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ORIGINAL ARTICLE

Plasma catecholamine and nephrine responses to brief intermittent maximal intensity exercise

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Abstract Catecholamines (noradrenaline, NA; adrenaline, AD; dopamine, DA) influence the metabolic and cardiovascular responses to exercise. However, changes in catecholamine metabolism during exercise are unclear. Plasma normetanephrine (NMET), metanephrine (MET) and catecholamine responses to a laboratory-based model of games-type exercise were examined. Twelve healthy men completed a resting control trial and a trial consisting of ten 6 s cycle ergometer sprints interspersed with 30 s recovery, in randomised order. Resting and post-sprint venous blood samples were taken. Plasma NA and AD increased after each sprint but DA was unaltered. Plasma nephrines increased significantly from sprint 4 onwards with peak NMET increasing 60% to 0.76 ± 0.19 nmol l^{-1} and MET 230% to 0.37 ± 0.16 nmol l^{-1} from resting values ($P < 0.05$). The results demonstrate increased catecholamine metabolism via elevated catechol-*O*-methyl transferase activity during intermittent sprinting. The results may aid regulation of the metabolic and cardiovascular responses to exercise by maintaining tissue adrenoceptor sensitivity to circulating catecholamines.

Keywords Metanephrine · Normetanephrine · Dopamine · Intermittent exercise

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Introduction

Paragraph 1

Plasma catecholamines (noradrenaline, NA; adrenaline, AD; dopamine, DA) provide a valuable measurement of whole body sympatho-adrenal activity during physical exercise (Kjær 1999). Acting as both neurotransmitters and hormones, they play important roles in the cardiovascular, metabolic and immune systems and in determining exercise capacity (Kaiser et al. 1986; Winder et al. 1987). The plasma catecholamine response to exercise results from an increase in sympathetic nerve activity and adrenal medulla chromaffin cell exocytosis alongside a small decrease in clearance rate (Esler et al. 1990; Goldstein et al. 2003; Kjær et al. 1985; Leuenberger et al. 1993).

Paragraph 2

The responses of catecholamine metabolic pathways to physical exercise have not been fully examined. Metabolism is important for clearance of catecholamines from the circulation and preventing prolonged stimulation of tissue adrenoceptors. *O*-methylation of NA and AD by catechol-*O*-methyltransferase (COMT) produces the 'nephrines' normetanephrine (NMET) and metanephrine (MET), respectively. The early work of Pequignot et al. (1978) demonstrated increased 24 h urinary nephrine

concentrations in subjects following ten minutes of continuous sub-maximal cycling. However, 24 h urinary nephrine concentrations only provide a rough measurement of nephrines and provide no information on time-course changes during or after exercise. In recent years with the advent of highly specific and sensitive methods, changes in the plasma nephrine response to exercise have been examined in more detail. Zamecnik (1997) found plasma nephrine concentrations increased five to sixfold from rest after two 30 min bouts of cycling performed at 50% VO_2max in healthy male subjects. Increased plasma NMET but not MET concentrations were reported by Raber et al. (2003) who examined healthy untrained subjects during 15 min of incremental cycling exercise that ended at a workload equivalent to 75% VO_2max. The above-mentioned studies provide information regarding the changes in catecholamine metabolism during exercise at sub-maximal exercise intensities but the plasma catecholamine and nephrine concentrations have not been examined during other types of exercise.

Paragraph 3

Sports and exercise activities often elicit high intensity bouts and are frequently interspersed with limited recovery periods. Such 'intermittent activities' are seen in a wide variety of racquet sports (tennis, squash, badminton), court games (basketball, volleyball, netball) and field games (football, rugby, hockey). Researchers have modelled intermittent activities in the laboratory and demonstrated 18-fold increases in plasma NA and AD concentrations when subjects performed an intermittent exercise model consisting of ten 6 s non-motorised sprints with a 30 s rest period between each sprint (Brooks et al. 1990; Gaitanos et al. 1993). Such high values are similar to those seen in some pathophysiological conditions e.g. phaeochromocytoma patients (Lenders et al. 1995). However, little is known about the catecholamine metabolic pathways in response to this common form of exercise.

