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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5

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·kg$^{-1}$·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

## Introduction

Octopamine is a naturally occurring amine structurally similar to the neurotransmitter noradrenaline.[1] It was first isolated from the salivary glands of the octopus[2] 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 processes[5] 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 $\beta_3$ 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: $24 \pm 2$ y; body mass: $78.4 \pm 8.7$ kg; height: $1.81 \pm 0.07$ m;

58     $VO_{2peak}$: $50.5 \pm 6.8$ mL·kg$^{-1}$·min$^{-1}$; peak power output: $295 \pm 41$ 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·L$^{-1}$; octopamine: 4.4 ± 0.5 mmol·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 $1.9 \pm 6.6$ kJ ($0.5 \pm 1.9\%$; 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 $\mu$M.[23] These values are greater than the serum concentrations reported in the present study (0.95 to

198 1.24 $\mu$M), 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 $\beta_3$

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 $\mu$M 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·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

*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

*Practical applications*

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

235    conditions.

236 • At the dose prescribed in the present study, octopamine does not appreciably influence

237 markers of fat metabolism, hormonal concentrations, heart rate or perceived exertion during

238 exercise.

239 • Given the lack of research, individuals should refrain from consuming octopamine until more

240 studies have investigated whether this stimulant can influence endurance performance or

241 metabolism.

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245 *Acknowledgements*

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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·min$^{-1}$) | 2.46 ± 0.35 | 2.46 ± 0.37 | 2.44 ± 0.38 | 2.51 ± 0.33 |
| Fat ox (g·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·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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318

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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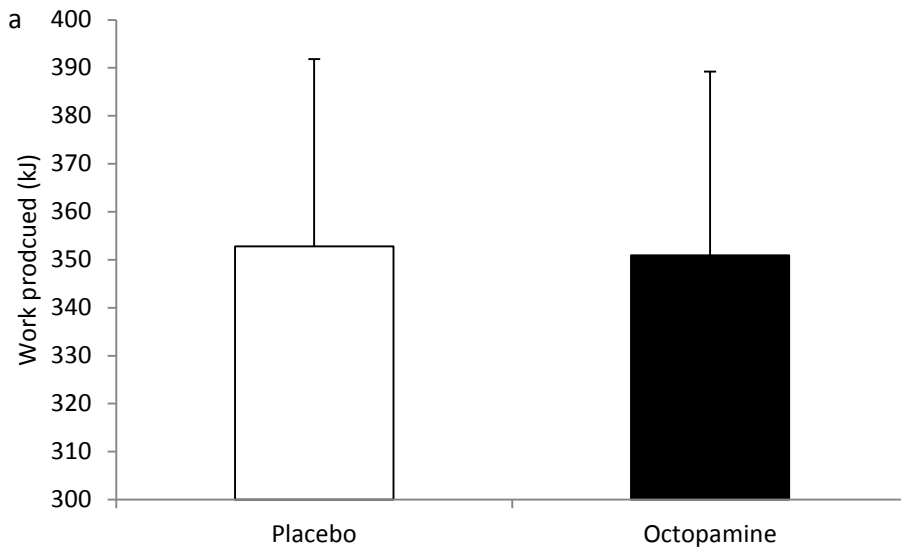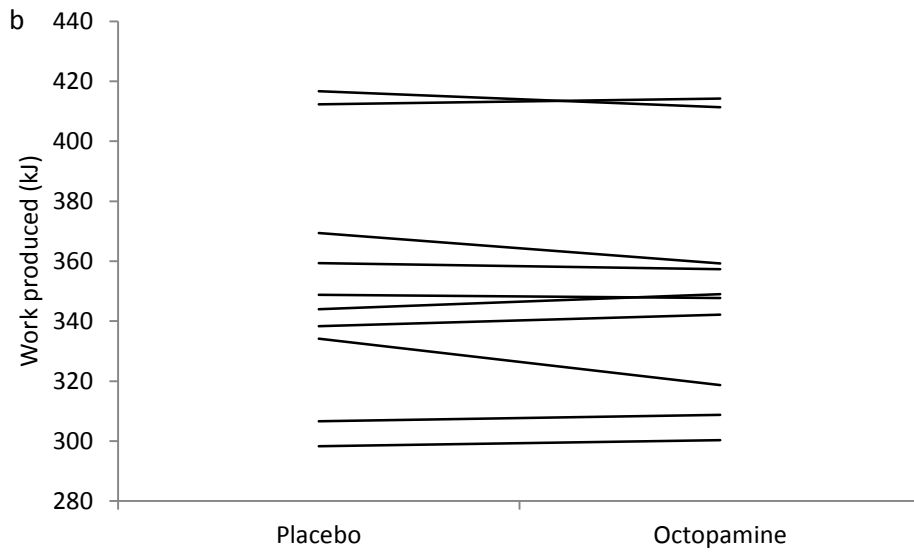
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338    Figure 1



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347    Figure 2



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