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ORIGINAL ARTICLE

# Oral tyrosine supplementation improves exercise capacity in the heat

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**Abstract** Increased brain dopamine availability improves prolonged exercise tolerance in the heat. It is unclear whether supplementing the amino-acid precursor of dopamine increases exercise capacity in the heat. Eight healthy male volunteers [mean age 32 ± 11 (SD) years; body mass 75.3 ± 8.1 kg; peak oxygen uptake ($\dot{V}O_{2peak}$) 3.5 ± 0.3 L min$^{-1}$] performed two exercise trials separated by at least 7 days in a randomised, crossover design. Subjects consumed 500 mL of a flavoured sugar-free drink (PLA), or the same drink with 150 mg kg body mass$^{-1}$ tyrosine (TYR) in a double-blind manner 1 h before cycling to exhaustion at a constant exercise intensity equivalent to 68 ± 5% $\dot{V}O_{2peak}$ in 30°C and 60% relative humidity. Pre-exercise plasma tyrosine:large neutral amino acids increased 2.9-fold in TYR ($P < 0.01$), while there was no change in PLA ($P > 0.05$). Subjects cycled longer in TYR compared to PLA (80.3 ± 19.7 min vs. 69.2 ± 14.0 min; $P < 0.01$). Core temperature, mean weighted skin temperature, heart rate, ratings of perceived exertion and thermal sensation were similar in TYR and PLA during exercise and at exhaustion ($P > 0.05$) despite longer exercise time in TYR. The results show that acute tyrosine supplementation is associated with increased endurance capacity in the heat in moderately trained subjects. The results also suggest for the first time that the availability of tyrosine, a nutritional dopamine precursor, can influence the ability to subjectively tolerate prolonged submaximal constant-load exercise in the heat.

**Keywords** Central fatigue · Amino acid · Mild hyperthermia

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## Introduction

It is clear that prolonged exercise capacity (Galloway and Maughan 1997) and performance (Tatterson et al. 2000) are impaired in the heat when compared with temperate conditions. This fatigue, defined as an inability to maintain a given work rate or power output, appears to involve changes within the central nervous system (Nybo and Nielsen 2001), secondary to an increase in body (i.e. core, muscle and brain) temperature per se (Nielsen et al. 1993). Hyperthermia may reduce the will or the "drive" to exercise (Brück and Olschewski 1987), and fatigue during prolonged exercise in the heat may therefore represent reduced arousal or motivation to continue exercise (Nielsen et al. 2001). In support of this, a slowing of frontal cortex electroencephalogram waves has been noted with increasing body (oesophageal) temperature during prolonged exercise in the heat, indicative of reduced arousal (Nielsen et al. 2001), and this correlates strongly with linear increases in subjective exertion (RPE; Nybo and Nielsen 2001). However, to date no study has been able to identify a precise neurobiological cause for this "central fatigue".

Alterations in brain neurotransmitter availability may provide a possible cause and some suggest that a high ratio of brain dopamine:5-Hydroxytryptophan (5-HT) augments prolonged exercise and a low ratio induces lethargy and reduced motivation (Davis and Bailey 1997). This may be

due in part, to the well documented role of dopamine in motor initiation and control and increased motivation and endurance (Chandler and Blair 1980; Freed and Yamamoto 1985). Notably, increasing brain dopamine activity via bupropion administration improves exercise performance in warm (30°C) but not in temperate (18°C) conditions (Watson et al. 2005), suggesting a specific role for dopamine in subjective tolerance of exercise with heat stress. It seems that increasing dopamine availability with bupropion dampens inhibitory signals within the central nervous system, allowing subjects to tolerate a higher core temperature (*Tcore*) and heart rate than placebo, but at the same RPE (Watson et al. 2005). Brain dopamine availability may therefore provide a plausible neurobiological mechanism for fatigue in the heat, and subsequently any substance which will increase dopamine availability should hypothetically prolong exercise in the heat. Acute consumption of tyrosine, a nutritional dopamine precursor, increases the ratio of tyrosine to other large neutral amino acids (LNAA; leucine, isoleucine, valine, methionine, threonine, lysine, tryptophan) (Glaeser et al. 1979). Tyrosine shares a common transport molecule with LNAA at the blood–brain barrier (Pardridge 1983), therefore an increase in the tyrosine ratio causes an increase in brain tyrosine and a decrease in LNAA. Brain dopamine formation is primarily rate-limited by the saturation of tyrosine hydroxylase with its substrate tyrosine (Cooper et al. 2003). However, in vivo measurements in animals suggest that tyrosine hydroxylase is only 75% saturated (Carlsson and Lindqvist 1978), and therefore frequent neuronal firing may make tyrosine hydroxylase more susceptible to tyrosine availability, or may deplete tyrosine within nerve terminals (Lovenberg et al. 1975; Weiner et al. 1978). Brain catecholamine synthesis may therefore be limited by tyrosine availability under conditions which increase the activity of brain catecholamine neurons above normal (Sved et al. 1979; Melamed et al. 1980).

No study has directly assessed the effect of nutritionally altering brain dopamine availability in humans using a nutritional precursor, and subsequent fatigue during prolonged exercise in the heat. Acute tyrosine consumption in humans does improve measures of mood, and dopamine dependant cognition, psychomotor performance, and behaviour, across a variety of dissimilar stressors which are theorised to increase brain catecholamine activity (Dollins et al. 1995; Banderet and Lieberman 1989; Mahoney et al. 2007; Neri et al. 1995; O'Brien et al. 2007). One study has measured a net brain uptake of tyrosine during prolonged exercise (Nybo et al. 2003); however, acute tyrosine supplementation did not improve either prolonged exercise capacity (Strüder et al. 1998) or performance (Chinevere et al. 2002) in temperate conditions. Exercise in the heat on the other hand, represents a specific demand on the brain dopamine which is not apparent in temperate conditions (Watson et al. 2005; Bridge et al. 2003). Therefore brain tyrosine requirement may be greater with the cumulative demands of exercise and heat stress, and may become limiting to dopamine synthesis and release.