Paragraph 4

The plasma DA response to physical exercise is presently unclear. Studies have shown increased plasma DA concentrations with exercise protocols of varying intensity and duration (Sakai et al. 1995; Tidgren et al. 1991) whereas other researchers have not (Bracken et al. 2005; Sagnol et al. 1990). Plasma DA originates from sympathetic nerves, adrenal medullae, kidneys and the sulfoconjugated pool (Strobel et al. 1990) with approximately 1–3% of plasma DA existing in an active free form (Miura et al. 1995) whilst the remainder is sulfoconjugated. Dopamine helps modulate sympatho-adrenal activity by increasing the synthesis of NA and AD in sympathetic nerves and adrenal medulla chromaffin cells, as well as through increased peripheral dopaminergic activity (Miura et al. 1995). Stimulation of D_1 receptors by increased DA concentrations mediates vasodilatation of vascular smooth muscle cells facilitating improved oxygen delivery to muscle whilst on the other hand, D_2 receptors on postganglionic presynaptic nerve terminals reduce NA release and accordingly, heart rate and vascular resistance (Murphy 2000). Therefore, a role may exist for DA in helping to mediate some cardiovascular changes with exercise. However, the importance of plasma DA during intermittent exercise, an exercise form that places significant importance on oxygen delivery to muscle in attenuating fatigue (Balsom et al. 1994), has not been studied. Therefore, an examination of the plasma DA response to this type of exercise is warranted.

Paragraph 5

Furthering our knowledge of the factors responsible for determining the plasma catecholamine concentration is important given the significance of catecholamines in determining the magnitude of metabolic, cardiorespiratory and hormonal responses to exercise and in determining performance. In an attempt to further our understanding of plasma catecholamine regulation the aim of this research was to examine the magnitude of the plasma catecholamine (NA, AD, DA) and nephrine (NMET, MET) responses to a laboratory-based model of brief intermittent maximal intensity cycle exercise.

Materials and methods

Subjects

Paragraph 6

The experimental protocol was approved by the University Research Ethics Committee. Twelve healthy, non-specifically trained male subjects volunteered to take part in this study. After receiving a full explanation of the protocol, all subjects completed written informed consent and medical history forms prior to participation in the study. The physical and physiological characteristics of the subjects were age 21 ± 2 years, stature 176 ± 5 cm, body mass 80.9 ± 9.1 kg and estimated body fat 17.2 ± 3.6%.

Preliminary testing

Paragraph 7

Subjects attended the laboratory on three separate occasions. On the first visit subjects carried out preliminary

anthropometric tests and were familiarised to the cycle ergometer (Monark, 824e). Using a crossover design with independent measures to examine the effects of repeated sprinting on catecholamine and nephrine concentrations, subjects attended the laboratory for either a control (CON) or an exercise (EX) trial in a randomised order. For the CON trial, all data were collected at the same time of day and at the same respective time points as the exercise trial except that no exercise was performed on this occasion. There was at least one week between experimental trials.

Main exercise trial procedures

Paragraph 8

Before arriving at the laboratory for the experimental trials, subjects were instructed to abstain from strenuous activity and to avoid foods containing high biogenic amine content or caffeinated drinks for 24 h prior to testing (Bracken et al. 2005). On arrival to the laboratory height and mass were determined and following this a heart rate monitor was placed around the subject's chest (Polar Accurex *Plus* HRM, Polar Instruments Ltd). Heart rate was recorded every 5 s. Subjects were then seated whilst a 21-gauge catheter was placed in an antecubital vein. Saline was infused periodically to keep the catheter patent. Twenty minutes later resting blood samples were taken. Following this, subjects sat quietly on the cycle ergometer for 5 min before either continuing to rest or, on another occasion, beginning the cycle test which consisted of ten maximal 6 s sprints against a workload equivalent to 0.075 kg kg^{-1} body mass with a 30 s recovery period between each sprint. Power was calculated using the methods of Lakomy (1986). Flywheel velocity was continuously monitored with an electrical d.c. generator driven by the cycle ergometer flywheel, which gave an analogue signal proportional to the angular velocity of the flywheel. This signal was then logged via an analogue to digital converter by the computer. Concurrently, a timing signal derived from the computers internal clock was also stored. With the moment of inertia of the flywheel constantly changing, corrections for the change in velocity were made by determining the work done in accelerating the flywheel i.e. acceleration balancing load or the frictional load required to stop the subject accelerating the flywheel. The power output was determined as follows;

$$\begin{aligned}&\text{Corrected Power Output (Watts)}\\&\quad = \text{Flywheel Velocity (rev min}^{-1}\text{)}\\&\qquad \times \text{ Flywheel Circumference (m)} \times [R_F(N)\\&\qquad + \text{ABL(N)}].\end{aligned}$$

