The effects of moderate alterations in adrenergic activity on acute appetite regulation in obese women: a randomised crossover trial

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Abstract:

Background: Previous evidence demonstrated that serum leptin correlated with appetite with, but not without, modest exercise.

Aim: The present experiments investigated the effects of exogenous adrenaline and α/β adrenoceptor blockade in combination with moderate exercise on serum leptin concentrations, appetite/satiety sensations and subsequent food intake in obese women.

Methods: Ten obese women [(mean ± SEM), age: 50 (1.9) y, body mass index: 36 (4.1) kg/m^2, waist: 104.8 (4.1) cm] participated in two separate, double-blind randomised experimental (EXP) trials. EXP-1: moderate exercise after α/β adrenergic blocker (labetalol, 100mg orally) vs. moderate exercise plus placebo; EXP-2: adrenaline infusion for 20 min vs. saline infusion. Appetite/satiety and biochemistry were measured at baseline, pre- and immediately post-intervention, 1-h post-intervention (i.e., before dinner). Food intake was assessed via ad libitum buffet style dinner.

Results: No differences were found in appetite/satiety, subsequent food intake, or serum leptin in any of the studies (EXP-1 or EXP-2). In EXP-1, blood glucose was higher ($p < 0.01$) and plasma FFA lower ($p = 0.04$) vs. placebo. In EXP-2, plasma FFA ($p < 0.05$) increased after adrenaline vs. saline infusion.

Conclusion: Neither inhibition of exercise-induced adrenergic activity by combined α/β adrenergic blockade, nor moderate increases in adrenergic activity induced by intravenous adrenaline infusion affected acute appetite regulation.

Key words: appetite regulation, adrenaline infusion, adrenergic blockade, moderate exercise, obesity

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Introduction

Obesity, is the most prevalent single disease in the world (ICD.10 code E.66), with more than 2.1 billion adults overweight (Ng et al., 2013). Better understanding of the mechanisms that regulate food intake, energy expenditure (EE), and energy balance, is critical for the prevention and management of obesity. Physical activity has been implicated in appetite and body mass regulation; appetite also seems to be 'coupled' to body weight control in individuals undertaking moderate physical activity (Shook et al., 2015). While physical activity tends to increase food intake (Westerterp et al. 2015), habitual exercisers are able to closely match food intake to EE (Martins et al., 2008); however, the mechanism underpinning the coupling between physical exercise and food intake regulation has yet to be explained.

In experimental rodent models and in cases of congenital obesity, leptin is a key regulator promoting satiety (Farooqi et al., 2009). In humans, leptin concentration is closely correlated to total fat mass (Considine et al., 1996) and physical activity strongly predicts circulating leptin concentrations independently of body fat mass suggesting a plausible role of physical activity in leptin sensitivity (Chu et al., 2001). Raised circulating leptin concentrations do not appear to prevent overeating in obese humans; who are considered 'leptin resistant' (Lean et al., 2016). Indeed, most models of diet-induced obesity in rodents have presented evidence that obesity causes central and peripheral leptin resistance whereby anorexigenic/orexigenic neurons fail to signal satiety in response to high circulating leptin (Morris and Rui, 2009). Leptin's transport across the blood brain barrier is also reduced concurrently with increasing adiposity (Banks and Farrell, 2003). As human obesity is

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48   associated with impaired appetite control, this implies that other factors may influence the

49   anorexic effects of leptin.

50   Several studies have demonstrated the acute regulation of circulating leptin turnover

51   by adrenergic agents and catecholamine (Keller et al., 2005; Scriba et al., 2000) and the role

52   of endogenous catecholamine in the hypothalamic paraventricular nucleus (PVN) has been

53   related to eating or satiety (Wellman, 2000). Activation for example, of α2-adrenoceptors in

54   the PVN enhances eating, whereas activation of α1-adrenoceptors inhibits eating (Wellman et

55   al., 1993). Moreover, an acute effect of elevated adrenaline levels on enhanced leptin

56   transport into the brain through activation of predominantly α1-adrenoceptors was found in

57   rats (Banks et al., 2001). A link between obesity, inactivity and raised circulating leptin

58   concentrations has been clearly demonstrated (Chu et al., 2001), which suggests that high

59   circulating leptin concentrations are ineffective in regulating appetite and body mass when

60   physically inactive. Studies in lean and obese rats suggested that acute and chronic exercise

61   improved the antiorexigenic action of leptin, as well as hypothalamic leptin signalling

62   (Krawczewski et al., 2011; Ropelle et al., 2010).

63   We also reported an association between circulating leptin and appetite suppression in

64   obese individuals, but only following an acute bout of moderate-intensity exercise (Tsofliou

65   et al., 2003). These studies support a role of exercise in mediating the action of leptin on

66   appetite regulation in the short term. As even light exercise is known to produce a marked

67   stress-response in sedentary individuals (Salvadori et al., 2003), the increase in

68   catecholamines that normally accompanies such a response might be responsible for the

69   coupling of leptin and appetite. Adrenaline may facilitate leptin transport into the brain

70   through stimulation of α-adrenoceptors located at the blood side of the blood brain barrier

71   (Banks, 2001). The purpose therefore of the current study was to investigate the effects of

increased circulating adrenaline concentrations by exogenous intravenous administration, and the effects of moderate exercise performed during α/β-adrenoceptor blockade, on our primary outcomes, appetite-satiety measures and on subsequent food intake in obese women. We also investigated the impact of these interventions on biological markers such as circulating leptin, glucose and free fatty acids (FFA) concentrations, using the association between serum leptin and appetite/satiety sensations as an indirect index of leptin sensitivity.

