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 ± SD; age: 24 ± 2 y; body mass: 78.4 ± 8.7 kg;
11 VO_{2peak}: 50.5 ± 6.8 $mL{\cdot}kg^{-1}{\cdot}min^{-1}$) completed one VO_{2peak} 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

30 Introduction

31 Octopamine is a naturally occurring amine structurally similar to the neurotransmitter noradrenaline.[1]
32 It was first isolated from the salivary glands of the octopus[2] and is synthesised from the amino acid
33 tyrosine with tyramine as an intermediate.[3] The function of octopamine is well-characterised in
34 invertebrates, where it modulates signal transduction processes through the activation of octopamine
35 receptors.[1] Vertebrates, including humans, are absent of these receptors, which led to the suggestion
36 that endogenous octopamine exerts no major role in human physiology.[1] However, low circulating
37 concentrations are observed in plasma,[4] leading octopamine to being classified as one of the primary
38 trace amines.[5] A unique group of G protein-coupled receptors known as trace amine-associated
39 receptors (TAAR) have been identified in recent years.[6] Importantly, octopamine can bind to the
40 TAAR1 subtype,[6] a receptor which modulates the release of monoamines from presynaptic terminals
41 in the brain.[7] This confirms previous reports of the presence of octopamine in mammalian nerve
42 tissues and brain.[8] Furthermore, octopamine is suggested to play a role in the pathogenesis of
43 Parkinson's disease.[4] Therefore, octopamine may, in part, modulate normal and abnormal
44 neurophysiological processes[5] and possess stimulant-like properties capable of influencing exercise
45 performance.[9]

46 Octopamine was studied as a therapeutic agent to treat hypotensive disorders, with doses of 450-600
47 $mg \cdot day^{-1}$ resulting in mild increases in blood pressure without the presence of adverse effects.[10]
48 Subsequent studies demonstrated the ability of octopamine to activate β_3 adrenoreceptors and
49 stimulate lipolysis,[11] suggesting octopamine could influence fat metabolism. Furthermore,
50 intracerebroventricular administration of octopamine increased locomotor activity in rats.[12] Despite
51 these observations, no human study has examined the influence of octopamine on exercise
52 performance or substrate metabolism. Therefore, the aim of this investigation was to determine
53 whether a low dose of octopamine could influence endurance performance and/or exercise
54 metabolism in a group of healthy volunteers.

55

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 VO_{2peak}: 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 VO_{2peak} (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

82 telemetry band (Polar Beat, Kempele, Finland) was positioned. Participants then rested in a seated
83 position for 15 min before a 21-g cannula was inserted into an antecubital vein to enable repeated
84 blood sampling; this was flushed with a small volume of saline after each sample to ensure patency. A
85 baseline venous sample (12 mL) was collected before participants ingested a capsule containing either
86 150 mg of starch (placebo) or 150 mg of octopamine (Blackburn Distributions, Lancashire, UK) with
87 a small volume of water (50 mL). The purity of octopamine was certified at >99% (HFL Sport
88 Science, Fordham, UK; Ref: LGC255966). The 150 mg dose was chosen to avoid hypertensive effects
89 reported after oral intakes of 450-600 mg in hypotensive patients.[10] All capsules were visually
90 identical and blinded by an external party not involved in any stage of data collection. Following
91 ingestion of the capsules, participants rested in a comfortable environment for 60 min; this timeframe
92 is sufficient to elicit peak octopamine concentrations in the blood.[13] After the rest period, a second
93 venous sample (12 mL) was collected before participants began cycle exercise for 30 min at a
94 workload corresponding to 55% W_{max}. During this period heart rate and rating of perceived exertion
95 (RPE) were recorded every 5 and 10 min, respectively.[14] Expired gas samples (1 min) were collected
96 into Douglas bags at 15 and 30 min to determine the rates of fat and carbohydrate oxidation.[15] Oxygen
97 and carbon dioxide concentrations in each bag were determined with a paramagnetic analyser
98 (Servomex 1400, Sussex, UK) calibrated against gases of known concentration on the morning of
99 each trial. Total volume was quantified (Harvard Dry Gas Meter, Harvard Apparatus, USA) and gas
100 values were expressed as STPD. Following the collection of each sample, participants were provided
101 with 100 mL of plain water. After the 30 min, a third venous sample (12 mL) was collected while
102 participants remained seated on the ergometer.