The flywheel speed and determination of acceleration balancing load were calculated before each and every EX trial. The following definitions for performance indices were used: (1) *Pedal revolution rate:* The pedal speed (rev min^{-1}) over single or successive sprints. (2) *Peak power output* (PPO, Watts): The product of the maximum 1 s integral of flywheel speed and the effective load. (3) *Mean power output* (MPO, Watts): The product of the mean 1 s integral of flywheel speed and the effective load. (4) *Minimum power output* (MinPO, Watts): The product of the end 1 s integral of flywheel speed and the effective load. (5) Work done (J): The product of the mean 1 s integral of flywheel speed, effective load and the duration of each sprint. (6) Total work (kJ): The product of the mean 1 s integral of flywheel speed, effective load, the duration of each sprint and the total number of sprints. (7) Between sprint fatigue rate (%): The percentage difference between the mean power output in sprints 1 and 10, i.e.

$$[(\text{MPO}_{\text{sprint1}} - \text{MPO}_{\text{sprint10}}) \div \text{MPO}_{\text{sprint1}}] \times 100.$$

Blood samples treatment

Paragraph 9

At rest and immediately following each sprint one ml of heparinised syringe of venous blood was obtained, immediately capped and analysed for blood pH (Radiometer ABL-5). Furthermore, a 10 ml blood sample was taken and immediately dispensed into lithium-heparinised tubes. From these samples, duplicate aliquots (100 µl) of blood were immediately deproteinised in 1 ml of ice-cold perchloric acid (2.5% v/v) and analysed later for lactate according to the method of Maughan (1982). Haemoglobin concentration was determined using the cyanomethaemoglobin technique and packed cell volume was determined using the capillary centrifuge technique from which changes in plasma volume were estimated (Dill and Costill 1974). A one ml aliquot of plasma was stored frozen (−70°C) for later determination of NMET and MET concentration using a commercially available enzyme linked immunoassay kit (Labor Diagnostica Nord, Germany).

Paragraph 10

To the remainder of the blood sample 200 µl of 0.1 mol l^{-1} of both ethylene glycol bis-(β-aminoethyl ether)-*N*′,*N*′,*N*′,*N*′-tetraacetic acid (EGTA) as anticoagulant and glutathione as antioxidant were added. Samples were centrifuged for 10 min at 3,000 rev min^{-1} with plasma separated and stored at −80°C for later analysis of catecholamines. Plasma NA, AD and DA concentrations were determined using an alumina extraction method involving HPLC with electrochemical detection [Gilson HPLC pump, model 307: Gilson electrochemical detector, model 141] (Bracken et al. 2005). Briefly, 100 µl of sample was

injected into a HPLC column (Spherisorb S5 ODS 2) and eluted with a mobile phase (monochloroacetic acid 11.14 g l^{-1}, NaOH 3.37 g l^{-1}, NaOS 0.74 g l^{-1} and EDTA 0.09 g l^{-1}, pH 3.0). Before separating catecholamines in the HPLC column, a standard solution containing 100 nmol l^{-1} NA, AD, dihydroxybenzalamine (DHBA) and DA was injected several times until retention times and peak heights were identical and the base line was stable. The flow rate was set at 2 ml min^{-1}. The chromatogram was analysed by computer integration (Chromjet, Thermo Separation Products). The coefficients of variation for ten samples of the plasma catecholamines were; NA 4.8%, AD 5.2% and DA 4.4%; for the plasma nephrines NMET 6.9% and MET 7.5%; blood lactate 3.1% and pH 0.9%.

Statistical analysis

Paragraph 11

Data were analysed using SPSS software (version 11; SPSS Inc., Chicago, IL, USA). Data were presented as the mean ± SD, with significance level set at $P < 0.05$. All data were assessed for normality (Shapiro–Wilk's test). Data were analysed using a two way repeated measures ANOVA with a Tukey post-hoc test where appropriate. Mauchly's test was consulted and Greenhouse–Geisser correction applied if the assumption of sphericity was violated. Relationships were investigated using Pearson's product moment correlation. Catecholamine and nephrine concentrations were adjusted for changes in plasma volume.