Material and methods

This study is reported according to the CONSORT guidelines (Schulz et al., 2010) (Figure S1 and Table S1 in Supplementary Files)

Participants

Ten (n=10) obese but otherwise healthy, premenopausal women (Table 1) gave written informed consent to participate in the study, which was conducted in accordance with the declaration of Helsinki. The sample size used in this study was based on the primary outcomes of interest such as appetite ratings and ad libitum intake. Using a paired design and a power of 0.8, a minimum of 9 participants would be needed to detect a 10 mm difference in postprandial ratings and to detect a 100 kcal difference in ad libitum EI (Lara et al., 2010; Horner et al., 2014). The protocol was approved by the the Glasgow Royal Infirmary Research Ethics Committee, (01HU009, 02HU002). All participants were in good physical and mental health with normal blood pressure ($\leq$ 140 / $\leq$ 90 mmHg), non-smokers, on no medication known to affect appetite, not known to be anaemic or hyperlipidemic and not on a special diet. Following eligibility screening and familiarisation with methodological procedures, using a double-blind, cross-over design, participants were randomised to intervention for each experiment (EXP-1 & EXP-2) using an online random number generator

96 (http://www.randomization.com). The order of the trials for each experiment was randomised

97 separately. There was an interval of at least seven days between trials. In EXP-1 (exercise

98 with either α/β-adrenoceptor blocker or placebo) all 10 eligible participants took part in the

99 study procedures and data analysis while in EXP-2 (adrenaline vs. saline infusion) results are

100 presented from nine participants; one participant did not continue after EXP-1.

101   Concealed treatment allocation was implemented; a person, unrelated to the trial

102 prepared the treatment allocation using sealed opaque envelopes. Both participants and

103 researchers evaluating the impact of the experiments were blinded to treatment. Intervention

104 agents were dispensed at each visit by two members of the staff not involved in the study.

105

106 **Experimental design and procedures**

107   Adrenaline was infused (MacCarthy et al., 1983; Centers for Disease Control, 2007),

108 raising circulating adrenaline levels to those typically seen during moderate exercise (Lean et

109 al., 1996). On a separate occasion, labetalol, which blocks α_1-, β_1- and β_2-adrenoceptors,

110 MacCarthy et al., 1983; McLoughlin et al., 1992) was administered prior to moderate

111 exercise. Participants visited the laboratory on four occasions to participate in four acute

112 interventions with an interval of at least seven days between trials (Figure 1); EXP-1:

113 moderate-intensity exercise with either α/β-adrenoceptor blocker or placebo, and EXP-2:

114 adrenaline infusion vs. saline infusion. Participants kept diet and physical activity records for

115 two days preceding the first experimental trial. These food and activity patterns were

116 replicated before all subsequent trials. Household measures (i.e., glasses, cupfuls,

117 tablespoons, slices, etc.) were used to quantify food and fluid consumption. For each

118 experiment, participants visited the laboratory approximately 5-h after a standard lunch and

119 this time duration was standardised within subject. Upon arrival at the laboratory, weight,

120   waist and hip circumference were measured using calibrated scales and inextensible tapes

121   with bone landmarks for anthropometry (Centers for Disease Control, 2007). Body fat

122   percentage was predicted from waist (Lean et al., 1996). Arterialised-venous blood samples

123   (McLoughlin 1992) were collected from an 18G indwelling catheter placed by percutaneous

124   puncture into a vein on the dorsum of a heated hand and a baseline sample (-60 min) was

125   taken. Serial blood samples (10 ml) were then drawn at 0, 20 and 80 min. Following each

126   blood sample, participants completed a set of self-rating 100-mm visual analogue scales for

127   hunger, desire to eat, prospective food consumption, satiety and fullness (Stubbs et al., 2000).

128   Throughout each trial, participants were seated in a comfortable environment watching food-

129   related digital versatile DVDs for 60 min. Food-related DVDs were intended to direct

130   participants' attention towards food and eating, to stimulate a familiar form of home

131   entertainment which might reduce anxiety and eating restraint (Bellisle et al., 2001).

132   After watching the food-related DVDs, participants took part in one of the following

133   interventions on each of the four study-days; EXP-1: 60 min prior to each of the two exercise

134   trials, participants were given either 100mg labetalol (Generics UK)] or placebo (calcium

135   carbonate). Then the participants were required to walk at a moderate pace (5km/h) on a

136   motorised treadmill for 20 minutes. This is in line with a previous study of our group that

137   found acute leptin coupling with appetite/satiety measures after a bout of moderate intensity

138   exercise in obese women (Tsofliou et al., 2003).

139   In EXP-2: a single dose of either adrenaline hydrochloride (i.e., a 1:10,000) diluted in

140   normal saline, or normal saline, was infused intravenously at a rate of 12.5ng min/kg ideal

141   body mass, via a pump for 20 min (Webber et al., 1994), to yield a plasma level not exceeding

142   1nmol/L. This dosage ensures that the plasma catecholamine concentration will not exceed the

143   level typically measured following moderate-intensity exercise (Gustafson et al., 1990). This

144   dosage aimed to maintain catecholamine concentrations similar to the levels attained by the 20

145   min of moderate exercise (McLoughlin et al. 1992). The DVD was switched off for 20 min

146   during each infusion.

