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

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ABSTRACT

TUMILTY, L., G. DAVISON, M. BECKMANN, and R. THATCHER. Failure of Oral Tyrosine Supplementation to Improve Exercise Performance in the Heat. *Med. Sci. Sports Exerc.*, Vol. 46, No. 7, pp. 1417–1425, 2014. **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**: After familiarization, 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 d. Subjects ingested 150 mg·kg^{-1} body mass tyrosine (TYR) or an isocaloric quantity of whey powder (PLA) in 500 mL of sugar-free flavored water in a randomized, double-blind fashion. Sixty minutes after drink ingestion, the subjects cycled for 60 min at 57% ± 4% peak oxygen uptake ($\dot{V}O_{2peak}$) and then performed a simulated cycling time trial requiring completion of an individualized target work quantity (393.1 ± 39.8 kJ). **Results**: The ratio of plasma tyrosine plus phenylalanine (tyrosine precursor) to amino acids competing for brain uptake (free-tryptophan, leucine, isoleucine, valine, methionine, threonine, and 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 preexercise ($P = 0.004$). Time-trial power output ($P = 0.869$) and performance (34.8 ± 6.5 and 35.2 ± 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. **Conclusions**: 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. **Key Words:** AMINO ACIDS, CATECHOLAMINES, MILD HYPERTHERMIA, CENTRAL FATIGUE, PROLONGED EXERCISE

Exercise performance is clearly impaired in high ambient temperature compared with 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, also likely contribute to this (6,28). 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 that 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 (38). 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 the augmentation of cerebral catecholamine release after systemic tyrosine injection, while under basal conditions, is generally limited in magnitude and duration (12). In animals exposed to extreme environmental stress that 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,

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exploratory behavior, memory, and coping behavior in rats exposed to acute, experimentally induced stress, such as tail shock and 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 before exposure to the environmental conditions (1,19,20). Evidence supporting an enhancement of exercise in humans after 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 before 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) after an acute administration of 150 $mg \cdot kg^{-1}$ body mass 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 to neutral amino acids that compete for brain uptake after 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. Because of the self-paced nature of a performance time trial, which will be highly influenced by motivation and arousal, it is plausible that any improvements after tyrosine supplementation would be more apparent during this type of exercise trial compared with 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 because of injury, and the corresponding data were 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 yr, range = 26 yr; median stature = 1.83 m, range = 0.13 m; mean ± SD for body mass = 77.9 ± 11.7 kg; peak oxygen uptake ($\dot{V}O_{2peak}$) = 60 ± 11 $mL \cdot kg^{-1} \cdot min^{-1}$; peak power output attained during ramp test on a cycle ergometer to elicit $\dot{V}O_{2peak}$ = 389 ± 44 W; maximal heart rate attained during ramp test = 186 ± 8 bpm), 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 1 month before the commencement of testing. The study was approved by Aberystwyth University Research Ethics Committee.

Experimental procedures. Subjects visited the laboratory on five separate occasions: an initial ramp test to determine peak power output, maximal heart rate, and $\dot{V}O_{2peak}$ using an online breath-by-breath system (Jager Oxycon Pro, Hoechberg, Germany); two familiarization visits, and two main experimental trials. No strenuous or unaccustomed exercise was permitted for 24 h before each test. Subjects were instructed to sleep for ≥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 $W \cdot s^{-1}$ until the subject reached volitional exhaustion, and $\dot{V}O_{2peak}$ 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 leveling 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 familiarization visit, and at least 7 d separated each of the remaining four trials. The familiarization 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 with the main experimental trials except that blood samples were not taken and the placebo drink (see below) was administered for both familiarizations. All of the familiarization and main trials commenced between 0630 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 before the first familiarization, and this enabled duplication before each subsequent