Results

Performance parameters

Paragraph 12

The performance responses are shown in Fig. 1. The first sprint elicited the highest peak power output and greatest mean power output ($P < 0.05$). Power parameters declined from sprint 1 onwards with the lowest mean power output occurring in the final sprint. This represented a fatigue rate of 26 ± 7% ($P < 0.05$). Subjects completed 48.7 ± 5.8 kJ of work in total over the ten sprints of which 54.0 ± 1.1% was performed in the first 5 sprints.

Plasma catecholamines

Paragraph 13

The plasma catecholamine concentrations during CON and EX are shown in Fig. 2. During EX, plasma NA increased

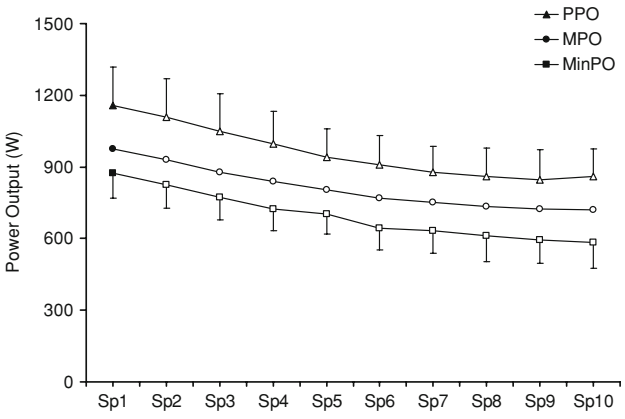

Fig. 1 Peak (*PPO*), mean (*MPO*) and minimum (*MinPO*) power outputs of subjects performing ten 6 s sprints (mean ± SD, $n = 12$). *Empty symbols* indicate power output values significantly lower than Sprint 1, ($P < 0.05$)

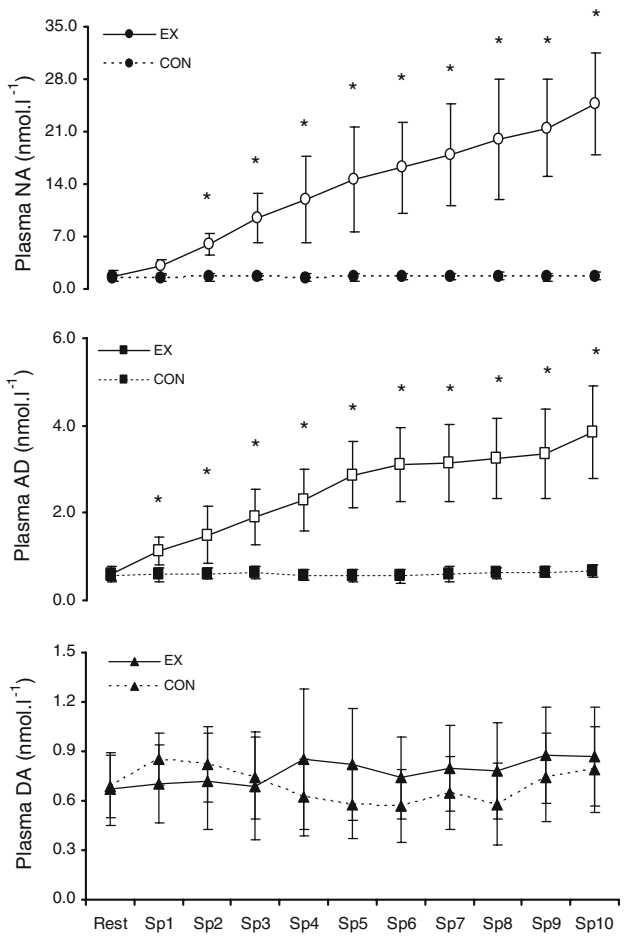

Fig. 2 Plasma noradrenaline (*NA*), adrenaline (*AD*) and dopamine (*DA*) concentrations of subjects at rest (*CON*) and following each 6 s sprint (*EX*) (mean ± SD, $n = 12$). *Empty symbols* indicate significant increase from resting values and * indicates a significant difference from equivalent CON value ($P < 0.05$)

following a single 6 s sprint and reached a peak value of 24.7 ± 9.9 nmol l^{-1} after sprint 10 ($P < 0.05$). This represented a 14.5-fold increase in plasma NA from CON. Resting plasma AD concentrations increased 6.3-fold following sprint 10 ($P < 0.05$). Resting plasma DA concentrations were unaltered during EX (*NS*).