147   Following each intervention, participants continued watching food-related DVDs for

148   another 1-h. They were then offered a buffet-type dinner comprising 11 food items: chicken

149   breast roasted (200g), baby potatoes roasted (160g), onion stuffing (60g), boiled peas (126g),

150   boiled carrots (116g), boiled corn (118g), tuna cucumber sandwich (176g), chicken and salad

151   sandwich (178g), banana (100g), 2 apple pies (120g), potato crisps (26g) and orange juice

152   (500ml), and were asked to eat as much as they wanted within 1h. Each person's selection

153   from the buffet dinner was analysed for energy intake and macronutrient content using a

154   computerised version of McCance and Widdowson's (revised by Holland et al., 1993) food

155   composition tables and elative energy intake (REI) was calculated for both exercise trials in

156   EXP-1 as energy intake minus the energy cost of the exercise (Douglas et al., 1982).

157   Rating of perceived exertion (breathlessness and leg exertion) (Borg, 1982) and heart

158   rate (HR) (Polar Sport Tester, Polar Electro Oy, Finland) were recorded every 10 min during

159   the moderate exercise and the infusion interventions. For EXP 1, expired gas was collected in

160   Douglas bags for 5 min at rest, and thereafter 1 min collections were obtained every 10 min

161   during the moderate exercise interventions. Expired gases were analysed within 5 min of

162   collection for $[O_2]$ (Servomex 570A, East Sussex, UK) and $[CO_2]$ (Servomex 1400 B4, East

163   Sussex, UK), volume (dry gas meter, Harvard Apparatus Ltd., Hertfordshire, UK) and

164   temperature (C6600 10-Channel Microprocessor, Comark, Hertfordshire, UK). Barometric

165   pressure was measured using a standard mercury barometer. Oxygen uptake ($\dot{V}O_2$), carbon

166   dioxide production ($\dot{V}CO_2$) and respiratory exchange ratio (RER, i.e. $\dot{V}O_2 / \dot{V}CO_2$) were

167   subsequently evaluated and the percentages of fuel oxidation were determined. Energy

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168 expenditure (kcal·min^{-1}) (Ravussin et al., 1985) and the rates of fat and carbohydrate

169 oxidation (g·min^{-1}) (Alkahtani et al., 2014) were calculated by standard equations: Energy

170 expenditure = $\{4.686 + [(RER - 0.707) / 0.293] \times 0.361\} \times VO_2$; Fat oxidation = $(1.67 \times VO_2)$

171 $- (1.67 \times VCO_2)$; Carbohydrate oxidation = $(4.55 \times VCO_2) - (3.21 \times VO_2)$.

172

173 **Blood treatment and analyses**

174   Venous blood was collected into K$_3$EDTA vacutainers for the measurement of blood

175 glucose, plasma free fatty acids (FFA) (colorimetric method, Boehringer Mannheim

176 Biochemica, London, UK) and into clot activator vacutainers for serum leptin measurement.

177 Duplicate aliquots (400 µl) of whole blood from the K$_3$EDTA tube were rapidly

178 deproteinised in 800 µl of 0.3 mol·l^{-1}perchloric acid; following centrifugation the supernatant

179 was used for the measurement of glucose (Maughan, 1982). Plasma supernatant was separated

180 and plasma (500 µl) was mixed with 50 µl EGTA-glutathione and stored at -70°C for

181 subsequent determination of adrenaline and noradrenaline (Forster, 1999). The remaining

182 plasma was stored at -20°C and later used for the measurement of FFA (colorimetric method,

183 Boehringer Mannheim Biochemica, London, UK). Blood collected into the clot activator

184 vacutainer was allowed to clot for 10 min. Following centrifugation, the serum was stored at -

185 70°C and subsequently analysed for leptin by radioimmunoassay.

186

187 **Statistical analysis**

188   Statistical analyses were carried out with IBM SPSS v22 for Windows. To assess the

189 impact of interventions statistical analysis of the data was carried out using General Linear

190 Model (GLM) with repeated measures followed by pairwise analysis with Bonferroni

191   adjustment. Results are presented as estimated marginal means ± SEM. Correlation analysis

192   was also carried out between serum leptin concentrations and appetite measures (for each time

193   point separately) and adiposity indices. Statistical significance was taken as $p < 0.05$.

194

195   **Results**

196   **Effects on self-reported appetite-satiety ratings and subsequent dietary intake**

197   Profiles of hunger, desire to eat, prospective food consumption (PFC), fullness and

198   satiety throughout each intervention in both experiments are shown in Figures 2a and 2b. In

199   both EXP-1 and EXP-2, a main time effect was observed in all appetite-satiety measures and

200   there were no significant differences on appetite/satiety measures between interventions.

201   In EXP 1: General Linear Model showed a significant time effect for huger ratings (p

202   = 0.003), satiety, desire to eat, and for PFC ratings ($p = 0.002$). No differences were found

203   over time in prospective food consumption or fullness ratings (Figure 2a). In EXP-2: there

204   was a significant time effect for hunger, satiety, fullness, PFC and for the desire to eat ($p <$

205   0.001).

206   Self-selected food intake at dinner did not differ significantly between trials in either EXP-1

207   or EXP-2 (Table 2).

208

209   **Effects on biochemical measures in both experiments**

210   In EXP-1: There was no effects of intervention ($p = 0.6$) and time by intervention

211   interaction ($p = 0.4$) for serum leptin. Significant differences were found in blood glucose and

212   plasma FFA between the two moderate exercise interventions. Blood glucose concentrations

213   were significantly higher and plasma FFA were significantly lower for 1h after the Moderate

214   exercise plus α/β blocker intervention compared to Exercise plus placebo (Table 3).

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215   In EXP-2: There was no significant difference on serum leptin concentrations and blood

216   glucose concentrations between the adrenaline and the saline infusions or over time,

217   throughout the trials ($p > 0.05$). Plasma concentrations of FFA were significantly higher

218   immediately after the adrenaline infusion compared to saline infusion (FFA $p = 0.032$). In

219   addition, plasma NA concentrations showed a borderline significant difference between

220   treatments (Table 4).