Therefore, the aim of the present study was to determine the effect of oral tyrosine supplementation on exercise capacity in the heat. It was hypothesized that following acute tyrosine supplementation, subjects would cycle for longer at a constant-load, submaximal power output in a warm (30°C) environment.

## Methods

### Subjects

Eight healthy male subjects [mean age 32 ± 11 (SD) years; stature 1.80 ± 0.08 m; body mass 75.3 ± 8.1 kg; peak oxygen uptake ($\dot{V}O_{2peak}$) 3.5 ± 0.3 L min$^{-1}$] volunteered to take part in the study. All subjects were regular exercisers involved in competitive team and endurance sports. All were familiar with cycling exercise, and none were specifically acclimated to exercise in the heat. All testing took place in the Autumn and Winter months within the UK when air temperature is typically in the mid teens or lower. All subjects were permanently resident in the UK for at least one month before commencing the study. Subjects were provided with verbal and written information about the study before giving their written informed consent and the study had prior approval granted by Aberystwyth University Research Ethics Committee.

### Experimental procedure

All subjects visited the laboratory on five separate occasions. During the first visit subjects carried out a 30 W min$^{-1}$ ramp test on a electrically braked cycle ergometer (Lode Excalibur Sport, Groningen, The Netherlands) to determine $\dot{V}O_{2peak}$ and gas exchange threshold (Beaver et al. 1986) using an online breath-by-breath system (Jager Oxycon Pro, Hoechberg, Germany). The remaining four visits comprised two familiarisation visits in order to accustom subjects to cycling to exhaustion in the heat, and two main trials. At least 4 days elapsed between the initial ramp test and the first familiarisation visit, and at least 7 days separated each of the three remaining visits. Subjects refrained from alcohol and unaccustomed exercise for 24 h prior to each of the familiarisations and main trials, and completed a food diary during this period in order to repeat their diet prior to all subsequent trials. Subject's sleep amounts were not

specifically monitored; however, they were instructed to have a good night sleep of around 8 h on the evening before each visit, to ensure that they arrived at the laboratory fresh and well rested. All participants verbally confirmed that they had adhered to this on each laboratory visit. Following an overnight fast of $\geq$ 8 h subjects consumed 500 mL of ordinary tap water 2 h before arriving at the laboratory between 0700 and 0900 h, and at precisely the same time for each individual's visits.

On arrival at the laboratory subjects provided a urine sample, the volume of which was recorded before an aliquot ($\sim$1.0 mL) was removed and immediately refrigerated for subsequent measurement of osmolality using freezing point depression (Osmostat 030, Gonotec, Berlin). Subjects were then weighed nude to the nearest 0.1 kg (Seca 645, Seca GMB and Co., Hamburg, Germany), and stature recorded (Holtain Ltd, Stadiometer, Crymych, UK). Subjects then positioned a rectal thermistor (Grant Instruments, Cambridge, UK) 10 cm beyond the anal sphincter, to enable core temperature measurement (*Tcore*), and fitted a short-range heart rate telemetry band (Polar S610i, Polar Electro Oy, Tampere, Finland). Skin thermistors (Grant Instruments) were attached using adhesive medical tape to the upper arm, chest, thigh and calf. Core and skin (*Tskin*) temperature were recorded using an electronic data logger (Squirrel SQ2020, Grant Instruments).

Subjects sat for at least 10 min in a thermoneutral (21.0 $\pm$ 1.0°C; 38 $\pm$ 6% relative humidity) environment, before a baseline blood sample (Rest) was taken from an antecubital vein. Subjects then consumed 500 mL of either a placebo [ordinary tap water with 20% sugar-free lemon squash (Morrisons, Bradford, UK) (PLA)], or the experimental drink [same as PLA but with the addition of 150 mg kg body mass$^{-1}$ tyrosine (Myprotein.co.uk, Cheadle, Cheshire, UK) in powder form (TYR)]. Pilot work showed that this dose elicited a large and long-lasting rise in the plasma tyrosine concentration. This dose has also been used effectively in previous studies in man, when placed under conditions which increase the demand on central catecholamine metabolism (Mahoney et al. 2007; Neri et al. 1995). The drinks were coded and prepared by a separate drinks supervisor to ensure they were blinded to both subject and primary researcher, and were randomly administered in a crossover fashion between the two main trials. The drinks were also served in opaque water bottles and shaken before being given to the subjects to disguise the consistency, and were indistinguishable in taste to the subjects. Subjects then rested for 60 min before a second blood sample (Pre) was taken. The subjects then entered the climate chamber (Design Environmental, Gwent, Wales, UK) which was maintained at 30.0 $\pm$ 0.6°C; 60 $\pm$ 0% relative humidity; 0.3 $\pm$ 0.1 m s$^{-1}$ mean air velocity, and mounted the cycle ergometer, before cycling to volitional exhaustion at a constant power output of 25% $\Delta$ (power output at gas exchange threshold, plus 25% of the difference between the gas exchange threshold and $\dot{V}O_{2peak}$). This ensured that all subjects were exercising in the 'heavy' intensity domain, defined as an intensity above the lactate threshold but below critical power threshold (Jones and Poole 2005), and at the same relative exercise intensity (Roston et al. 1987). This was equivalent to 161 $\pm$ 17 W or 68 $\pm$ 5% $\dot{V}O_{2peak}$ for this group. No warm up was performed before the cycle to exhaustion. Fluid (ordinary tap water with 20% sugar-free lemon squash) was provided to all subject at a rate of 2 mL kg body mass$^{-1}$ every 15 min throughout exercise. When it was evident from visual signs that participants were nearing exhaustion (e.g. cadence falling dramatically, slumping on the cycle ergometer), then verbal encouragement in the form of standardised motivating statements to continue exercise, were given to each subject. At the point of volitional exhaustion, which for practical purposes was defined as an inability to maintain a cadence above 60 rpm for three consecutive 10 s periods despite verbal encouragement, subjects were quickly moved to a seat just outside the climate chamber in order that a final blood sample (Exhaustion) could be obtained. A maximum of 3 min elapsed between the end of exercise and the last blood sample being drawn. Subjects were monitored for 15 min to ensure that core temperature decreased, they then showered and towelled dry to exclude effective sweat losses from any body mass calculations. A further urine sample was taken and volume and osmolality measured as previously described before subjects were re-weighed nude.