Plasma normetanephrine and metanephrine

Paragraph 14

The plasma NMET and MET responses to CON and EX are outlined in Fig. 3. Prior to exercise, resting plasma NMET concentrations of 0.45 ± 0.06 nmol l^{-1} increased following ten 6 s sprints to 0.76 ± 0.19 nmol l^{-1} ($P < 0.05$). Plasma MET concentrations at rest were 0.16 ± 0.7 nmol l^{-1}, and rose 2.3-fold to 0.37 ± 0.16 nmol l^{-1} in response to ten 6 s sprints ($P < 0.05$). Significant increases in both nephrines were evident following sprint 4 onwards ($P < 0.05$).

Blood acid-base status

Paragraph 15

The responses of blood acid–base variables are shown in Table 1. There was a significant decrease in resting blood pH after sprint 1 ($P < 0.05$) with lowest values observed following completion of the nine sprints (7.08 ± 0.08, $P < 0.05$). The resting blood lactate concentration of 0.2 ± 0.1 mmol l^{-1} increased to a peak value following sprint 10 (9.6 ± 2.1 nmol l^{-1}) and represented a 43-fold increase overall ($P < 0.05$).

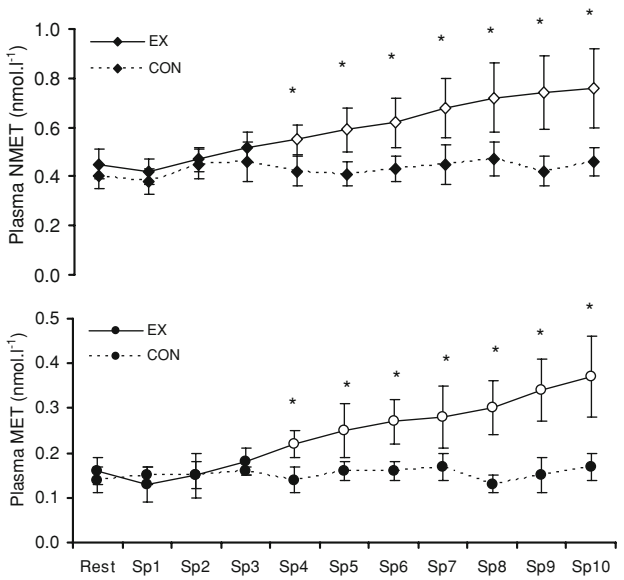

Fig. 3 Plasma normetanephrine (*NMET*) and metanephrine (*MET*) concentrations of subjects at rest (*CON*) and following each 6 s sprint (*EX*) (mean ± SD, $n = 12$). *Empty symbols* indicate significant increase from resting values and * indicates a significant difference from equivalent CON value ($P < 0.05$)

Heart rate

Paragraph 16

During EX, the resting heart rate of 73 ± 13 beats min^{-1} increased to 142 ± 15 beats min^{-1} following the first sprint ($P < 0.05$) and progressively increased to a maximum value of 173 ± 9 beats min^{-1} following sprint 10 ($P < 0.05$, Table 1).

Estimated changes in plasma volume

Paragraph 17

The resting haemoglobin concentration increased from 15.0 ± 2.0 to 15.3 ± 0.9 g dl^{-1}, ($P < 0.05$) following the final sprint. Furthermore, the haematocrit percentage increased from 45 ± 4 to 51 ± 4% during the same time period ($P < 0.05$). These figures were used to estimate the change in plasma volume which significantly decreased from sprint 1 values to 18.1 ± 4.0% ($P < 0.05$) following the tenth sprint (Table 1).

Catecholamine, nephrine, performance and acid–base balance relationships

Paragraph 18

Correlations between plasma catecholamines, nephrines, total work, blood lactate, pH and heart rate are reported in Table 2. Plasma NA and AD concentrations following sprint 10 were significantly correlated with total work done, blood lactate, pH and peak heart rate ($P < 0.05$). There were no significant relationships found between the plasma DA concentration and any blood parameters. Plasma NMET and MET concentrations were related to their parent amines, NA and AD, respectively and both nephrines showed modest but significant relationships with the total work done ($P < 0.05$).

