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Supplementation with a low-dose of octopamine does not influence endurance cycling performance in recreationally active men

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1 **Full title:** Supplementation with a low-dose of octopamine does not influence endurance cycling
2 performance in recreationally active men

Running title: Octopamine and endurance performance

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6 Abstract

7 *Objectives:* The aim of this study was to examine the influence of octopamine supplementation on
8 endurance performance and exercise metabolism.

9 *Design:* Double-blind cross-over study.

10 *Methods:* Ten healthy, recreationally active men (Mean $\pm$ SD; age: 24 ± 2 y; body mass: 78.4 ± 8.7 kg;
11 $\text{VO}_{2\text{peak}}$: $50.5 \pm 6.8 \text{ mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) completed one $\text{VO}_{2\text{peak}}$ test, one familiarisation trial and two
12 experimental trials. After an overnight fast, participants ingested either a placebo or 150 mg of
13 octopamine 60 min prior to exercise. Trials consisted of 30 min of cycle exercise at 55% peak power
14 output, followed by a 30 min performance task whereby participants completed as much work (kJ) as
15 possible.

16 *Results:* Performance was similar between the experimental trials (placebo: 352.8 ± 39.0 kJ;
17 octopamine: 350.9 ± 38.3 kJ; Cohen's d effect size=0.05; $p=0.380$). Substrate oxidation and
18 circulating concentrations of free fatty acids, prolactin and cortisol were similar between trial
19 conditions (all $p>0.05$). There were also no differences across trials for heart rate or perceived
20 exertion during exercise (both $p>0.05$).

21 *Conclusions:* Acute supplementation with a low dose of octopamine did not influence endurance
22 cycle performance, substrate oxidation or circulating hormonal concentrations, which could be due to
23 the low serum octopamine concentrations observed. Future studies should investigate the influence of
24 larger doses of octopamine in recreationally active and well-trained individuals during prolonged
25 exercise in temperate and high ambient conditions.

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29 Key words: Fatigue; exercise; stimulants; supplements; substrate oxidation

Introduction

Octopamine is a naturally occurring amine structurally similar to the neurotransmitter noradrenaline.¹ It was first isolated from the salivary glands of the octopus² and is synthesised from the amino acid tyrosine with tyramine as an intermediate.³ The function of octopamine is well-characterised in invertebrates, where it modulates signal transduction processes through the activation of octopamine receptors.¹ Vertebrates, including humans, are absent of these receptors, which led to the suggestion that endogenous octopamine exerts no major role in human physiology.¹ However, low circulating concentrations are observed in plasma,⁴ leading octopamine to being classified as one of the primary trace amines.⁵ A unique group of G protein-coupled receptors known as trace amine-associated receptors (TAAR) have been identified in recent years.⁶ Importantly, octopamine can bind to the TAAR1 subtype,⁶ a receptor which modulates the release of monoamines from presynaptic terminals in the brain.⁷ This confirms previous reports of the presence of octopamine in mammalian nerve tissues and brain.⁸ Furthermore, octopamine is suggested to play a role in the pathogenesis of Parkinson's disease.⁴ Therefore, octopamine may, in part, modulate normal and abnormal neurophysiological processes⁵ and possess stimulant-like properties capable of influencing exercise performance.⁹

Octopamine was studied as a therapeutic agent to treat hypotensive disorders, with doses of 450-600 mg·day⁻¹ resulting in mild increases in blood pressure without the presence of adverse effects.¹⁰ Subsequent studies demonstrated the ability of octopamine to activate β_3 adrenoreceptors and stimulate lipolysis,¹¹ suggesting octopamine could influence fat metabolism. Furthermore, intracerebroventricular administration of octopamine increased locomotor activity in rats.¹² Despite these observations, no human study has examined the influence of octopamine on exercise performance or substrate metabolism. Therefore, the aim of this investigation was to determine whether a low dose of octopamine could influence endurance performance and/or exercise metabolism in a group of healthy volunteers.