## Measurements

Heart rate, *Tcore* and *Tskin* were recorded every 10 min throughout the resting and exercise periods. Measurements of *Tskin* were used to calculate body heat content (Burton 1935) and the weighted mean skin temperature using the method of Ramanathan (1964). Every 15 min throughout exercise RPE was recorded (Borg 1982), as was thermal sensation using a 21 point scale, ranging from $-$10 (cold impossible to bear) to +10 (heat impossible to bear) (adapted from Parsons 2003). Mean air velocity within the climate chamber was calculated every 15 min throughout exercise as the mean of four measurements taken in front, behind, and at left and right sides of the subjects' head, using a hand-held anemometer (Kestrel 1000, Richard Paul Russell Ltd, Lymington, UK). Body mass losses during each exercise trial were calculated as the difference between pre- and post-exercise body mass, after adjusting for the volume of fluid consumed, and any urine output following initial weighing.

### Blood sampling

All blood samples collected during the experimental trials were obtained by venipuncture from an antecubital vein, with minimal stasis, and were drawn into vacutainer tubes (BD Vacutainer Systems, Plymouth, UK) comprising 6 mL into a heparinized vacutainer and 4.5 mL into a $K_3$EDTA treated vacutainer. Whole blood from the $K_3$EDTA vacutainer was used to measure haemoglobin, haematocrit, blood glucose and blood lactate and the remaining blood ($K_3$EDTA and heparin tubes) was immediately centrifuged at 1,500*g* for 10 min at 4°C. The plasma was removed, and stored at −84°C for later analysis of amino acids. Blood glucose and blood lactate were measured using an automated analyser (2300 Stat Plus, Yellow Spring Instrument Co., OH, USA), and haemoglobin concentrations were measured using an automated haematology analyser (ABX Pentra 120, Horiba ABX Diagnostics, Northampton, UK). Haematocrit was measured using microcentrifugation; whole blood was drawn into micro-capillary tubes, spun for 5 min using a Hawksley microcentrifuge, and the separated red cell volume was measured using a Hawksley haematocrit reader. Plasma volume changes were estimated using the equations of Dill and Costill (1974). All blood analyses were carried out in duplicate, except haematocrit measurements, which were carried out in triplicate. The coefficient of variation for blood lactate measurement was 1.5%, for blood glucose 1.7%, for haemoglobin 0.3%, and for haematocrit 0.8%. The coefficient of variation for measurement of individual amino acids was: leucine, 8.5%; isoleucine, 9.7%; valine, 5.8%; methionine, 11.4%; threonine, 8.7%; lysine, 5.0%; tryptophan, 5.2%; and tyrosine, 6.5%.

### Amino acid analysis

Plasma amino acids were analysed using gas-chromatography mass spectrometry. From on ice, 200 μL thawed heparinised plasma samples were transferred into micro tubes (2 mL Eppendorf) containing ~500 μL glass beads, diluted with 1,520 μL degassed helium and chilled (−20°C) methanol/chloroform (4:1, v/v), vortexed (10 s), shaken for 15 min at 4°C and centrifuged for 5 min at 4°C and 14,240*g* (Hettich EBA 12R). For gas chromatography–mass spectrometry analysis 420 μL of the supernatant were transferred into new 2 mL micro tubes and dried in vacuo (speed vac, Univapo 150 H). Sample tubes containing supernatant were then stored at –80°C.

The carbonyl moieties were protected by methoximation using 60 μL of a 20 mg $mL^{-1}$ solution of methoxyamine hydrochloride (Fluka) in pyridine (Fluka) at 30°C for 90 min, to enable two-step derivatization of dried samples. Acidic protons were subsequently derivatized with 60 μL *N*-methyl-*N*-trimethylsilyltrifluoride (MSTFA, Mand N) at 37°C for 30 min. Following this, 60 μL were transferred into 200 μL glass vials (Chromacol) and 1 μL was injected split-less into a Leco Pegasus III GC-tof–MS system (St. Joseph, USA) consisting of a Focus autosampler (Anatune), an Agilent 6890N gas chromatograph equipped with a DB5-MS column (20 m × 0.25 mm ID × 0.25 μm film). Injector temperature was 250°C, the transferline was set to 260°C and the ion source temperature held at 230°C. Helium flow was 1.2 mL $min^{-1}$. After 1 min at 80°C, oven temperature was increased by 30°C $min^{-1}$ to 330°C, held at 330°C for 3 min and cooled to 80°C. Automated deconvolution and peak finding was performed using ChromaTof software (Leco, St. Joseph, USA).