221   Baseline serum leptin concentrations correlated significantly with body mass index (BMI

222   ($kg \cdot m^{-2}$), fat mass (FM (%) and waist circumference (BMI $r = 0.78$, $p = 0.01$, FM $r = 0.63$, $p=$

223   0.04, Waist $r = 0.71$ $p = 0.02$). No significant associations were found between serum leptin

224   concentrations and appetite-satiety measures at any time point in the two experiments ($p >$

225   0.05).

226   **Physiological responses to treadmill walking and to adrenaline infusion**

227   HR, perceived breathlessness and leg-tiredness during the moderate exercise and the

228   infusion interventions are show in Table 5; there was no significant difference in HR between

229   trials in either EXP-1 or EXP-2 (Table 5). The average energy expenditure (EE) of

230   participants was 136 kcal ($\pm$ 30) and 128 ($\pm$ 40) in exercise plus placebo and exercise plus α/β

231   blocker respectively; the EE was not significantly different between exercise trails. In both

232   EXP-1 and EXP-2, oxygen uptake ($\dot{V}_{O_2}$), carbon dioxide production ($\dot{V}_{CO_2}$), respiratory

233   exchange ratio (RER) and fuel oxidation rates were not significantly different between trials (

234   Table 6).

235

236   **Discussion**

237   In the current study, we examined the effects of exogenous adrenaline and α-/β-

238   adrenoceptor blockade in combination with moderate exercise on serum leptin concentration,

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239   appetite/satiety sensations and food intake in obese women. It was envisaged that this

240   approach would allow us to identify whether adrenergic stimulation mediates the central

241   effect of leptin on appetite regulation. The novel result of the current study is that moderate

242   manipulation of adrenergic activity via adrenaline infusion or α/β-adrenoceptor blockade

243   using 100 mg labetalol during moderate intensity exercise was not found to affect post-

244   exercise appetite/satiety sensations and subsequent energy intake in obese women.

245   Previous studies have shown impaired catecholamine responses to physical exercise in

246   obese individuals (Salvadori et al. 2003). In the current study, plasma noradrenaline

247   concentration increased to 2.3nmol·l⁻at the end of the adrenaline infusion[1] (only borderline

248   significance was found though), typical of the suppressed levels found during exercise in

249   obesity; substantial variation was reported in noradrenaline concentration during intense or

250   exhaustive exercise in obese, young individuals (from 4.28 to 5.9 nmol·l⁻¹) (Zouhal et al.

251   2013). HR tended to increase towards the end of the adrenaline infusion (82b.min⁻¹) at similar

252   levels with previous adrenaline infusion studies in obese women (Walsh et al. 1998) but we

253   did not observe significant differences; plasma FFA reached concentrations of 1.09mmol·l⁻¹,

254   which is indicative of adrenaline-stimulated lipolysis (Webber et al. 1994). We were not able

255   to determine post adrenaline infusion values of circulating adrenaline concentrations due to

256   unresolved peaks co-eluting with adrenaline. However, the plasma FFA profiles would be

257   consistent with responses to plasma adrenaline concentrations above 0.6nmol·l⁻¹ (~0.8nmol·l⁻¹

258   during 20 min of 12.5ng per kg IBW per minute adrenaline infusion), a level that would

259   stimulate lipolysis (Webber et al. 1994).

260   Catecholamines have long been implicated in appetite regulation as clinical appetite

261   suppressants in obese patients (Lean and Finer, 2006) and it is demonstrated that they exert

262   regulatory effects upon the expression of mRNA leptin and circulating leptin concentrations

263   (Ricci and Fried, 1999). The current study, is the first study though to investigate the role of

264   short-term increases in adrenergic activity in the acute appetite response following exercise in

265   humans. It was observed that 20min of adrenaline infusion did not affect acute appetite or

266   serum leptin concentration and leptin concentrations did not also change after 20min of

267   moderate intensity exercise. This is in agreement with others that found decreases in leptin

268   only after prolonged moderate intensity exercise in trained men (Zaccaria et al. 2013) and

269   overweight women (Tiryaki-Sonmez et al., 2013) or a delayed leptin reduction in active

270   individuals within a 24h timeframe post-exercise (King et al., 2015). Notably, exercise-

271   induced noradrenaline increase, but not other biochemical factors (i.e. cortisol or FFA), was

272   suggested to account for the reduction in post-exercise circulating leptin (Zaccaria et al.

273   2013). However these studies did either not measure subsequent effects on appetite/satiety

274   feelings post exercise or found no compensatory appetite response (King et al., 2015). As the

275   exercise-induced appetite regulatory response, both hormonal and behavioural, might diverge

276   in the presence of obesity (Heden et al. 2013) whether there is interplay between adrenergic

277   activity, leptin response and appetite expression after exercise remains to be clarified utilising

278   different modes of exercise in individuals with different body weights.

279   Furthermore, research in physical exercise and appetite regulation has shown that

280   single bouts of exercise might suppress the orexigenic ghrelin while simultaneously elevating

281   anorexigenic signals peptide YY (PYY), glucagon-like peptide-1 (GLP-1), cholecystokinin

282   (CCK) and pancreatic polypeptide (PP) (Zouhal et al.2019). These observations have been

283   reported mainly in lean, physically active males while evidence in females and particularly

284   in individuals with obesity is sparse and contradictory. It is also suggested that exercise

285   training in women with obesity might influence the regulation of food intake via improved

286   leptin sensitivity (Martins et al., 2013). New evidence from animal studies indicates that

287 leptin might enhance the effects of gut satiety hormones highlighting the importance of

288 interactions among the feeding-related hormones which probably lead into an integrated

289 anorectic signal (Akieda-Asai et al., 2014). Future studies need to measure leptin in

290 conjunction with the other appetite-regulating peptides (acylated ghrelin, PYY, GLP-1, CCK

291 and PP) to enable a better understanding of how exercise-induced responses to appetite-

292 regulating hormones might differ in obesity (Dorling et al., 2018).