56 Methods

57 Ten healthy, recreationally active men (age: 24 ± 2 y; body mass: 78.4 ± 8.7 kg; height: 1.81 ± 0.07 m;
 58 $\text{VO}_{2\text{peak}}$: 50.5 ± 6.8 mL·kg⁻¹·min⁻¹; peak power output: 295 ± 41 W) participated in this study, which
 59 employed a double-blind, randomised, cross-over design. Before the study, all participants received
 60 written and verbal information regarding the nature of the investigation. Following an opportunity to
 61 ask questions, a written statement of consent was signed. All participants were free from chronic
 62 disease and deemed eligible to take part following the completion of a health screen questionnaire.
 63 The experimental protocol was approved by the Ethics Approvals (Human Participants) Sub-
 64 Committee of Loughborough University, UK (Ref: R15-P072).

65 All participants completed one incremental maximal exercise test, one familiarisation trial and two
 66 experimental trials. The initial visit consisted of incremental cycle exercise to volitional exhaustion on
 67 an electronically braked cycle ergometer (Lode Corival, Groningen, Holland) to determine peak
 68 power output at $\text{VO}_{2\text{peak}}$ (W_{max}) and the power output required to elicit 55% and 75% of W_{max} .
 69 Following this, participants completed a familiarisation trial. This was undertaken to ensure all
 70 participants were accustomed to the procedures employed during the investigation and to minimise
 71 any learning or anxiety effects. This visit was identical to the experimental trials in all respects, with
 72 the exception of no treatment being administered. All visits to the laboratory were separated by 5-7 d
 73 and were performed at the same time of day to minimise circadian-type variance. Participants were
 74 instructed to record their dietary habits and physical activity patterns during the 24 hr before the
 75 familiarisation trial and to replicate this in the 24 hr preceding the subsequent experimental trials.
 76 Additionally, no strenuous exercise, alcohol ingestion or excessive caffeine consumption (i.e. above
 77 habitual intake) was permitted during the 24 hr before each experimental trial. Compliance to these
 78 measures was verified upon arrival at the laboratory, prior to any data collection.

79 Participants arrived at the laboratory in the morning (7-9 am) following an overnight fast (8-12 hr)
 80 with the exception of ingesting 500 mL of plain water approximately 90 min before arrival. Post-void
 81 nude body mass was recorded upon arrival (Adam AFW-120K, Milton Keynes, UK) and a heart rate

telemetry band (Polar Beat, Kempele, Finland) was positioned. Participants then rested in a seated position for 15 min before a 21-g cannula was inserted into an antecubital vein to enable repeated blood sampling; this was flushed with a small volume of saline after each sample to ensure patency. A baseline venous sample (12 mL) was collected before participants ingested a capsule containing either 150 mg of starch (placebo) or 150 mg of octopamine (Blackburn Distributions, Lancashire, UK) with a small volume of water (50 mL). The purity of octopamine was certified at >99% (HFL Sport Science, Fordham, UK; Ref: LGC255966). The 150 mg dose was chosen to avoid hypertensive effects reported after oral intakes of 450-600 mg in hypotensive patients.¹⁰ All capsules were visually identical and blinded by an external party not involved in any stage of data collection. Following ingestion of the capsules, participants rested in a comfortable environment for 60 min; this timeframe is sufficient to elicit peak octopamine concentrations in the blood.¹³ After the rest period, a second venous sample (12 mL) was collected before participants began cycle exercise for 30 min at a workload corresponding to 55% $W_{\max}$. During this period heart rate and rating of perceived exertion (RPE) were recorded every 5 and 10 min, respectively.¹⁴ Expired gas samples (1 min) were collected into Douglas bags at 15 and 30 min to determine the rates of fat and carbohydrate oxidation.¹⁵ Oxygen and carbon dioxide concentrations in each bag were determined with a paramagnetic analyser (Servomex 1400, Sussex, UK) calibrated against gases of known concentration on the morning of each trial. Total volume was quantified (Harvard Dry Gas Meter, Harvard Apparatus, USA) and gas values were expressed as STPD. Following the collection of each sample, participants were provided with 100 mL of plain water. After the 30 min, a third venous sample (12 mL) was collected while participants remained seated on the ergometer.