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

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

115 All venous samples were drawn directly into dry syringes. A small volume (2 mL) was dispensed into
116 tubes containing K_2EDTA. Duplicate 100 μL aliquots were rapidly deproteinised in 1 mL of ice-cold
117 0.3N perchloric acid. These were centrifuged and the resulting supernatant used to determine blood
118 glucose concentrations (GOD-PAP, Randox Ltd, UK). Haemoglobin (cyanmethemoglobin method)
119 and haematocrit (microcentrifugation) values were used to estimate percentage changes in blood and
120 plasma volumes relative to the resting sample.[20] A separate 5 mL was dispensed into tubes containing
121 K_2EDTA and a further 5 mL was dispensed into tubes containing clotting activator; both aliquots
122 were left on ice for 60 min prior to centrifugation at 1750 g for 10 min at 4°C. The resulting plasma
123 from the K_2EDTA treated blood was stored at -21°C for the subsequent determination of free fatty
124 acids (FFA; Randox laboratories Ltd, Crumlin, UK) by colorimetric methods. The resulting serum
125 from the clotted blood was stored at -21°C for the subsequent determination of prolactin and cortisol
126 with ELISA (DRG diagnostics, Germany) and octopamine with a modified reverse-phase HPLC
127 method as previously described.[21]

128 All data were analysed using IBM SPSS statistics version 21.0. Normality was assessed with the
129 Shapiro Wilk test. To evaluate differences in exercise performance, pre-exercise nude body mass, and
130 fasting plasma glucose across trial conditions, a paired *t*-test was employed. Cohen's *d* effect size (ES)
131 for differences in total work produced during the performance task was determined ([mean 1 - mean
132 2]/pooled SD) and interpreted as trivial (0-0.19), small (0.2-0.49), medium (0.5-0.79) or large (>0.8)
133 as previously described.[22] Variables measured throughout each trial were analysed using a two-way
134 (trial x time) repeated-measures ANOVA. Where the assumption of sphericity had been violated, the
135 degrees of freedom were corrected with a Greenhouse-Geisser as appropriate. Main effects and

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

139

140 Results

141 Mean environmental temperature was similar between trials (placebo: 20.0 ± 0.8°C; octopamine: 20.0
142 ± 0.8°C; p=0.903). There were no differences across trials for pre-exercise nude body mass (placebo:
143 78.6 ± 8.8 kg; octopamine: 78.7 ± 8.9 kg; p=0.602) or fasting plasma glucose (placebo: 4.4 ± 0.5
144 $mmol \cdot L^{-1}$; octopamine: 4.4 ± 0.5 $mmol \cdot L^{-1}$; p=0.483), suggesting that participants began each trial in a
145 similar physiological state.

146 All ten participants completed both experimental trials, no adverse effects were reported. There was
147 no clear difference in total work produced during the performance task, with mean values of 352.8 ±
148 39.0 kJ and 350.9 ± 38.3 kJ recorded during the placebo and octopamine trials, respectively (ES=0.05;
149 p=0.380; Figure. 1a).

150 Serum octopamine concentrations remained below the limit of detection for all time points during the
151 placebo trial and for the baseline sample during the octopamine trial. During the octopamine trial
152 serum concentrations increased ($p<0.05$), with mean values of 0.95 ± 0.50, 1.11 ± 0.25 and 1.24 ±
153 0.18 µM recorded at 60, 90 and 120 min post-capsule ingestion, respectively. No pair-wise differences
154 were identified from 60 to 120 min post-ingestion ($p>0.725$).

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

161 effect of time ($p<0.05$), with higher values at 30 and 60 min compared with baseline ($p<0.05$; Figure
162 2d). No main effect of trial ($p=0.240$) or interaction effect ($p=0.704$) was apparent. There were main
163 effects of time for blood and plasma volume ($p<0.05$), but no main effects of trial ($p>0.231$) or trial x
164 time interactions ($p>0.504$).