### Statistical analyses

Data were analysed using a computerised statistical package (SPSS version 17.0, SPSS inc., Chicago, IL, USA) and are presented as mean ± SD unless otherwise stated. A repeated measures two-factor (time × trial) ANOVA was used to compare trials. Where a significant interaction was found in ANOVA, post hoc analysis was carried out using paired student's *t* tests with the Bonferroni correction to highlight pairwise differences. Exhaustion values were analysed separately using paired student's *t* test to account for the different exercise duration between subjects and trials. Simple linear regression was used to analyse the slope values of *Tcore*, RPE, and thermal sensation responses in relation to time. Statistical significance was accepted at the $P < 0.05$ level.

## Results

### Time to exhaustion

The coefficient of variation for exercise time to exhaustion between the second familiarisation trial and the placebo trial was 10.7%, and there was no effect of trial order ($P = 0.771$). Subjects exercised for 15 ± 11% longer in TYR compared to PLA (80.3 ± 19.7 min vs. 69.2 ± 14.0 min; $P = 0.006$; Fig. 1) at a power output of 161 ± 17 W (68 ± 5% $\dot{V}O_{2peak}$).

### Blood measures

All amino acid measurements were corrected for plasma volume changes from baseline. Significant time × trial interaction ($P < 0.01$), main effect of time ($P < 0.01$) and trial ($P < 0.01$) were evident for changes in tyrosine concentration (Fig. 2a). Mean plasma tyrosine concentrations increased in TYR from Rest to Pre-, and this was

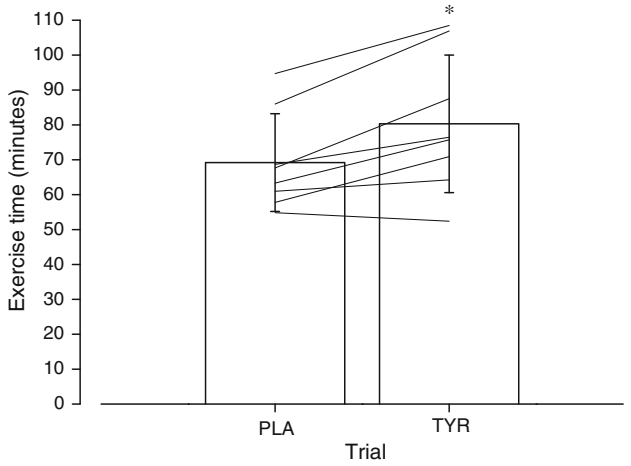


**Fig. 1** Mean (±SD) (*columns*) and individual (*lines*) exercise times to exhaustion ($n = 8$) in tyrosine (TYR) and placebo (PLA) conditions. *Mean value significantly higher than PLA ($P = 0.006$)

maintained throughout exercise ($P < 0.01$). The ratio of tryptophan:LNAA did not show an interaction effect ($P = 0.998$) and did not change over time ($P = 0.149$; Fig. 2b). There was a time × trial interaction for tyrosine:LNAA ($P = 0.001$) and the ratio changed significantly over time ($P < 0.01$; Fig. 2c). Post hoc analysis showed no difference at rest ($P = 0.344$), however tyrosine:LNAA increased almost threefold at 1 h following supplementation in TYR ($P = 0.004$) and this was maintained until exhaustion ($P < 0.01$).

Blood glucose concentrations showed no time × trial interaction ($P = 0.469$) and no change over time ($P = 0.268$). Subjects were euglycemic (4.1 ± 0.8 mmol in PLA and 4.4 ± 0.8 mmol in TYR; Table 1) at exhaustion. Blood lactate concentrations exhibited no interaction ($P = 0.186$); however, there was a main effect of time ($P = 0.005$). Post hoc analysis showed that blood lactate concentrations at exhaustion (2.1 ± 1.2 mmol for PLA and 1.8 ± 0.9 mmol for TYR; Table 1) had increased from Rest and Pre-values ($P < 0.05$).

## Hydration status

Two-way ANOVA revealed no time × trial interaction effect for urine osmolality ($P = 0.531$). Urine osmolality values suggest that subjects arrived at the lab euhydrated (380 ± 255 mosmol $L^{-1}$ in PLA and 419 ± 198 $L^{-1}$ in TYR) and there was no change over time ($P = 0.354$). At end of exercise, urine osmolality was 283 ± 168 mosmol $L^{-1}$ in PLA and 388 ± 251 mosmol $L^{-1}$ in TYR. Exercise induced similar body mass losses in PLA and TYR (1.3 ± 0.2 and 1.5 ± 0.3 kg, respectively; $P = 0.225$), which equated to 1.8 ± 0.4 and 2.0 ± 0.6% of body mass for PLA and TYR, respectively. Sweat rates during exercise