Discussion

Paragraph 19

This study examined the plasma NMET, MET and catecholamine responses to a laboratory-based model of brief intermittent maximal cycle exercise. Our data demonstrated

Table 1 Heart rate, blood lactate, blood pH, haematocrit (Hct), haemoglobin (Hb) and estimated changes in plasma volume (PV) of subjects at rest and in response to ten 6 s sprints ($n = 12$, mean ± SD)

	Rest	Sprint 1	Sprint 2	Sprint 3	Sprint 4	Sprint 5	Sprint 6	Sprint 7	Sprint 8	Sprint 9	Sprint 10
HR bpm											
CON	70 ± 11	72 ± 12	71 ± 9	73 ± 13	69 ± 12	69 ± 13	72 ± 12	73 ± 13	71 ± 13	70 ± 10	73 ± 13
EX	73 ± 13	142 ± 15*†	158 ± 9*†	164 ± 8*†	167 ± 9*†	170 ± 8*†	172 ± 8*†	171 ± 8*†	172 ± 8*†	172 ± 8*†	173 ± 9*†
Lactate (mmol l^{-1})											
CON	0.3 ± 0.1	0.3 ± 0.2	0.3 ± 0.1	0.4 ± 0.2	0.3 ± 0.2	0.3 ± 0.1	0.4 ± 0.1	0.5 ± 0.2	0.4 ± 0.2	0.4 ± 0.1	0.5 ± 0.2
EX	0.2 ± 0.1	0.5 ± 0.2*†	1.4 ± 0.5*†	2.9 ± 1.3*†	4.8 ± 1.5*†	6.1 ± 1.7*†	6.8 ± 1.4*†	7.5 ± 1.6*†	8.0 ± 1.8*†	8.9 ± 2.1*†	9.6 ± 2.0*†
pH											
CON	7.36 ± 0.03	7.35 ± 0.02	7.35 ± 0.03	7.36 ± 0.02	7.34 ± 0.02	7.34 ± 0.03	7.35 ± 0.03	7.33 ± 0.02	7.34 ± 0.03	7.35 ± 0.03	7.34 ± 0.04
EX	7.34 ± 0.02	7.32 ± 0.02*†	7.27 ± 0.03*†	7.22 ± 0.04*†	7.18 ± 0.06*†	7.15 ± 0.08*†	7.13 ± 0.05*†	7.12 ± 0.06*†	7.10 ± 0.07*†	7.08 ± 0.08*†	7.09 ± 0.09*†
ΔPV %											
CON		2.2 ± 0.4	1.1 ± 0.3	2.5 ± 0.7	0.4 ± 0.3‡	1.8 ± 0.5	−2.0 ± 0.7‡	2.9 ± 0.8	−1.3 ± 0.8‡	1.8 ± 0.6	−0.2 ± 0.1‡
EX		−1.2 ± 0.4*	−3.0 ± 0.5*‡	−5.5 ± 1.1*‡	−6.7 ± 1.7*‡	−7.3 ± 2.4*‡	−9.7 ± 3.1*‡	−10.9 ± 2.6*‡	−13.3 ± 3.1*‡	−15.7 ± 2.9*‡	−18.1 ± 4.0*‡

† Indicates a significant difference from rest values within each trial ($P < 0.05$). ‡ Indicates a significant difference from sprint 1 values within each trial ($P < 0.05$). * Indicates significant difference between CON and EX ($P < 0.05$)

large increases in plasma NA and AD and also revealed significant increases in plasma NMET and MET during exercise. There was, however, an unaltered plasma DA response to exercise. The results suggest an increased rate of catecholamine metabolism via catechol-*O*-methyl transferase (COMT) outside the sympathetic neuron during intermittent maximal intensity cycle exercise.

Paragraph 20

The performance parameters measured during the intermittent cycle sprint protocol were similar to those reported by other researchers using a similar method (Gaitanos et al. 1993; Brooks et al. 1990). The generation of power output over the repeated 6 s sprint test was derived from a high muscle ATP resynthesis rate predominantly supplied from phosphocreatine (PCr) and anaerobic glycolysis (Gaitanos et al. 1993). The large drop of 0.25 pH units and corresponding 9.4 mmol l^{-1} increase in blood lactate demonstrate the maximal nature of the brief intermittent maximal intensity cycle test.