Subsequently, there was a 2-3 min delay while the ergometer was set up for the performance task. Participants were instructed to complete as much work (kJ) as possible within 30 min. This method of measuring performance is consistent with previous studies which examined the performance benefits of stimulants such as caffeine.^{16,17} Furthermore, this performance test elicits a coefficient of variation of approximately 3% in recreationally active participants following one familiarisation trial,¹⁸ indicating a similar test-retest reliability to the energy-based time-trial protocols.¹⁹ Participants began

exercise at a workload corresponding to 75% $W_{\max}$, but were free to adjust their workload as desired from the outset. During this period participants received feedback regarding time elapsed and cadence, but no other information or verbal encouragement was provided and contact was limited to the recording of the physiological and perceptual variables. Heart rate was recorded every 5 min and RPE at 10 and 20 min, respectively. A final venous sample (12 mL) was collected upon completion of exercise while participants remained seated on the ergometer. After this, the cannula was removed.

All venous samples were drawn directly into dry syringes. A small volume (2 mL) was dispensed into tubes containing K₂EDTA. Duplicate 100 μ L aliquots were rapidly deproteinised in 1 mL of ice-cold 0.3N perchloric acid. These were centrifuged and the resulting supernatant used to determine blood glucose concentrations (GOD-PAP, Randox Ltd, UK). Haemoglobin (cyanmethemoglobin method) and haematocrit (microcentrifugation) values were used to estimate percentage changes in blood and plasma volumes relative to the resting sample.²⁰ A separate 5 mL was dispensed into tubes containing K₂EDTA and a further 5 mL was dispensed into tubes containing clotting activator; both aliquots were left on ice for 60 min prior to centrifugation at 1750 g for 10 min at 4°C. The resulting plasma from the K₂EDTA treated blood was stored at -21°C for the subsequent determination of free fatty acids (FFA; Randox laboratories Ltd, Crumlin, UK) by colorimetric methods. The resulting serum from the clotted blood was stored at -21°C for the subsequent determination of prolactin and cortisol with ELISA (DRG diagnostics, Germany) and octopamine with a modified reverse-phase HPLC method as previously described.²¹

All data were analysed using IBM SPSS statistics version 21.0. Normality was assessed with the Shapiro Wilk test. To evaluate differences in exercise performance, pre-exercise nude body mass, and fasting plasma glucose across trial conditions, a paired *t*-test was employed. Cohen's *d* effect size (ES) for differences in total work produced during the performance task was determined ($[\text{mean } 1 - \text{mean } 2] / \text{pooled SD}$) and interpreted as trivial (0-0.19), small (0.2-0.49), medium (0.5-0.79) or large (>0.8) as previously described.²² Variables measured throughout each trial were analysed using a two-way (trial x time) repeated-measures ANOVA. Where the assumption of sphericity had been violated, the degrees of freedom were corrected with a Greenhouse-Geisser as appropriate. Main effects and

interactions were followed up with Bonferroni adjusted paired *t*-tests for normally distributed data or Bonferroni adjusted Wilcoxon Signed Rank tests for non-normally distributed data. Data are presented as means $\pm$ SD throughout. Statistical significance was accepted at $p < 0.05$.

Results

Mean environmental temperature was similar between trials (placebo: $20.0 \pm 0.8^\circ\text{C}$; octopamine: $20.0 \pm 0.8^\circ\text{C}$; $p=0.903$). There were no differences across trials for pre-exercise nude body mass (placebo: 78.6 ± 8.8 kg; octopamine: 78.7 ± 8.9 kg; $p=0.602$) or fasting plasma glucose (placebo: 4.4 ± 0.5 mmol $\cdot\text{L}^{-1}$; octopamine: 4.4 ± 0.5 mmol $\cdot\text{L}^{-1}$; $p=0.483$), suggesting that participants began each trial in a similar physiological state.

All ten participants completed both experimental trials, no adverse effects were reported. There was no clear difference in total work produced during the performance task, with mean values of 352.8 ± 39.0 kJ and 350.9 ± 38.3 kJ recorded during the placebo and octopamine trials, respectively (ES=0.05; $p=0.380$; Figure. 1a).

Serum octopamine concentrations remained below the limit of detection for all time points during the placebo trial and for the baseline sample during the octopamine trial. During the octopamine trial serum concentrations increased ($p < 0.05$), with mean values of 0.95 ± 0.50 , 1.11 ± 0.25 and 1.24 ± 0.18 μM recorded at 60, 90 and 120 min post-capsule ingestion, respectively. No pair-wise differences were identified from 60 to 120 min post-ingestion ($p > 0.725$).