visit. After arriving at the laboratory after 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 milliliter before a 1.0-mL aliquot was frozen at −80°C for later measurement of osomolality, in duplicate, using freezing point depression (Osmostat 030; Gonotec, Berlin, Germany). Nude, postvoid body mass was measured to the nearest 0.1 kg (Seca 645; Seca GMB and Co., Hamburg, Germany), and stature was recorded (stadiometer; Holtain Ltd., Crymych, UK). A rectal thermistor (Grant Instruments, Cambridge, England, UK) 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) were attached to the calf, thigh, chest, and triceps 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), 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 heparinized vacutainer and 4.5 mL into a K_3EDTA-treated vacutainer (BD Vacutainer Systems, Plymouth, UK). The experimental or placebo drink were administered in a randomized, double-blind and counterbalanced manner before subjects were seated in a quiet, comfortable environment (20.5°C ± 0.4°C, 44% ± 6% relative humidity) for 1 h. The experimental drink (TYR) contained 150 mg·kg^{-1} body mass tyrosine (SHS international Ltd., Liverpool, UK) and 7 g vanilla flavoring (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 hydrolyzed whey protein (Myprotein.co.uk) instead of tyrosine (equating to 11.6 ± 1.6 g tyrosine or 6.1 ± 0.8 g whey protein; 22 ± 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. Previous pilot work with three volunteers who did not participate in this study confirmed that the drinks were indistinguishable in color, 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). The temperature and the relative humidity of the chamber were maintained at 30.1°C ± 0.2°C and 60% ± 0%, respectively, and the mean air velocity within the chamber was 0.26 ± 0.1 m·s^{-1} during all trials. Once inside the chamber, subjects commenced cycling, without a warm-up, at a constant power output equivalent to 10% Δ (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 the $\dot{V}O_{2peak}$ [16]; 156 ± 24 W or 57% ± 4% $\dot{V}O_{2peak}$ in this subject group) for 60 min. The purpose of this exercise period was to induce hyperthermia before the 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^{-1} body mass 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 were standardized 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 that would be completed during 30 min of cycling at 60% of the power output eliciting $\dot{V}O_{2peak}$ during the initial ramp test (393.1 ± 39.8 kJ in this group). The cycle ergometer was set in linear mode (workload increases as pedaling rate increases) during the time trial so that the power output and hence the completed work were directly related to pedaling 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 nontransparent 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 standardized for each individual subject during subsequent trials. Subjects emptied their bladder, and urine volume was again recorded to the nearest milliliter 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, toweled dry, and then reweighed nude.

Physiological measurements and blood analysis. Heart rate, T_{core}, and T_{skin} were recorded every 10 min throughout 60 min of rest and 60 min of submaximal exercise. Power output, heart rate, T_{core}, and T_{skin} 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 reference 22), were recorded every 10 min throughout the 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 and 50 min of the constant-load exercise period. Oxygen and carbon dioxide concentrations were measured using a combined paramagnetic oxygen analyzer and infrared carbon dioxide analyzer (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 utilization and energy expenditure (23). Final measurements of heart rate, T_{core}, T_{skin}, 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 the front, from behind, and to the left and right of each subject at head height while seated on the cycle ergometer, using a handheld anemometer (Kestrel 1000; Richard Paul Russell Ltd., Lymington, UK).

Blood from the K_3EDTA vacutainer was used to measure hematocrit, hemoglobin, lactate, and glucose. Whole blood was drawn into microcapillary tubes, spun for 5 min at 14,000*g* using a Hawksley microcentrifuge (Haematospin 1400; Hawksley, Lancing, UK), and the separated red cell volume was measured using a Hawksley hematocrit tube reader, with a coefficient of variation of 0.8% for 10 repeated measurements on the same sample. Hemoglobin was measured using an automated hematology analyzer (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 $g \cdot L^{-1}$ hemoglobin. Hematocrit and hemoglobin measurements were used to estimate plasma volume changes (11). Blood glucose and blood lactate concentrations were measured using an automated analyzer (2300 Stat Plus; Yellow Spring Instrument Co., OH), calibrated with standard concentrations for glucose (0.00 and 50.00 $mmol \cdot L^{-1}$) and lactate (0.00 and 30.00 $mmol \cdot L^{-1}$). The coefficient of variation for 10 repeated measurements on a sample standard for blood glucose (6.14 $mmol \cdot L^{-1}$) and blood lactate (5.35 $mmol \cdot L^{-1}$) is 1.7% and 1.5%, respectively. The heparinized blood was immediately centrifuged at 1500*g* for 10 min at 4°C, and then the plasma was 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 as follows: 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%. Hematocrit 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 analyze 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 two-factor (time × trial) 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 analyzed separately using Student's paired *t*-test to account for the different exercise duration between subjects and trials. Nonnormally distributed data are presented as median (range) and were analyzed using Friedman's tests, and where appropriate, *post hoc* tests were carried out using the Wilcoxon matched pairs tests with the Bonferroni correction. Statistical significance was set at $P < 0.05$.