Paragraph 21

There was a twofold increase in plasma NA following a single 6 s sprint and a 15-fold increase following completion of ten sprints. Performance of one sprint also increased plasma AD concentrations and there was a six-fold increase following ten sprints. It is likely that the increased plasma NA and AD concentrations were due to an increased spillover into the circulation from sympathetic nerve terminals and adrenal medulla chromaffin cells with contributions from an increased sympathetic nerve-mediated vasoconstriction causing a reduction in blood flow through the vascular beds of hepatomesenteric and renal organs (Kjær et al. 1985; Esler et al. 1990; Goldstein et al. 2003). A reduction in the clearance of NA from the circulation may have also contributed (Leuenberger et al. 1993). Our values are similar in magnitude to those reported in other studies (Brooks et al. 1990; Gaitanos et al. 1993) and demonstrate the large rise in plasma concentrations in spite of a rest period five times greater than the sprint duration. Given that the reported half life (t½) of catecholamines in plasma is 2–3 min (Goldstein et al. 2003) the 30 s rest period between each sprint was insufficient to allow full clearance from the circulation before commencement of the next sprint and facilitated a progressive increase in the concentration of NA and AD in the plasma. Therefore, the measured plasma catecholamine concentrations in our study represent the NA or AD, which has escaped rapid re-uptake by postganglionic neurons or chromaffin cells (Hagberg et al. 1979), binding via sulphoconjugation pathways (Strobel et al. 1999) or to specific

Table 2 Correlations between plasma catecholamines, nephrines, total work, blood lactate, pH and heart rate

	Peak plasma NA	Peak plasma AD	Peak plasma DA	Peak plasma NMET	Peak plasma MET
Peak plasma NA	–				
Peak plasma AD	**0.71**	–			
Peak plasma DA	0.21	0.03	–		
Peak plasma NMET	**0.62**	0.45	0.03	–	
Peak plasma MET	0.51	**0.58**	0.14	0.45	–
Total Work Done	**0.73**	**0.75**	0.17	**0.58**	**0.60**

Values in bold indicate significance (N-2 = 10 df, $P < 0.05$)

tissue adrenoceptors. Although, monoamine oxidase (MAO) plays an important role in intra-neuronal catecholamine metabolism (it also plays a minor role in extraneuronal metabolism, Goldstein et al., 2003) it is incapable of oxidatively deaminating all catecholamines in the outer mitochondrial membrane of the sympathetic nerve cells (Young and Landsberg 1998). Therefore, the plasma catecholamine concentration represented an elevated catecholamine spillover rate into the circulation. As muscle glycogenolytic rate is elevated during high intensity exercise (Febbraio et al. 1998), the correlations found between peak plasma NA and AD and the products of anaerobic metabolism i.e. lactate and H^+ suggest a relationship between sympatho-adrenal activity and anaerobic energy metabolism during intermittent exercise. Moreover, the peak plasma NA and AD concentrations were related to the maximum heart rate achieved by the subjects. Plasma NA provides a reliable indicator of sympatho-adrenal activity (Wallin et al. 1981) and has a facilitatory effect alongside AD (Esler et al. 1990) so our results suggest that peak plasma NA and AD concentrations of ~25 and 4 nmol l^{-1} were enough to alter the myocardium contractility rate thus augmenting oxygen supply to working muscles. Consistent with this view is the finding that cardiovascular effects can take place once plasma AD concentrations increase to 0.6 nmol l^{-1} or above (Weltman et al. 1994).