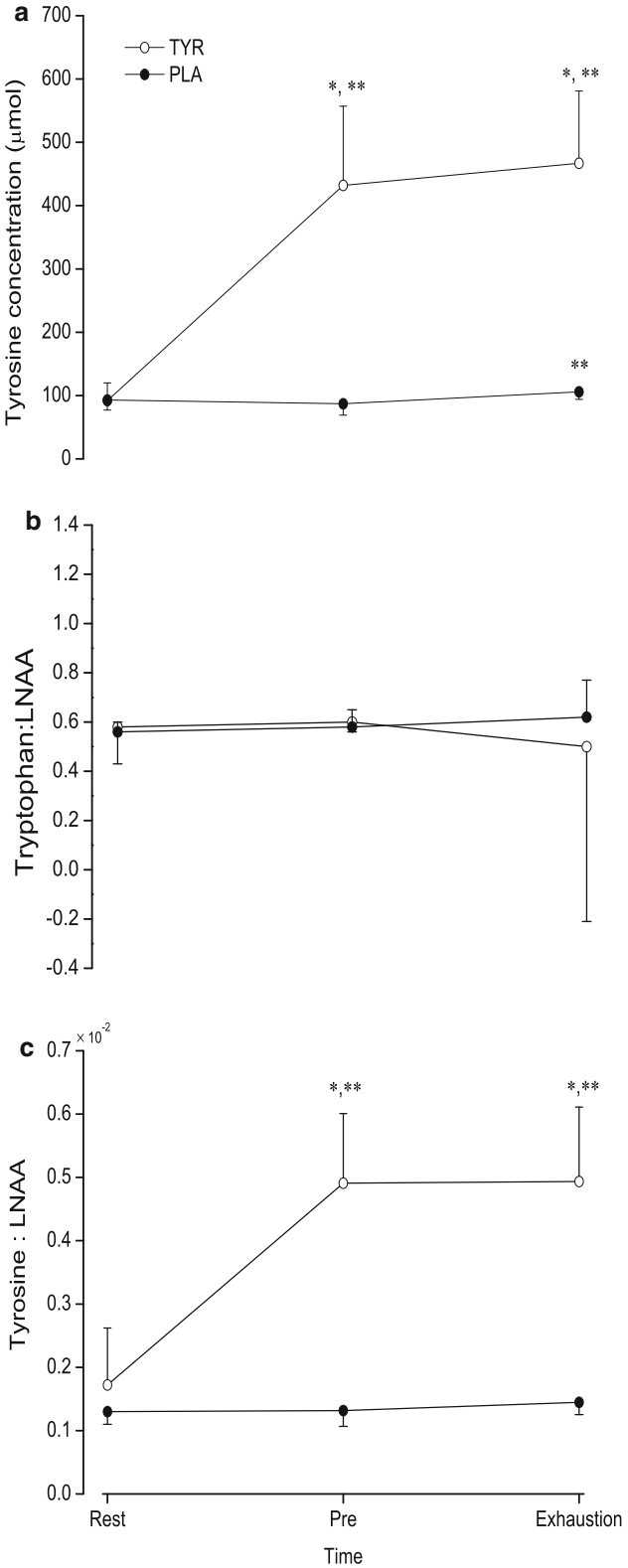


**Fig. 2** Plasma tyrosine concentration (**a**), tryptophan:LNAA (**b**) and tyrosine:LNAA (**c**) for tyrosine (*open circle*) and placebo (*filled circle*) conditions. Values are mean ± SD. *Significantly higher than PLA at same time point ($P < 0.05$) ($n = 8$); **significantly higher than Rest value (within trial) ($P < 0.05$) ($n = 7$)

**Table 1** Changes in plasma volume, blood glucose, and blood lactate

| | Rest | Pre | Exhaustion |
|---|---|---|---|
| Δ Plasma volume (%) | | | |
| PLA | – | –1.8 ± 3.8 | –3.6 ± 6.3 |
| TYR | – | –1.6 ± 1.2 | –6.7 ± 2.3 |
| Post hoc *t* test for time effect | | | * |
| Blood glucose (mmol) | | | |
| PLA | 4.6 ± 0.2 | 4.2 ± 0.2 | 4.1 ± 0.8 |
| TYR | 4.4 ± 0.5 | 4.3 ± 0.2 | 4.4 ± 0.8 |
| Post hoc *t* test for time effect | | | * |
| Blood lactate (mmol) | | | |
| PLA | 0.6 ± 0.2 | 0.5 ± 0.1 | 2.1 ± 1.2 |
| TYR | 0.6 ± 0.2 | 0.6 ± 0.2 | 1.8 ± 0.9 |
| Post hoc *t* test for time effect | | | * |

Values are mean ± SD

* Significantly different than Rest and Pre values ($P < 0.05$; $n = 8$)

were also similar for PLA and TYR (1.2 ± 0.3 and 1.1 ± 0.1 L $h^{-1}$, respectively, $P = 0.48$).

## Heart rate

During exercise, heart rate exhibited no time × trial interaction ($P = 0.666$) but did increase significantly over time ($P < 0.01$; Fig. 3). Heart rate pre-exercise was 65 ± 11 beats $min^{-1}$ for PLA and 65 ± 6 beats $min^{-1}$ for TYR, and was increased by 10 min of exercise up until exhaustion ($P < 0.01$; 177 ± 16 beats $min^{-1}$ for PLA and 174 ± 17 beats $min^{-1}$ for TYR).

## Temperature measures

Body heat content (data not shown) did not show an interaction ($P = 0.281$). There was however, a main effect of time ($P < 0.01$) with an increase in body heat content compared to the start of exercise evident after 10 min cycling, which was maintained until exhaustion ($P < 0.01$). *Tskin* showed no time × trial interaction ($P = 0.178$) but increased significantly after 10 min of exercise ($P < 0.01$; Fig. 4a) to 35.0 ± 0.8°C and 35.0 ± 0.9°C for PLA and TYR, respectively, at exhaustion. *Tcore* did not exhibit a time × trial interaction ($P = 0.144$) but increased after 10 min of exercise up until exhaustion ($P < 0.01$; Fig. 4b) when *Tcore* reached 39.0°C in PLA (±0.3°C) and TYR (±0.6°C). The mean slope values of *Tcore* versus time from 0 to 50 min (which represented the last common measurement time point) were the same in TYR and PLA (0.37 ± 0.06°C $min^{-1}$ and 0.39 ± 0.09°C $min^{-1}$; $P = 0.568$).