293 With regard to the effect of adrenaline infusion on acute appetite control in obese

294 women, previous studies reported reduced circulating leptin concentrations after 60min of

295 adrenaline infusion (0.010µg/kg fat free mass/min) suggesting that a decrease in obesity-

296 related leptinemia could stimulate a compensatory appetite response but this was not assessed

297 (Couillard et al., 2002). The lack of any significant adrenaline-induced decrease in serum

298 leptin concentrations in the present study may be due to the shorter period of adrenaline

299 infusion compared to previous studies which found reduced circulating leptin levels after

300 infusions of 60 to 180min (Couillard et al., 2002). Secondly, the large variability in leptin

301 response to adrenaline previously observed in human obesity, i.e low- and high-leptin

302 responders, could account for the present unchanged leptin concentrations during adrenaline

303 infusion and could indicate a potential heterogeneity in leptin sensitivity among obese

304 individuals (Couillard et al., 2002). It is possible that adrenaline-induced changes in leptin

305 could induce changes in appetite/satiety sensations and food intake in the short-term, but

306 additional work is necessary to understand the complexity of this physiological mechanism,

307 the timeframe of its action and whether there are differences in regulation of appetite and food

308 intake between low- and high-leptin responders to adrenaline.

309 The current study was not able to reproduce the association between leptin and

310 appetite sensations that was found in our earlier study (Tsofliou et al., 2003). There was no

311 evidence for a difference in energy intake (EI) 1h after the moderate exercise with placebo

312 (average 813kcal) compared to α/β-adrenergic blockade (average kcal 900) ($p = 0.2$). When

313 the relative EI (REI) was additionally calculated for the exercise trials, no difference in REI

314 incurred between exercise with placebo (677 kcal) and exercise with α/β-adrenergic blockade

315 (772 kcal). Previous data from walking studies reported no compensatory response in absolute

316 EI in lean and obese individuals and either no changes in relative EI or a significant decrease

317 when the median energy deficit of exercise was around 335kcal (Schubert et al., 2013). The

318 present findings indicate that α/β-adrenergic blockade was not able to induce a different

319 appetite response to exercise with placebo and did not trigger a compensatory response in EI

320 and appetite sensations after an acute exercise-induced energy deficit. These findings however

321 were derived from a small sample and require further verification.

322 In the present study, labetalol 100mg resulted in a lower plasma FFA concentration

323 immediately after and 1h after moderate exercise (0.49nmol.l^{-1}, 0.59nmol.l^{-1} respectively)

324 compared to placebo (0.74nmol.l^{-1}, 0.73nmol.l^{-1} respectively) possibly by blocking the β-

325 receptor mediated lipolysis (Ladage et al., 2013). The α/β-adrenergic blockade also induced a

326 significant increase in post-exercise blood glucose concentration (4.9mmol.l^{-1}) compared to

327 placebo (4.5mmol.l^{-1}). These results are supported by earlier studies (Hartling, 1980).

328 However, they are disputed by recent reports suggesting that β-blockers differ in terms of

329 their mechanism of action and their effects on glucose and lipid metabolism with respect to

330 their molecular pharmacological mechanisms (Ladage et al., 2013); and particularly,

331 nonvasodilating β-blockers are associated with even a worsening of glycemic and lipidic

332 control at rest (Fonseca, 2010). With regard to α- blockade, 100mg labetalol, did not produce

333 significant differences in resting and post-exercise HR. This is in line with previous studies

334 showing that labetalol at doses of 100, 200 and 400mg did not alter resting HR compared to

335 placebo in healthy males (Beachen et al., 2002). However, few evidence has indicated a dose-

336 dependent reduction in post-exercise HR at 1 and 2h (Tham et al. 1993).

337 The present findings suggest that combined α/β-adrenergic blockade during moderate-

338 intensity exercise does not influence appetite/satiety sensations or subsequent food intake

339 following exercise in obese women. The changes in blood glucose and plasma FFA suggest

340 that the 100mg of α/β adrenergic blocker were sufficient to induce β-adrenergic blockade.

341 Labetalol was chosen as a safe and well understood α/β blocker, however, it has greater

342 affinity for β- than α-adrenoceptors (MacCarthy et al., 1983). For this reason, any conclusions

343 with respect to α- adrenoceptor blockade should be drawn with caution. Labetalol decreased

344 circulating FFA and increased glucose concentrations, which indicate inhibition of

345 catecholamine-stimulated lipolysis and confirm the primarily β-adrenoceptor blockade. There

346 is no simple way to know if α-blockade was adequate. There is evidence which attributes the

347 anorexigenic effect of catecholamines to α-adrenoceptors in the brain (Wellman et al. 1993).

348 It is this effect that a popular class of antiobesity drugs exploit to reduce eating behaviour

349 (e.g. sibutramine) by blocking noradrenaline (NA) reuptake through activation of brain α_1-

350 adrenoceptor receptors (Lean, 2001).

351

352 **Study limitations** The monitoring period of appetite response was relatively brief in our

353 study. According to recent findings changes in appetite hormones could emerge over the

354 following 24 hrs (King et

355 al., 2015). Determining the energy intake response might also require multiple *ad libitum*

356 meals, rather than single feeding episodes (Deighton et al., 2014). In our study, all women

357 were premenopausal but menstrual cycle was not controlled for in the study design to account

358 for the perceived confounding effect of the menstrual cycle on appetite sensations, appetite-

15

359 regulating hormones and energy intake (Brennan et al. 2009). However, we did not find any

360 differences in appetite responses and energy intake between the interventions which could

361 have been confounded by cyclical changes in sex hormones in our women.