RESULTS

Time-trial performance. After the 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 second familiarization 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 ± 6.5 min in TYR and 35.2 ± 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 seven 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.

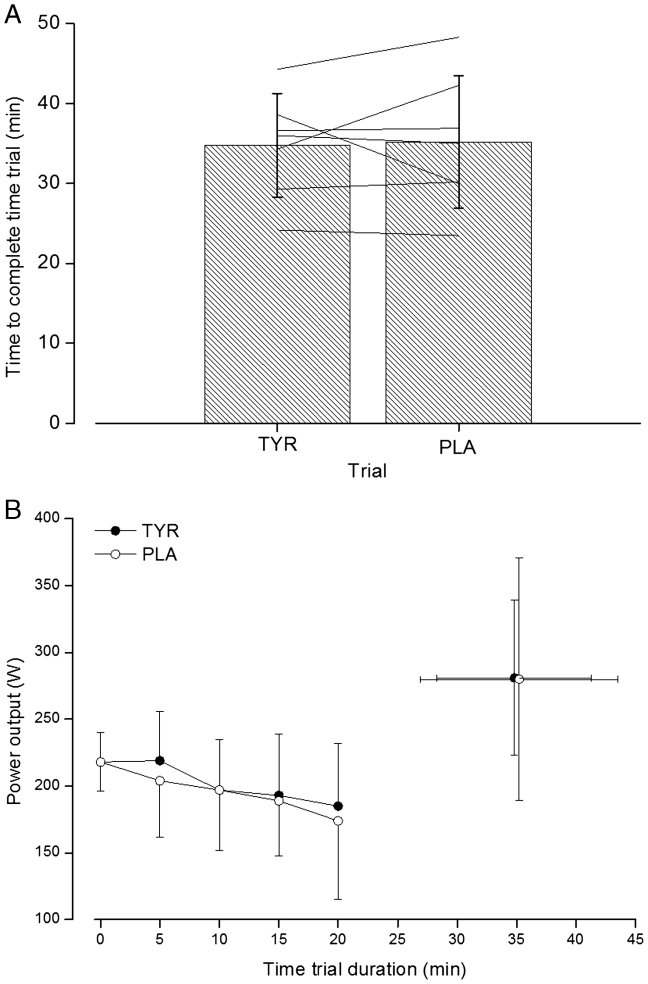

FIGURE 1—Mean ± SD (columns and vertical error bars) and individual (remaining individual lines) times to complete simulated cycling time trial (A) and power output during time trial, up to the last time point all subjects were exercising and value recorded at the end of the time trial (B), in tyrosine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

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 the completion of the time trial (P < 0.01). There was no difference between trials in the ratio of plasma tyrosine plus phenylalanine to Σ(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 more than 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 preexercise (P = 0.004). The plasma concentrations of the remaining individual amino acids analyzed 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 ± 0.9 mmol·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) mmol·L^{-1} in TYR and 4.1 (range, 4.9) mmol·L^{-1} in PLA]. Plasma volume progressively declined, and to a similar extent in both trials, after drink ingestion (P < 0.05) reaching −8.4% ± 6.0% in TYR and −9.1% ± 4.5% in PLA at end of the time trial.