## Subjective measures

ANOVA showed no time × trial interaction for thermal sensation ($P = 0.875$) but this increased significantly as

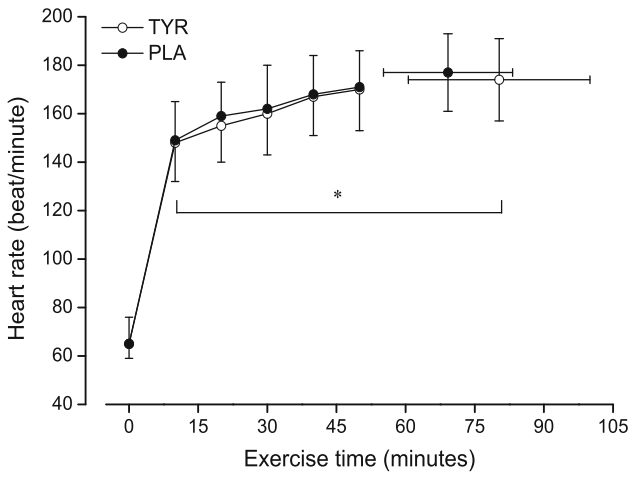


**Fig. 3** Heart rate responses to exercise ($n = 8$) in tyrosine (*open circle*) and placebo (*filled circle*) conditions. Values are mean ± SD. *Significantly higher than 0 min value ($P < 0.05$)

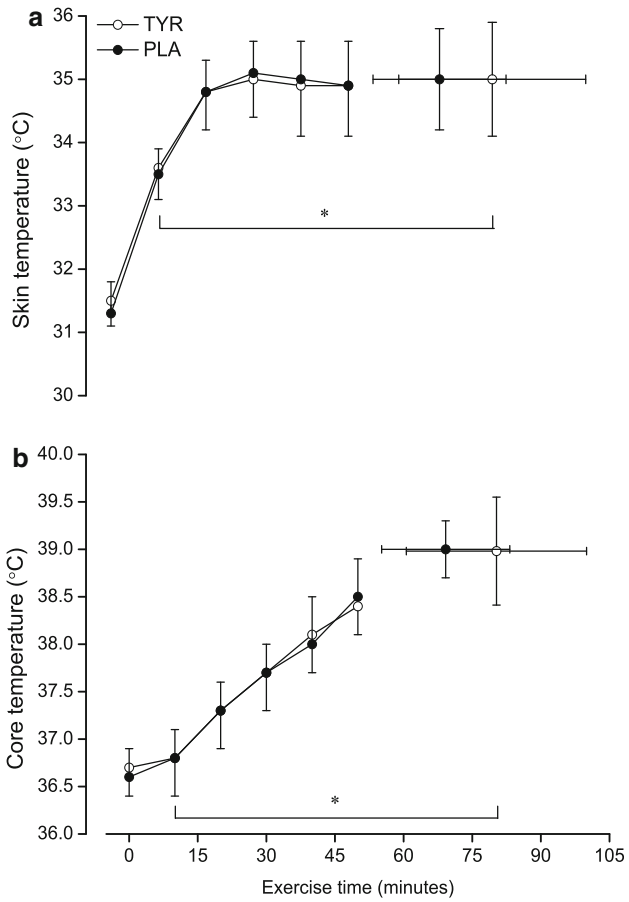


**Fig. 4** Mean weighted skin temperature (**a**) and core temperature (**b**) responses to exercise ($n = 8$) in tyrosine (*open circle*) and placebo (*filled circle*) conditions. Values are mean ± SD. *Significantly higher than 0 min value ($P < 0.05$)

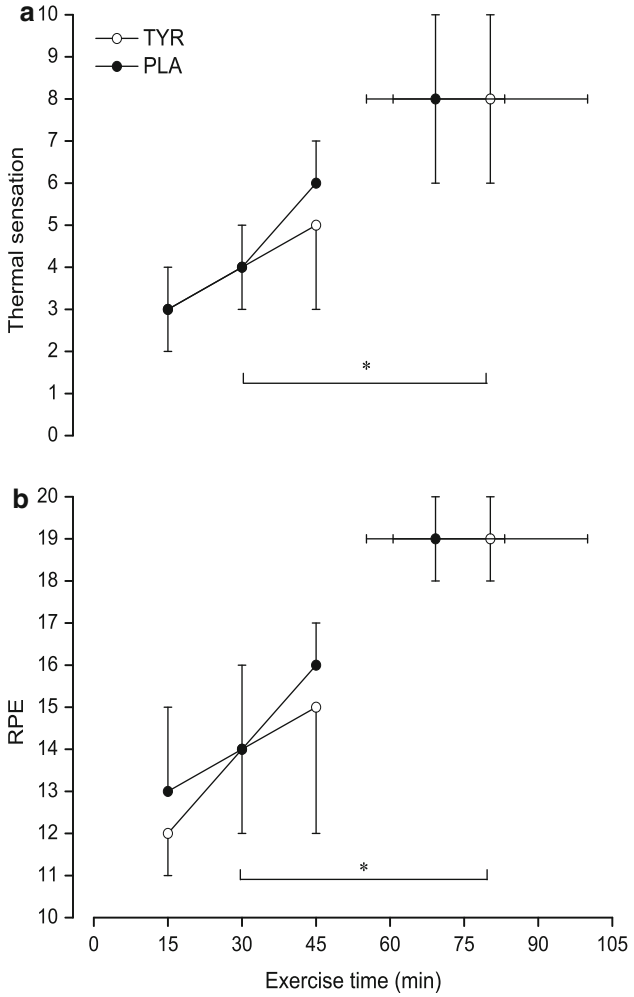


**Fig. 5** Ratings of thermal sensation (**a**) and perceived exertion (**b**) ($n = 8$) in tyrosine (*open circle*) and placebo (*filled circle*) conditions. Values are mean ± SD. *Significantly higher than 15 min value in both conditions ($P < 0.05$)

exercise progressed from 30 min onwards ($P < 0.01$; Fig. 5a). Exhaustion coincided with a thermal sensation of 8 ± 2, representing a subjective rating of "extremely hot, close to limit". Similarly, there was no interaction for RPE ($P = 0.550$; Fig. 5b), but ratings did increase during exercise from 30 min onwards ($P < 0.01$), to 19 ± 1 at exhaustion. There was no difference between TYR and PLA in the mean slope values for RPE (0.09 ± 0.07 and 0.10 ± 0.05 arbitrary units $min^{-1}$, respectively; $P > 0.05$) or thermal sensation (0.10 ± 0.07 and 0.10 ± 0.04 arbitrary units $min^{-1}$, respectively; $P > 0.05$) from 15 to 45 min. Assuming a linear increase from 45 min to exhaustion, the mean RPE slope values for TYR were significantly lower than PLA (1.94 ± 1.43 and 3.75 ± 1.67 arbitrary units $min^{-1}$, respectively, $P < 0.05$) but were similar for thermal sensation (0.07 ± 0.06 and 0.11 ± 0.06 arbitrary units $min^{-1}$, $P > 0.05$).