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

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

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

181

182 Discussion:

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

186 performance versus placebo, with a mean difference between trials of 1.9 ± 6.6 kJ (0.5 ± 1.9%; Figure
187 1a). While one participant produced 15.5 kJ (4.6%) less work during the octopamine trial compared
188 with placebo, the individual changes in performance by the remaining participants were consistent
189 and small (<3%; Figure 1b). Therefore, it seems likely that any variation in performance is
190 attributable to day-to-day variability in the performance test.[18] Furthermore, substrate oxidation rates
191 and the circulating concentrations of FFA's, prolactin and cortisol were similar between trials.

192 While the mechanism of action of octopamine is well-established in invertebrates,[1] its precise
193 function in humans remains elusive.[5] However, low concentrations have been observed in plasma[4]
194 and throughout the central nervous system.[5,7,8] Previous work demonstrated that octopamine binds to
195 TAAR1,[6] a receptor which modulates neurotransmitter release across several brain regions.[7] However,
196 the EC_{50} values for TAAR1 from human, rat and mouse transfected-cell lines are in the range of 2-20
197 μM.[23] These values are greater than the serum concentrations reported in the present study (0.95 to
198 1.24 μM), suggesting a larger dose of octopamine may be required to influence this receptor.
199 Furthermore, octopamine is rapidly metabolised after oral ingestion, with approximately eleven times
200 more conjugated octopamine present in the urine compared with intravenous infusion.[13] This might
201 explain the contrast between the present study and a previous animal model,[12] as octopamine was
202 directly introduced into the brain of rats and therefore not subjected to extensive hepatic first-pass
203 metabolism. Furthermore, endurance performance in the heat is influenced by pharmacological
204 manipulation of central catecholamines.[24] Hence, the provision of a larger dose of octopamine
205 coupled with a high ambient temperature could provide conditions by which octopamine might
206 enhance performance; this hypothesis warrants investigation in future studies.

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

213 not reflect the physiological responses observed after oral intake in humans. Furthermore, even
214 chronic ingestion (4 wk) of a dose approximately seven times greater than the present study (15.3
215 $mg \cdot kg^{-1}$) failed to induce higher FFA, glycerol or triglyceride concentrations in rats.[26] For an 80 kg
216 human, this corresponds to a daily dose of approximately 1,200 mg, which is twice the dose
217 demonstrated to induce hypertensive effects.[10] Hence, it is unlikely that acute low doses of
218 octopamine (~150 mg) influence fat metabolism in humans.

219

220 *Conclusion*

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

232

233 *Practical applications*

234 • An acute 150 mg dose of octopamine may not enhance endurance performance in temperate
235 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.

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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 ($g{\cdot}min^{-1}$)	2.46 ± 0.35	2.46 ± 0.37	2.44 ± 0.38	2.51 ± 0.33
Fat ox ($g{\cdot}min^{-1}$)	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 ($L{\cdot}min^{-1}$)	2.22 ± 0.30	2.29 ± 0.31*	2.21 ± 0.31	2.29 ± 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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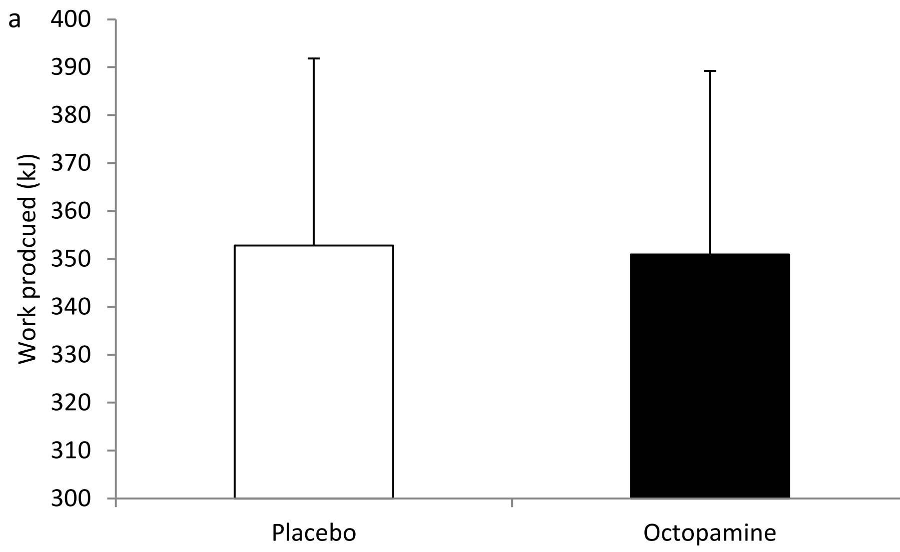
338 Figure 1