Circulating cortisol showed a main effect of time ($p < 0.05$), but no main effect of trial ($p=0.334$) or a trial x time interaction ($p=0.080$; Figure 2a). There was a main effect of time for serum prolactin ($p < 0.05$), with higher values recorded at 30 and 60 min compared with baseline ($p < 0.05$; Figure 2b). No main effect of trial ($p=0.833$) or interaction effect ($p=0.288$) was observed. FFA concentrations remained similar compared with baseline during both trials, with no main effect of time ($p=0.783$), trial ($p=0.351$) or trial x time interaction ($p=0.412$; Figure 2c). Glucose concentrations showed a main

effect of time ($p < 0.05$), with higher values at 30 and 60 min compared with baseline ($p < 0.05$; Figure 2d). No main effect of trial ($p = 0.240$) or interaction effect ($p = 0.704$) was apparent. There were main effects of time for blood and plasma volume ($p < 0.05$), but no main effects of trial ($p > 0.231$) or trial x time interactions ($p > 0.504$).

There was a main effect of time for fat oxidation ($p = 0.026$), but no main effect of trial ($p = 0.597$) or interaction effect ($p = 0.387$; Table. 1). For carbohydrate oxidation there was no main effect of trial ($p = 0.661$), time ($p = 0.148$) or a trial x time interaction ($p = 0.419$). Oxygen uptake showed a main effect of time ($p = 0.001$), with higher values at 30 min compared with 15 min ($p < 0.05$; Table. 1). No main effect of trial ($p = 0.927$) or interaction effect ($p = 0.382$) was observed. For RER there was no main effect of trial ($p = 0.775$), time ($p = 0.121$) or a trial x time interaction ($p = 0.366$; Table 1).

Heart rate showed a main effect of time during the fixed-intensity exercise ($p < 0.05$), with similar mean values across trials (placebo: 136 ± 5 bpm; octopamine: 135 ± 5 bpm; $p = 0.240$). No trial x time interaction was observed ($p = 0.893$). Heart rate showed a main effect of time during the performance task ($p < 0.05$). Mean values were similar between trials (placebo: 168 ± 7 bpm; octopamine: 168 ± 6 bpm; $p = 0.625$) and no interaction effect occurred ($p = 0.168$).

There was a main effect of time for RPE during the fixed-intensity exercise ($p = 0.010$). Mean values were similar between trial conditions (placebo: 12.6 ± 0.4 ; octopamine: 12.3 ± 0.5 ; $p = 0.343$) and no trial x time interaction was observed ($p = 0.241$). Similarly, there was a main effect of time for RPE during the performance task ($p < 0.05$). Mean values were similar between trials (placebo: 16.7 ± 0.6 ; octopamine: 16.5 ± 0.5 ; $p = 0.177$) and no interaction effect occurred ($p = 0.798$).

Discussion:

The present study was the first to examine whether a low dose of octopamine could influence endurance cycling performance or exercise metabolism in a group of healthy, recreationally active male participants. The present findings demonstrate that an acute 150 mg dose did not enhance

performance versus placebo, with a mean difference between trials of 1.9 ± 6.6 kJ ($0.5 \pm 1.9\%$; Figure 1a). While one participant produced 15.5 kJ (4.6%) less work during the octopamine trial compared with placebo, the individual changes in performance by the remaining participants were consistent and small ($<3\%$; Figure 1b). Therefore, it seems likely that any variation in performance is attributable to day-to-day variability in the performance test.¹⁸ Furthermore, substrate oxidation rates and the circulating concentrations of FFA's, prolactin and cortisol were similar between trials.

While the mechanism of action of octopamine is well-established in invertebrates,¹ its precise function in humans remains elusive.⁵ However, low concentrations have been observed in plasma⁴ and throughout the central nervous system.^{5,7,8} Previous work demonstrated that octopamine binds to TAAR1,⁶ a receptor which modulates neurotransmitter release across several brain regions.⁷ However, the EC₅₀ values for TAAR1 from human, rat and mouse transfected-cell lines are in the range of 2-20 μ M.²³ These values are greater than the serum concentrations reported in the present study (0.95 to 1.24 μ M), suggesting a larger dose of octopamine may be required to influence this receptor. Furthermore, octopamine is rapidly metabolised after oral ingestion, with approximately eleven times more conjugated octopamine present in the urine compared with intravenous infusion.¹³ This might explain the contrast between the present study and a previous animal model,¹² as octopamine was directly introduced into the brain of rats and therefore not subjected to extensive hepatic first-pass metabolism. Furthermore, endurance performance in the heat is influenced by pharmacological manipulation of central catecholamines.²⁴ Hence, the provision of a larger dose of octopamine coupled with a high ambient temperature could provide conditions by which octopamine might enhance performance; this hypothesis warrants investigation in future studies.