Paragraph 22

This study found significant changes in plasma nephrine concentrations in response to the intermittent sprint exercise protocol. With such large increases in plasma NA and AD, it is intriguing to examine one of the routes of catecholamine metabolism during this type of exercise. Resting concentrations measured in the plasma were a quarter that of their parent catecholamines and indicate the low level of COMT activity at rest. Both nephrines significantly increased after performance of four 6 s cycle sprints (interspersed with three 30 s rest periods; total duration 114 s). Given that the rate of spillover for NMET and MET has been estimated at 0.4 and 0.5 nmol min^{-1}, respectively and assuming a maximal appearance rate, a 6 s sprint would theoretically increase resting nephrine concentrations by 4–5% per sprint at most (figures based on Goldstein et al. 2003 and assuming a total body plasma volume of 3.3 l). Therefore, a number of sprints would need to be performed before significant increases in plasma nephrines were observed. Peak plasma NMET concentrations were elevated ~60% above resting values. With the enzyme catechol-*O*-methyltransferase present in red blood cells, lungs, liver and adrenal medullae (Young and Landsberg 1998) and blood flow to these regions increasing during exercise, this increase strongly suggests an increased COMT activity in these tissues during intermittent exercise and might explain the increased plasma NMET. Peak plasma MET concentrations also increased by 200% greater than resting values. Some studies have failed to observe any increases in plasma concentrations of MET during exercise (Raber et al. 2003). The different findings may be related to exercise intensity as the protocol of Raber et al. (2003) ended at an intensity of 75% VO_2max whereas our protocol consisted of maximal intensity cycle sprints. Therefore, exercise intensity may be an important factor in determining the rate of COMT activity and future studies should examine the changes in plasma nephrines with progressive intensity exercise protocols. The greater percentage increase in MET compared with NMET is probably not due to differences in blood flow or clearance rates as both nephrines were sampled at the same time and the half-lives of the nephrines are similar. Greater extraneuronal uptake and *O*-methylation of circulating AD compared to NA is one possibility (Goldstein et al. 2003) as adrenal medulla chromaffin cells possess high quantities of COMT and account for over 90% of the circulating MET concentration (Eisenhofer et al. 2004). Therefore, changes in plasma–MET concentrations represent alterations in the degradation rate of AD within the adrenal medulla. The magnitude of the increases in plasma nephrine concentrations was less than the rate of increase in catecholamines over the same time period. This may suggest an inability of COMT to degrade the increases of NA

and AD in the circulation but without access to time course measurements and because of the differing half-lives of the amines and amine metabolites, the validity of this point cannot be determined. Future studies should address this point more thoroughly employing a different exercise model.

Paragraph 23

The results of this study demonstrated no changes in plasma DA concentration with intermittent exercise. Our findings are similar to other research that have employed exercise intensities below (10 h triathlon and 24 h run; Sagnol et al. 1990) and above (110% $V\mathrm{O}_2$max for 2 min; Bracken et al. 2005) the maximal rate of oxygen consumption but are in contrast to other researchers' work that demonstrated increases in plasma DA (Odink et al. 1986; 3 bouts of cycle exercise at 45, 60, 75% $V\mathrm{O}_2$max and Sakai et al. 1995; a half marathon). Overall, this presents an unclear picture as to the exercise factors (i.e. exercise intensity or duration) responsible for altering the plasma DA concentration. It could be that organ level changes in DA release are not always picked up in the systemic circulation as Tidgren et al. (1991) found graded supine dynamic exercise ranging from 30 to 90% of maximal workloads caused significant increases in left renal vein plasma DA at 60 and 90% of maximum workload. This demonstrated an increased DA outflow from the kidneys, but concentrations taken from a brachial artery did not increase until after 10 min of exercise at the 90% maximum workload. Another reason why the plasma DA concentration was unaltered with exercise might relate to its increased delivery to lungs and kidneys during exercise. These organs are sites rich in phenolsulfotransferase activity, the enzyme responsible for increased sulphoconjugation of DA (Strobel et al. 1990). The influence of the enzyme arylsulfatase, which cleaves DA from the sulphoconjugated pool in an acid environment (Strobel et al. 1990), may be minor within the range of the exercise-induced change in blood acid–base status (blood pH 7.08 ± 0.08 and blood lactate 9.6 ± 2.1 mmol l^{-1}) or other factors such as local tissue conjugation may play a role. Future work should aim to discriminate the role of exercise intensity and/or duration as potential factors in altering the plasma DA concentration.

Paragraph 24

In conclusion, our data demonstrate large increases in plasma NA and AD but an unaltered plasma DA response to brief intermittent maximal intensity cycle exercise. Furthermore, there was an increased rate of catecholamine metabolism via elevated catechol-*O*-methyl transferase activity as evidenced by the resultant increases in plasma nephrine concentrations in response to exercise. The results shed more light on the factors, which regulate the circulating catecholamines; the amines that help determine the metabolic and cardiovascular responses to intermittent maximal intensity cycle exercise.

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