## Discussion

The main finding of the present study was that an acute dose of 150 mg kg body $mass^{-1}$ tyrosine, a nutritional dopamine precursor, consumed 1 h pre-exercise was associated with an increase in exercise capacity by 15 ± 11% during a constant-load cycling trial in a warm (30°C) environment. This novel finding shows that tyrosine availability contributes to the ability to continue exercise under such conditions, and indirectly supports the importance of brain dopamine to exercise tolerance in the heat (Watson et al. 2005; Bridge et al. 2003).

Evidence suggests that factors within the central nervous system may be involved in fatigue during prolonged exercise in the heat, which may arise due to inhibitory signals from the hypothalamus, secondary to a high brain temperature (Nielsen et al. 1993; Nybo and Nielsen 2001). One avenue of research suggests that fatigue in the heat may represent a reduced state of arousal (Nielsen et al. 2001) concomitant with a linear increase in RPE (Nybo and Nielsen 2001). Although an exact neurobiological cause for this is lacking, a high brain dopamine:5-HT ratio may preserve arousal levels during prolonged exercise (Davis and Bailey 1997), and there is strong evidence that increased brain dopamine availability is specifically involved in exercise tolerance in the heat (Watson et al. 2005; Bridge et al. 2003). The increased TYR:LNAA with no change in tryptophan:LNAA in the present study would increase the availability of tyrosine for brain dopamine synthesis and release, due to competitive uptake at the blood–brain barrier (Pardridge 1983). Brain dopamine has several roles including movement initiation, motor control, increased arousal, and endurance (Chandler and Blair 1980; Freed and Yamamoto 1985), whereas 5-HT is linked to reduced arousal and sleep, and both neurotransmitters are synthesized from their respective amino acid precursors tyrosine and tryptophan. In the present study an increased availability of a dopamine precursor may have maintained arousal levels or improved subjective tolerance of the exercise protocol in TYR, allowing exercise to continue for longer compared to PLA. The slope values for RPE provides some support for this, as they were similar in both conditions up to 45 min of exercise and at end of exercise, despite exhaustion occurring 11 ± 8 min later in TYR. This implies that either the rate of rise from 45 min to exhaustion (assuming a linear increase) was slower in the TYR trial as evidenced by the lower slope values, or subjects in TYR reached maximal subjective sensations at a similar rate as PLA, but tolerated them for longer. Either way, both these explanations are suggestive of an improved subjective tolerance of the exercise protocol following tyrosine supplementation. Perhaps a more frequent measurement of RPE and TS would better highlight any effect

of tyrosine supplementation, and future work is needed to fully address this. The dosage used in the present study has previously improved mood and arousal scores, and dopamine-dependant cognitive and psychomotor performance while under severe environmental stress theorised to increase brain catecholamine metabolism (Mahoney et al. 2007; Neri et al. 1995). These effects are consistent with an effect on the prefrontal cortex, a brain area which is rich in dopaminergic innervation, and which is particularly susceptible to tyrosine availability due to the inherently high firing rates of these neurons (Roth et al. 1988). Furthermore, changes in electricoencephalogram activity in this area are strongly correlated with increasing RPE during prolonged exercise in the heat, although the exact significance remains to be elucidated. Nonetheless, the present results extend findings from previous tyrosine studies, by suggesting an association between tyrosine availability and prolonged exercise tolerance in the heat, and this may reflect a maintenance of prefrontal cortex dopamine neuronal activity (Roth et al. 1988).