362

Conclusions

364   In conclusion, neither inhibition of exercise-induced adrenergic activity by combined

365 alpha/beta adrenergic blockade, nor moderate increases in adrenergic activity induced by

366 intravenous adrenaline infusion, significantly affected acute appetite ratings or ad-libitum

367 intake in obese premenopausal women. Testing with a more potent α-blockade may be

368 necessary to trigger a detectable effect and elucidate the role of adrenergic activity in

369 exercise-induced anorexia. In this way we could conclude with complete confidence that the

370 observed anorexic effect of exercise on appetite in obese women is not mediated by increased

371 adrenergic activity. Finally, to definitively exclude sympathetic system involvement in

372 exercise-related appetite regulation, the effects of more selective α-adrenergic stimulation on

373 leptin-mediated appetite sensitivity after exercise should be investigated.

374

380 **Author Contributions:** FT, YPP and MEJL conceived and designed the studies, oversaw

381 its implementation and contributed to the writing of the manuscript. MH supported

382 acquisition of data and contributed to the revision of the manuscript. AMW, JL and IAM

383   contributed in data analysis. FT wrote the first draft of the manuscript. All authors contributed

384   to the interpretation of data and approved the final manuscript.

Conflicting Interests

386   The authors have no conflicts or relevant interests to declare.

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Table 1. Subject characteristics, $n = 10$.

Age (years)	50.3 ± 1.9
Weight (kg)	90.2 ± 5.2
Height (cm)	158.0 ± 0.02
BMI (kg·m⁻²)	36.0 ± 4.1
Waist circumference (cm)	104.8 ± 4.1
Hip circumference (cm)	115.2 ± 3.1
Fat mass (%) predicted by waist	47.7 ± 1.7
Systolic Blood Pressure (mmHg)	129.6 ± 2.4
Diastolic Blood Pressure (mmHg)	89.2 ± 1.4

Values are mean ± SEM.

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Table 2. Buffet style dinner intake subsequent to all interventions.

Dietary intake	Exercise plus placebo, n=10	Exercise plus α/β blocker, n=10	*p*-Value	Adrenaline infusion, n=9	Saline infusion, n=9	*p*-Value
Energy intake (kcal)	812.7 ± 75.9	899.9 ± 64.7	0.23	1023.3 ± 81.2	1013.2 ± 79.7	0.85
Protein (g)	57.1 ± 6.6	59.2 ± 5.5	0.48	67.8 ± 7.6	65.2 ± 6.1	0.43
Protein (%)	28 ± 1.6	27 ± 2.3	0.67	26.5 ± 2.2	27 ± 2.1	0.92
Carbohydrate (g)	103.4 ± 8.2	112.8 ± 7.9	0.41	124.9 ± 9.6	120.2 ± 11.5	0.43
Carbohydrate (%)	50 ± 2.7	48 ± 3.1	0.62	47 ± 2.8	45 ± 1.7	0.10
Fat g	21.5 ± 2.7	26.1 ± 3.6	0.17	31.3 ± 3.4	33.1 ± 3.4	0.48
Fat %	22 ± 1.5	25 ± 6.8	0.30	26± 1.3	28 ± 4.2	0.06

Data are shown as mean ± SEM; no significant differences between interventions in both EXP-1 and EXP-2 (paired t-test).

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Table 3. Serum leptin, blood glucose, plasma free fatty acids (FFA) during the EXP-1, n=10.

	Interventions	(-60 min)	(0 min)	(20 min)	(80 min)	Time	Intervention	Intervention x Time
						p-Value		
Serum leptin (ng·ml^{-1})	Exercise plus placebo	62.28± 6.99	65.71 ± 8.39	73.01 ± 8.45	65.65± 7.41	$p = 0.0004$	$p = 0.694$	$p = 0.406$
	Exercise plus α/β blocker	62.75± 7.27	63.37 ± 7.33	68.90 ± 7.5	65.24± 7.84			
Blood glucose (mmol·l^{-1})	Exercise plus placebo	4.63 ± 0.16	4.53 ± 0.08	4.55 ± 0.09	4.52 ± 0.06	$p = 0.659$	$p = 0.0004$	$p = 0.028$
	Exercise plus α/β blocker	4.59 ± 0.16	4.83 ± 0.11	4.91 ± 0.07*	4.89 ± 0.06*			
Plasma FFA (mmol·l^{-1})	Exercise plus placebo	0.61 ± 0.13	0.65 ± 0.08	0.74 ± 0.09	0.73 ± 0.07	$p = 0.866$	$p = 0.101$	$p < 0.001$
	Exercise plus α/β blocker	0.67 ± 0.11	0.59± 0.07	0.49 ± 0.06*	0.59 ± 0.06*			

Values are estimated marginal means ± SEM. Analysis was conducted by GLM with repeated measures adjusted for multiple comparisons using Bonferroni corrections.
The superscript symbol * indicates significant differences between exercise interventions (Exercise plus α/β blocker vs Exercise plus placebo: glucose 20 min $p = 0.001$, 80 min (after dinner) $p < 0.001$; FFA 20 min $p = 0.02$, 80 min (after dinner) $p= 0.005$).

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Table 4. Serum leptin, blood glucose, plasma free fatty acids (FFA), plasma adrenaline and noradrenaline (NA) concentrations during the EXP-2, n=9.