Previous research demonstrated that octopamine can selectively and potently bind to β_3 adrenoreceptors and stimulate lipolysis in mammalian fat cells,¹¹ suggesting oral supplementation might influence fat metabolism in humans. However, no differences were observed between the two trials in the estimated rates of fat and carbohydrate oxidation or the peripheral concentrations of FFA. While these findings contrast with previous *in vitro* data,¹¹ the doses required to induce lipolysis in these experiments ranges from 10 μ M to 1 mM.^{11,25} Therefore, observations from *in vitro* models may

not reflect the physiological responses observed after oral intake in humans. Furthermore, even chronic ingestion (4 wk) of a dose approximately seven times greater than the present study (15.3 mg·kg⁻¹) failed to induce higher FFA, glycerol or triglyceride concentrations in rats.²⁶ For an 80 kg human, this corresponds to a daily dose of approximately 1,200 mg, which is twice the dose demonstrated to induce hypertensive effects.¹⁰ Hence, it is unlikely that acute low doses of octopamine (~150 mg) influence fat metabolism in humans.

Conclusion

Under the conditions of the present study, octopamine supplementation did not influence endurance performance, substrate oxidation or the peripheral concentrations of FFA's, cortisol and prolactin. These findings may be due to the low serum concentrations observed. As such, future studies should examine the performance and metabolic responses to larger intakes of octopamine (300-400 mg). Furthermore, given the training status of the participants in the present investigation (recreationally active), it would be of interest to investigate the effects of octopamine in well-trained individuals. As central catecholaminergic neurotransmission can modulate endurance performance in the heat,²⁴ the influence of a high ambient temperature on the ergogenic potential of octopamine should also be investigated. Nevertheless, the results of the present study may be of interest to the World Anti-Doping Agency, given octopamine is currently on the list of prohibited substances, meaning its use is banned in competition.⁹

Practical applications

- An acute 150 mg dose of octopamine may not enhance endurance performance in temperate conditions.

- At the dose prescribed in the present study, octopamine does not appreciably influence markers of fat metabolism, hormonal concentrations, heart rate or perceived exertion during exercise.
- Given the lack of research, individuals should refrain from consuming octopamine until more studies have investigated whether this stimulant can influence endurance performance or metabolism.

Word count: 2,784

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314 Table 1: Substrate oxidation and oxygen uptake during the fixed-intensity exercise

	Placebo		Octopamine	
	15	30	15	30
CHO ox ($\text{g} \cdot \text{min}^{-1}$)	2.46 ± 0.35	2.46 ± 0.37	2.44 ± 0.38	2.51 ± 0.33
Fat ox ($\text{g} \cdot \text{min}^{-1}$)	0.19 ± 0.08	0.23 ± 0.08	0.20 ± 0.04	0.21 ± 0.05
RER	0.95 ± 0.02	0.94 ± 0.02	0.94 ± 0.01	0.94 ± 0.01
VO2 ($\text{L} \cdot \text{min}^{-1}$)	2.22 ± 0.30	$2.29 \pm 0.31^*$	2.21 ± 0.31	$2.29 \pm 0.30^*$

315 CHO ox, carbohydrate oxidation; Fat ox, fat oxidation; RER, respiratory exchange ratio; VO2, Oxygen uptake. *Significant difference ($P < 0.05$) compared with the 15 min
 316 value.

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319 Figure Captions

320 Figure 1: Total work produced (a) and individual responses (b) during the experimental trials.

321 Figure 2: Circulating concentrations of cortisol (a), prolactin (b), free fatty acids (c) and
322 glucose (d) during the experimental trials. *denotes a significant difference ($P<0.05$)
323 compared with the -60 value.

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338 Figure 1

347 Figure 2