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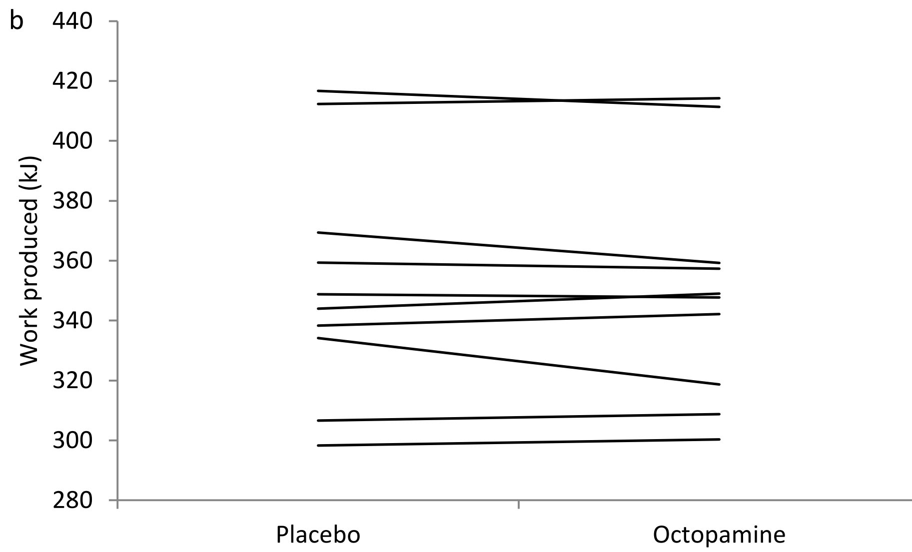

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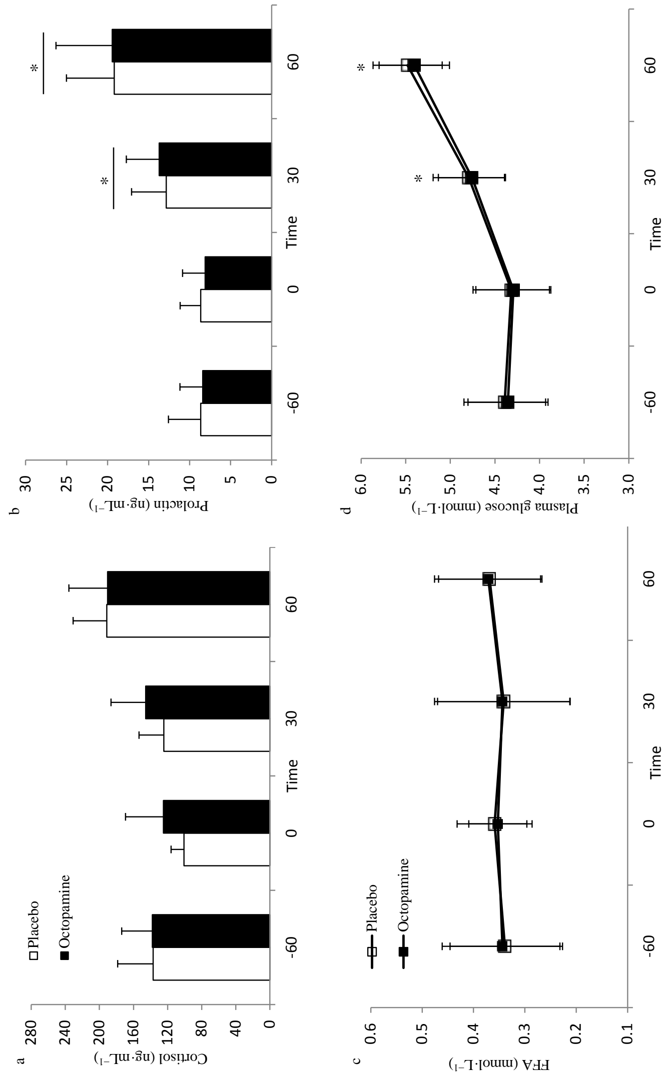
347 Figure 2



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