In contrast to the present results, tyrosine studies carried out in temperate conditions showed no improvement in either exercise capacity (Strüder et al. 1998) or performance (Chinevere et al. 2002). One study showed that 20 g tyrosine can affect hormonal markers of dopaminergic function, despite no effect on time to exhaustion during constant-load submaximal exercise in temperate conditions (Strüder et al. 1998). Nonetheless, the same study reported an improvement in 'drive' in psychometric testing suggesting that tyrosine can act centrally on cognitive affect following oral supplementation (Strüder et al. 1998). Different effects of tyrosine on exercise may be due to the present protocol placing a larger demand on the dopaminergic system (Bridge et al. 2003; Watson et al. 2005), and increasing the subjective demand of exercise compared to temperate conditions (Nybo and Nielsen 2001). Taken together, the available evidence suggests the combined demands of both exercise and heat stress increase tyrosine requirements and that the availability of tyrosine, a direct dopamine precursor, may limit prolonged exercise capacity in the heat. Furthermore, although central fatigue was not measured directly in the present study the available peripheral data do not adequately explain the difference in exercise time between TYR and PLA. Previous studies in which brain dopamine availability was directly increased with amphetamines in temperate conditions have noted longer cycling at a fixed submaximal RPE, and at a higher power output compared to placebo (Swart et al. 2009), and improved maximal intensity exercise tolerance (Chandler and Blair 1980). Notably, Watson et al. (2005) administered a dual dopamine/noradrenaline reuptake inhibitor (bupropion) and reported improvements in cycling performance in 30°C but not 18°C, suggesting a specific role of brain catecholamines in warm environmental conditions. It seems that increasing dopamine availability via bupropion dampened inhibitory signals within the central nervous system, allowing improved subjective tolerance of higher core temperature and heart rate than placebo, but at the same RPE (Watson et al. 2005). A similar exercise enhancing effect may have occurred in the present study following tyrosine supplementation. Maintenance of brain dopamine in TYR may have allowed subjects to temporarily override afferent thermoregulatory feedback, or central inhibitory signals so that exercise continued for longer (Swart et al. 2009; Watson et al. 2005). It should be noted that previous drug interventions would specifically increase the release, and prolong the action of dopamine at receptor sites (Swart et al. 2009; Watson et al. 2005), whereas tyrosine supplementation may be expected to maintain or prevent the drop in dopamine activity. Nonetheless, our study is the first to demonstrate that acute supplementation of the natural dopamine precursor is associated with increased exercise capacity in the heat. One accepted viewpoint states that under 'normal' conditions brain tyrosine concentrations are above the Michaelis constant ($K_m$) for tyrosine hydroxylase and therefore supplementary tyrosine would not be expected to augment dopamine synthesis (Cooper et al. 2003). However, direct in vivo measurements indicate that in animals, tyrosine hydroxylase is only 75% saturated (Carlsson and Lindqvist 1978), and supplementary tyrosine can increase dopamine release if brain neurons are firing more rapidly (Sved et al. 1979; Melamed et al. 1980). Furthermore, one human study has demonstrated a small net uptake of tyrosine by the human brain during prolonged exercise (Nybo et al. 2003) in the absence of tyrosine supplementation. It is possible that the longer exercise time in the present study occurred through increased brain noradrenaline concentrations, as tyrosine is a general catecholamine precursor. However this is unlikely as dopaminergic neurons are more susceptible to tyrosine depletion than noradrenergic neurons (McTavish et al. 1999), and increased brain noradrenaline concentrations tend to reduce exercise performance in the heat (Roelands et al. 2008).

Research into the effect of heat on fatigue during prolonged exercise has commonly proposed a "critical" core temperature of 40°C beyond which exercise cannot continue (Gonzalez-Alonso et al. 1999). Although the present subjects' end *Tcore* was considerably lower than this, these values are in agreement with previous studies involving moderately fit subjects (Cheung and McLellan 1998; Latzka et al. 1998). Furthermore, recent observations of athletes tolerating *Tcore* of greater than 40°C during athletic events have further questioned this threshold (Ely et al. 2009). One possible explanation for the difference in exercise time between TYR and PLA in

the present study is that similar maximal *Tcore* was reached in both conditions but maintained for 11 min longer in TYR, in line with an improved subjective tolerance of elevated *Tcore*. An alternative, albeit speculative explanation, is that the increased exercise capacity in TYR occurred due to improved thermoregulatory responses by reducing the rate of rise in *Tcore* from 50 min up to exhaustion via a direct effect on hypothalamic dopamine transmission. Dopamine has a well documented role in heat loss mechanisms in the preoptic anterior hypothalamus area (Hasegawa et al. 2000), which is the main thermoregulatory centre in the brain (Boulant 2000). Also, drugs which augment dopamine metabolism in this area have a vasodilatory effect and cause a fall in core and brain temperature, whereas dopamine antagonists have the opposite effect (Nunes et al. 1991). If the supplementary tyrosine in the present study maintained dopamine metabolism in the hypothalamus thus reducing the rate of *Tcore* increase, then this was not reflected by a lower body heat content and higher sweat rates and body mass losses in TYR. Although it was not addressed in the present study, a measurable increase in skin blood flow following supplementation would provide stronger evidence of a thermoregulatory effect of TYR.

Separate reports suggest that fatigue during prolonged exercise in the heat may occur primarily due to high cardiovascular strain rather than central nervous system factors per se (Kenefick et al. 2010), occurring secondary to hyperthermia invoked tachycardia and dehydration induced decrease in stroke volume (Gonzalez-Alonso et al. 2008). This may seem at odds with the present hypothesis and results; however, if cardiovascular strain did limit exercise in the present study, then tyrosine seemingly allowed subjects to maintain maximal heart rate for longer compared to placebo. This is consistent with the proposal of improved arousal and/or motivation in TYR. Also, fluid was provided during exercise, which would have limited the effects of some of these cardiovascular changes on fatigue. It is possible that the additional tyrosine may have directly affected cardiovascular function by acting on sympathetic neurons. Dollins et al. (2005) noted maintenance of pulse pressure and greater tolerance of lower body negative pressure exposure following acute tyrosine supplementation, indicative of increased catecholamine output from peripheral (and perhaps central) sympathetic neurons, or adrenomedullary cells. Tyrosine administration also exhibits a vasopressor effect in hypotensive animals (Conlay et al. 1985), consistent with the idea that neurons must be activated for tyrosine to affect catecholamine synthesis and release (Sved et al. 1979). The increase in blood pressure seems to occur due to increased peripheral (and perhaps central) catecholamine outflow, as adrenal epinephrine levels are increased (Conlay et al. 1985), and pre-treatment with an α-adrenergic receptor blocker prevents the increase (Conlay et al. 1985). Unfortunately, the available data in the present study do not allow us to draw firm conclusions in relation to this.

## Conclusions

In summary, the present study indicates for the first time, that supplementing a nutritional dopamine precursor 1 h pre-exercise is associated with increased exercise capacity in the heat, and show that tyrosine availability at least in part, may influence prolonged exercise tolerance with heat stress in moderately trained individuals. Future studies should include direct measurements of central fatigue and confirm to what extent there is an increased brain extraction of tyrosine in man following supplementation, while exercising in a warm environment.

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