Temperature measurements. Exercise caused a gradual increase in T_{core} (P = 0.003) at a similar rate in both trials (P = 0.131; Fig. 3A). At the end of the time trial, T_{core} was 39.0°C ± 0.7°C in TYR and 38.9 ± 0.6 in PLA (P = 0.474). T_{skin} also increased during exercise (P = 0.012), plateauing after 20 min of constant-load exercise and after 15 min of the time trial (Fig. 3B). The drink ingested had no influence on T_{skin} (P = 0.822), and there was no difference between

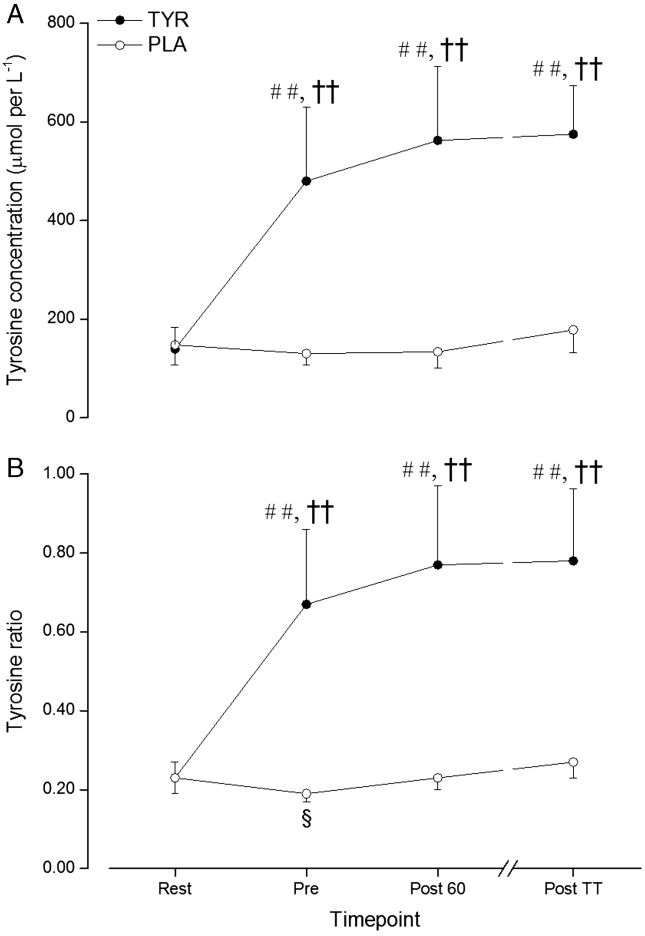

FIGURE 2—Changes in plasma tyrosine concentration (A) and tyrosine ratio [plasma concentration ratio of tyrosine plus phenylalanine and Σ(f-tryptophan; valine, leucine, isoleucine, threonine, methionine, lysine)] (B) in tyrosine (TYR) and placebo (PLA) trials. TT, time trial. ##Significant difference 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 ± 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 (μmol·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)
Σ (mmol·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 presented as median (range).
*Significantly different from Rest in same trial ($P < 0.05$, $n = 7$).
**Significant difference between trials ($P < 0.05$, $n = 7$).
f-Tryptophan, free-tryptophan; Σ, Σ(valine, leucine, isoleucine, threonine, methionine, lysine).

trials in T_{skin} at end of the time trial (34.8°C ± 0.8°C in TYR and 35.0°C ± 1.3°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% ± 4% and 99% ± 4% of maximum in TYR and PLA, respectively ($P = 0.729$).

Urine analysis, fluid intake, and body mass losses. Preexercise urine osmolality suggested that subjects were similarly hydrated before both trials ($P = 0.571$; 329 ± 136 mOsm·kg^{-1} in TYR and 369 ± 148 mOsm·kg^{-1} in PLA). After the time trial, urine osmolality was similar in each trial (198 ± 56 mOsm·kg^{-1} in TYR and 325 ± 296 mOsm·kg^{-1} in PLA; $P = 0.218$) and unchanged from preexercise ($P = 0.202$). Subjects drank similar volumes of fluid during the time trial in both trials (311.1 ± 114.4 mL in TYR and 363.0 ± 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 postexercise body mass adjusted for fluid intake and urine output after initial weighing (2.9% ± 0.7% and 3.1% ± 1.1% of preexercise body mass in TYR and PLA, respectively; $P = 0.528$), and the rate of body mass loss

