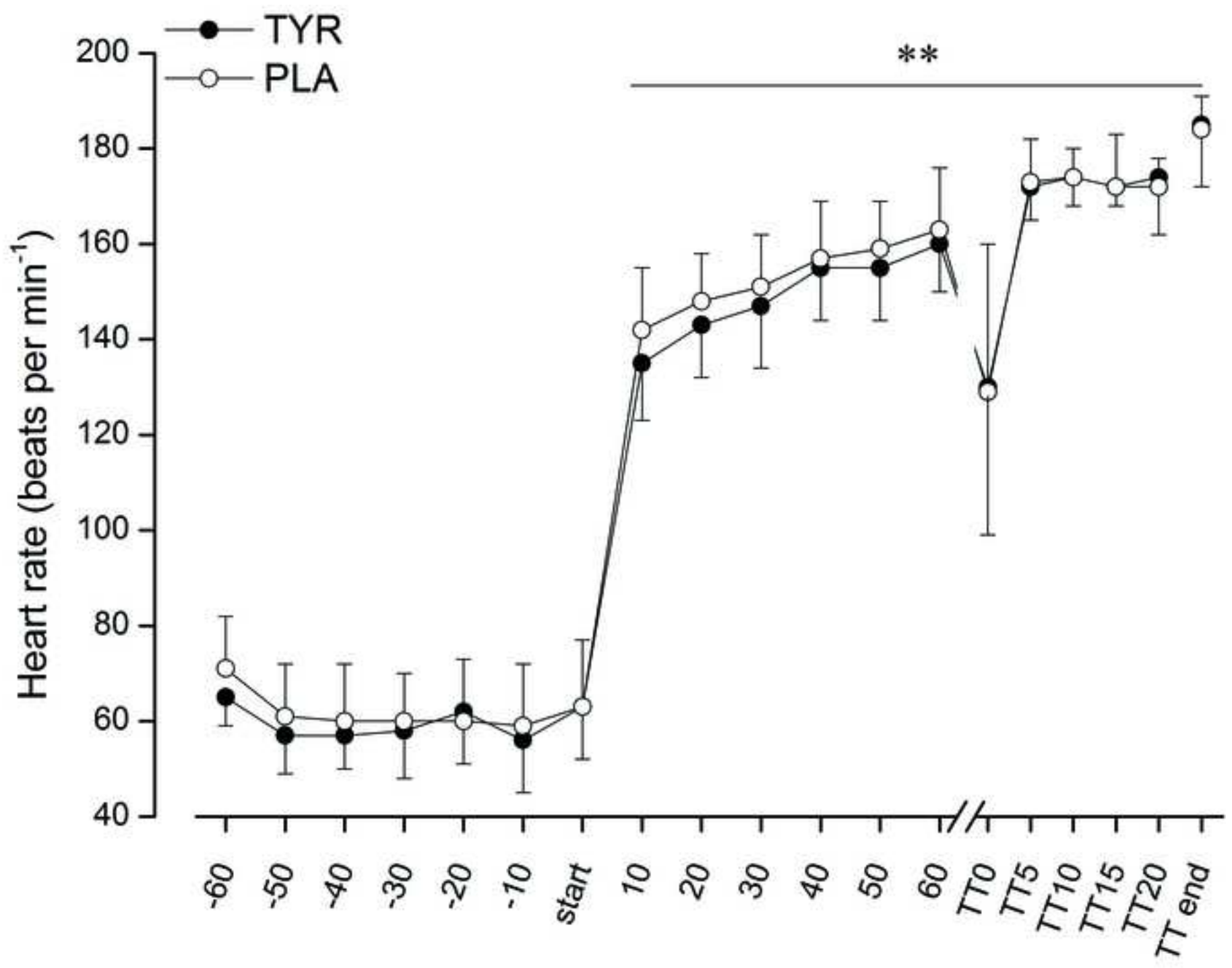


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