	Interventions	(-60 min)	(0 min)	(20 min)	(80 min)	Time	Intervention	Intervention x Time
						p-Value		
Serum leptin (ng·ml^{-1})	Adrenaline infusion	63.68 ± 7.77	63.20 ± 8.11	61.98 ± 8.58	67.70 ± 10.49	$p = 0.068$	$p = 0.688$	$p = 0.961$
	Saline infusion	65.80 ± 8.15	65.86 ± 8.07	65.31 ± 9.18	68.90 ± 7.73			
Blood glucose (mmol·l^{-1})	Adrenaline infusion	4.79 ± 0.34	4.59 ± 0.09	4.76 ± 0.82	4.530 ± 0.07	$p = 0.136$	$p = 0.696$	$p = 0.532$
	Saline infusion	5.03 ± 0.27	4.72 ± 0.06	4.60 ± 0.05	4.575 ± 0.03			
Plasma FFA (mmol·l^{-1})	Adrenaline infusion	0.75 ± 0.15	0.84 ± 0.13	1.09 ±0.17*	0.82 ± 0.11	$p = 0.010$	$p = 0.025$	$p = 0.083$
	Saline infusion	0.56 ± 0.11	0.57 ± 0.13	0.65 ± 0.15	0.70 ± 0.10			
Plasma Adrenaline (nmol·l^{-1})	Adrenaline infusion	-	0.17 ± 0.26	-	-			
	Saline infusion	-	0.16± 0.20	-	-			
Plasma NA (nmol·l^{-1})	Adrenaline infusion	-	1.59 ± 0.19	2.32± 0.19	-	$p = 0.010$	$p = 0.063$	$p = 0.060$
	Saline infusion	-	1.49 ± 0.26	1.61 ± 0.26	-			

Values are estimated marginal means ± SEM. Analysis was conducted by ANOVA with repeated measures adjusted for multiple comparisons using Bonferroni corrections. The superscript symbol * indicates significant differences between infusion trials (Adrenaline infusion *vs* Saline infusion: at 20 min FFA; $p = 0.032$) (pairwise comparisons, adjustment for multiple comparisons: Bonferroni). Post adrenaline infusion values of circulating adrenaline concentrations were not determined due to unresolved co-eluting peaks with Adrenaline.

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Table 5. Heart rate, perceived breathlessness and leg-tiredness during the exercise and infusion interventions in both experiments.

Time (min)

p-value

	Interventions	Rest	5	10	15	20	Time	Intervention	Intervention x Time
Heart rate (beats.min^{-1})	Exercice plus placebo	79.83 ± 7.39	121.33 ± 9.93	132.17 ± 11.13	131.17 ± 8.87	134.33 ± 10.94	<0.001	0.572	0.146
	Exercise plus α/β blocker	86.50 ± 6.24	119.83 ± 18.43	128.17± 10.62	125.83 ± 7.56	130.67± 11.02			
	Adrenaline infusion	76.60 ± 5.28	75.40 ± 4.93	78.00± 4.95	81.20 ± 3.63	81.80 ± 3.99	0.016	0.098	0.053
	Saline infusion	76.00 ± 5.21	73.50 ± 4.87	75.00 ± 6.04	74.20 ± 5.61	75.40 ± 4.53			
Perceived breathlessness (rating(0-20))	Exercise plus placebo	7.83± 0.70	9.83 ± 0.54	11.17± 0.65	11.50 ± 0.81	12.33 ± 0.53	<0.001	0.468	0.758
	Exercise plus α/β blocker	7.17 ± 0.17	10.00 ± 0.76	11.00 ± 0.67	12.17 ± 0.48	12.33 ± 0.33			
	Adrenaline infusion	8.29 ± 0.78	7.71 ± 0.64	8.00 ± 0.66	7.71 ± 0.64	7.71 ± 0.644	0.461	0.458	0.394
	Saline infusion	7.86 ± 0.63	8.00 ± 0.66	8.00 ± 0.66	7.86 ± 0.63	7.857 ±0.634			
Perceived leg-tiredness (rating (0-20))	Exercise plus placebo	7.33 ± 0.42	10.67 ± 0.67	11.50 ± 0.56	12.33 ± 0.72	12.50 ± 0.34	<0.001	0.475	0.490
	Exercise plus α/β blocker	8.00 ± 0.63	10.33 ± 0.61	11.83 ± 0.83	12.83 ± 0.60	13.33 ± 0.76			
	Adrenaline infusion	7.50 ± 0.46	7.50 ± 0.46	7.75 ± 0.62	7.63 ± 0.53	7.63 ± 0.53	0.252	0.039	0.732
	Saline infusion	8.50 ±0.66*	8.75 ±0.73*	8.75 ± 0.73	8.75 ± 0.73*	8.75 ± 0.73*			

589 Values are estimated marginal means ± SEM. Analysis was conducted by General Linear Model (GLM) with repeated measures adjusted for multiple comparisons using the
590 Bonferroni corrections. The superscript symbol * indicates significant differences between infusion interventions (Adrenaline infusion *vs* Saline infusion: Perceived leg-
591 tiredness (rest *p* = 0.033, 5 min, 15 min and 20 min *p* = 0.038).