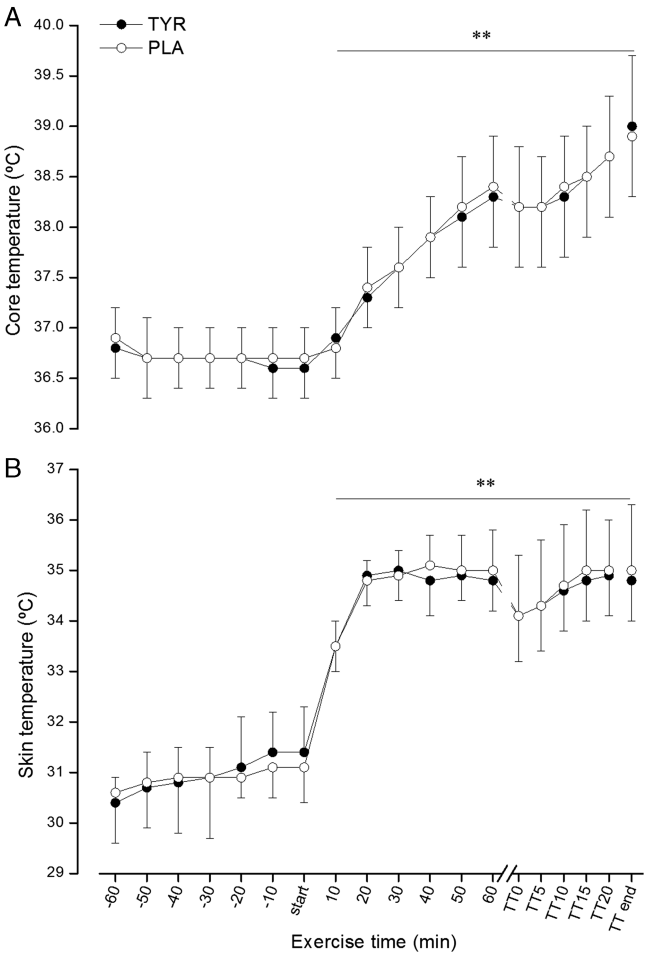

FIGURE 3—Core temperature (A) and mean weighted skin temperature (B) responses during a 1-h rest, 60 min of constant-load submaximal-intensity exercise and simulated cycling time trial (TT), up to the last time point that all subjects were exercising and value recorded at end of TT, in tyrosine (TYR)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

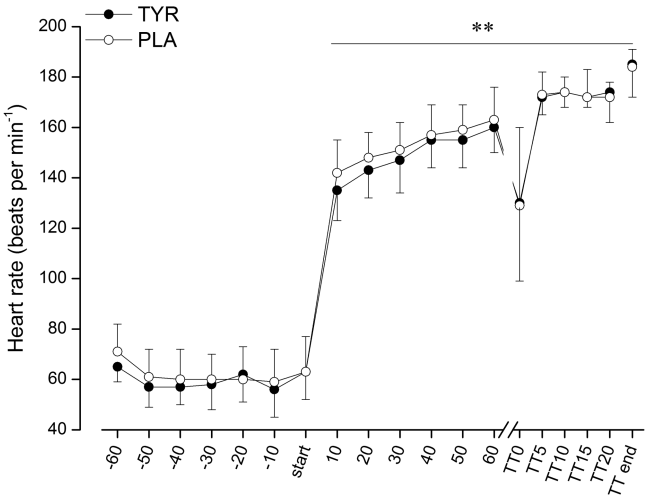

FIGURE 4—Heart rate responses during the 1-h rest, 60 min of constant-load submaximal-intensity exercise and simulated cycling time trial (TT), up to the last time point that all subjects were exercising and value recorded at end of TT, in tyrosine (TYR), and placebo (PLA) trials. **Significantly different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

($P = 0.436$; 4.0 ± 1.0 kg·h^{-1} in TYR and 4.3 ± 1.6 kg·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 ($P > 0.05$). The 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 ($P > 0.05$). The 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." The 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-intensity exercise, and were similar in both trials ($P > 0.05$).

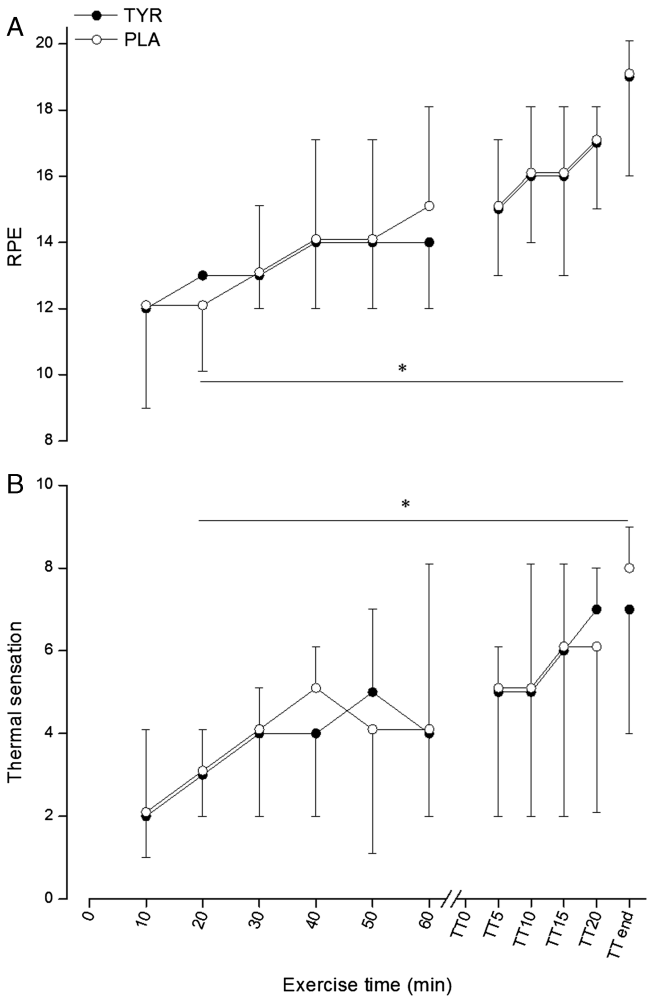

FIGURE 5—RPE (A) and thermal sensation ratings (B) during 60 min of constant-load submaximal-intensity cycling and simulated cycling time trial (TT), up to the last time point that all subjects were exercising and value recorded at end of TT, in tyrosine (TYR) and placebo (PLA) trials. *Significantly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

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, an acute administration of tyrosine did not improve self-paced exercise performance in the heat compared with a placebo containing an isocaloric quantity of hydrolyzed 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). In addition, 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 with 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 before performance of a time trial in the heat. The clear impairment in exercise performance carried out in warm compared with 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 reduced will or "drive" to exercise (21). Changes in central catecholamine activity are implicated in these processes because of their intrinsic connection with increased arousal and motivation (4), thermoregulation (9,15), and motor initiation and control (14). In addition, the 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 after an acute administration of a noradrenaline reuptake inhibitor, reboxetine, compared with a lactose placebo (25). Enhanced noradrenaline activity in the present study after tyrosine administration may have constrained any performance effect during the simulated time trial.

The current protocol was used 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 to amino acids competing for brain uptake after tyrosine administration was similar in magnitude to one study in which exercise capacity in the heat was prolonged by 15%, 1 h after an administration of 150 $mg \cdot kg^{-1}$ tyrosine (34). This would favor 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 after 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 before prolonged exercise in the heat provides support for the idea that exercise tolerance in the heat is enhanced after prior oral tyrosine administration (34). However, a separate study using 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 the benefit of tyrosine administration before 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 earlier. This may also explain why studies examining acute tyrosine administration in man before 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). Although 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 after 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 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 with an isocaloric quantity of whey protein. Furthermore, the present data suggest that the subjective interpretation of the exercise cannot be manipulated by a plasma amino acid profile, following the ingestion of the experimental mixture, which would favor 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 with 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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