592 **Table 6**. Gas exchange, energy expenditure and substrate oxidation in EXP 1 (at rest and during 20min of exercise) and in EXP 2 (at rest and during 20 min of
593 adrenaline/saline infusion)

	Trials	**Rest**	**20 min intervention**
VO_2 (L.min^{-1})	Exercise plus placebo	0.3 ± 0.04	1.4 ± 0.3
	Exercise plus α/β blocker	0.3 ± 0.06	1.3 ± 0.4
	Saline infusion	0.2 ± 0.09	0.3 ± 0.05
	Adrenaline infusion	0.3 ± 0.06	0.3 ± 0.05
VCO_2 (L.min^{-1})	Exercise plus placebo	0.2 ± 0.06	1.1 ± 0.2
	Exercise plus α/β blocker	0.2 ± 0.07	1.1 ± 0.3
	Saline infusion	0.2 ± 0.07	0.2 ± 0.04
	Adrenaline infusion	0.2 ± 0.05	0.2 ± 0.03
Energy Expenditure (kcal·min^{-1})	Exercise plus placebo	1.3 ± 0.1	6.8 ± 1.5
	Exercise plus α/β blocker	1.3 ± 0.3	6.4 ± 2.0
	Saline infusion	1.1 ± 0.4	1.2 ± 0.2
	Adrenaline infusion	1.3 ± 0.3	1.4 ± 0.2
CHO oxidation (g·min^{-1})	Exercise plus placebo	0.08 ± 0.33	0.58 ± 0.45
	Exercise plus α/β blocker	0.10 ± 0.15	0.55 ± 0.27
	Saline infusion	0.06 ± 0.15	0.07 ± 0.09
	Adrenaline infusion	0.08 ± 0.12	0.03 ± 0.15
Fat oxidation (g·min^{-1})	Exercise plus placebo	0.10 ± 0.13	0.48 ± 0.21
	Exercise plus α/β blocker	0.09 ± 0.04	0.45 ± 0.15
	Saline infusion	0.10 ± 0.06	0.15 ± 0.07
	Adrenaline infusion	0.10 ± 0.06	0.14 ± 0.07

594 Values are estimated marginal means ± SEM. No significant differences were found between trials in EXP-1 or EXP-2

Table A1. CONSORT Checklist of information about the present randomised controlled study.

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomised trial in the title	1
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	1
Introduction			
Background and	2a	Scientific background and explanation of rationale	2
objectives	2b	Specific objectives or hypotheses	2
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	3
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	3
Participants	4a	Eligibility criteria for participants	3
	4b	Settings and locations where the data were collected	3
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	3,4
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	3,4,5
	6b	Any changes to trial outcomes after the trial commenced, with reasons	NA
Sample size	7a	How sample size was determined	Pages 1 & 4
	7b	When applicable, explanation of any interim analyses and stopping guidelines	NA
Randomisation:			
Sequence	8a	Method used to generate the random allocation sequence	3
generation	8b	Type of randomisation; details of any restriction (such as blocking and block size)	3
Allocation concealment	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	3

		mechanism	
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	3
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those assessing outcomes) and how	3
	11b	If relevant, description of the similarity of interventions	NA
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	5
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	5

Results

Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome	Figure S1-Supplementary material
	13b	For each group, losses and exclusions after randomisation, together with reasons	Figure S1
Recruitment	14a	Dates defining the periods of recruitment and follow-up	NA
	14b	Why the trial ended or was stopped	NA
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	Pg.3
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	FS1
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	6-11 Tables 2-4
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	NA
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	NA
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	NA

Discussion

Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	12-14
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	12-14
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	12-14

Other information

Registration	23	Registration number and name of trial registry		3
Protocol	24	Where the full trial protocol can be accessed, if available		NA
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders		14

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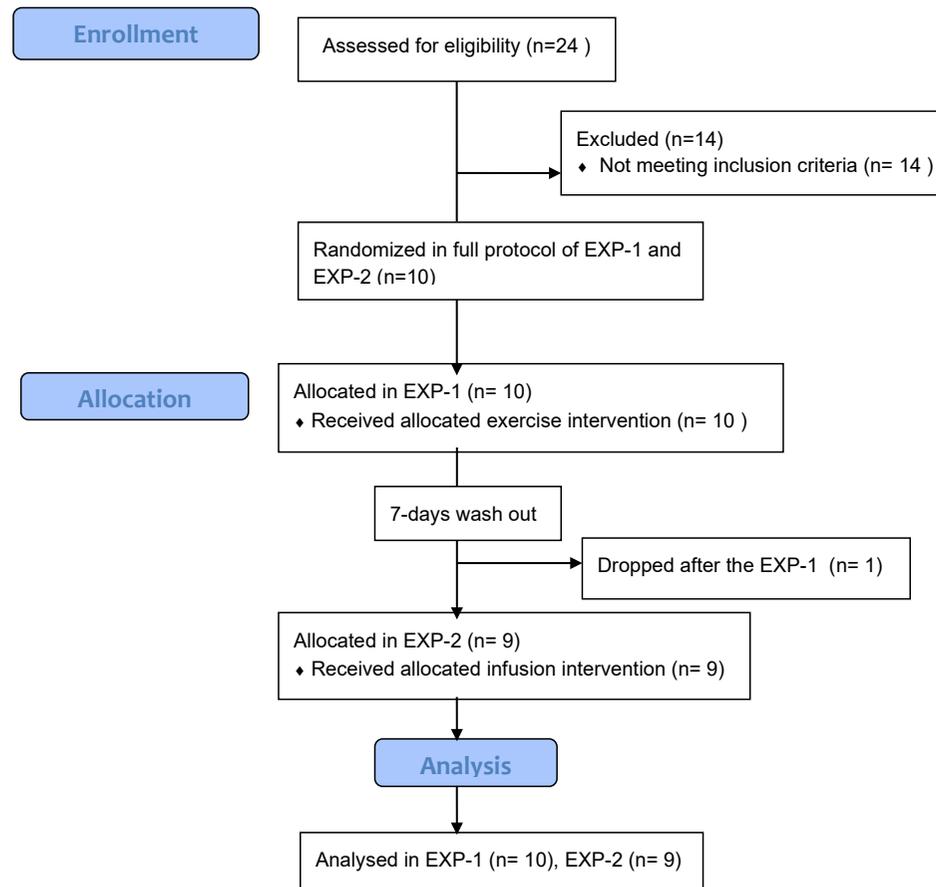602
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Figure S1. Participant flow diagram.

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