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

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

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 (≤ 140 / ≤ 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

(http://www.randomization.com). The order of the trials for each experiment was randomised separately. There was an interval of at least seven days between trials. In EXP-1 (exercise with either α/β-adrenoceptor blocker or placebo) all 10 eligible participants took part in the study procedures and data analysis while in EXP-2 (adrenaline vs. saline infusion) results are presented from nine participants; one participant did not continue after EXP-1.

Concealed treatment allocation was implemented; a person, unrelated to the trial prepared the treatment allocation using sealed opaque envelopes. Both participants and researchers evaluating the impact of the experiments were blinded to treatment. Intervention agents were dispensed at each visit by two members of the staff not involved in the study.

Experimental design and procedures

Adrenaline was infused (MacCarthy et al., 1983; Centers for Disease Control, 2007), raising circulating adrenaline levels to those typically seen during moderate exercise (Lean et al., 1996). On a separate occasion, labetalol, which blocks α_1-, β_1- and β_2-adrenoceptors, MacCarthy et al., 1983; McLoughlin et al., 1992) was administered prior to moderate exercise. Participants visited the laboratory on four occasions to participate in four acute interventions with an interval of at least seven days between trials (Figure 1); EXP-1: moderate-intensity exercise with either α/β-adrenoceptor blocker or placebo, and EXP-2: adrenaline infusion vs. saline infusion. Participants kept diet and physical activity records for two days preceding the first experimental trial. These food and activity patterns were replicated before all subsequent trials. Household measures (i.e., glasses, cupfuls, tablespoons, slices, etc.) were used to quantify food and fluid consumption. For each experiment, participants visited the laboratory approximately 5-h after a standard lunch and this time duration was standardised within subject. Upon arrival at the laboratory, weight,

waist and hip circumference were measured using calibrated scales and inextensible tapes with bone landmarks for anthropometry (Centers for Disease Control, 2007). Body fat percentage was predicted from waist (Lean et al., 1996). Arterialised-venous blood samples (McLoughlin 1992) were collected from an 18G indwelling catheter placed by percutaneous puncture into a vein on the dorsum of a heated hand and a baseline sample (-60 min) was taken. Serial blood samples (10 ml) were then drawn at 0, 20 and 80 min. Following each blood sample, participants completed a set of self-rating 100-mm visual analogue scales for hunger, desire to eat, prospective food consumption, satiety and fullness (Stubbs et al., 2000). Throughout each trial, participants were seated in a comfortable environment watching food-related digital versatile DVDs for 60 min. Food-related DVDs were intended to direct participants' attention towards food and eating, to stimulate a familiar form of home entertainment which might reduce anxiety and eating restraint (Bellisle et al., 2001).

After watching the food-related DVDs, participants took part in one of the following interventions on each of the four study-days; EXP-1: 60 min prior to each of the two exercise trials, participants were given either 100mg labetalol (Generics UK)] or placebo (calcium carbonate). Then the participants were required to walk at a moderate pace (5km/h) on a motorised treadmill for 20 minutes. This is in line with a previous study of our group that found acute leptin coupling with appetite/satiety measures after a bout of moderate intensity exercise in obese women (Tsofliou et al., 2003).

In EXP-2: a single dose of either adrenaline hydrochloride (i.e., a 1:10,000) diluted in normal saline, or normal saline, was infused intravenously at a rate of 12.5ng min/kg ideal body mass, via a pump for 20 min (Webber et al., 1994), to yield a plasma level not exceeding 1nmol/L. This dosage ensures that the plasma catecholamine concentration will not exceed the level typically measured following moderate-intensity exercise (Gustafson et al., 1990). This

dosage aimed to maintain catecholamine concentrations similar to the levels attained by the 20 min of moderate exercise (McLoughlin et al. 1992). The DVD was switched off for 20 min during each infusion.

Following each intervention, participants continued watching food-related DVDs for another 1-h. They were then offered a buffet-type dinner comprising 11 food items: chicken breast roasted (200g), baby potatoes roasted (160g), onion stuffing (60g), boiled peas (126g), boiled carrots (116g), boiled corn (118g), tuna cucumber sandwich (176g), chicken and salad sandwich (178g), banana (100g), 2 apple pies (120g), potato crisps (26g) and orange juice (500ml), and were asked to eat as much as they wanted within 1h. Each person's selection from the buffet dinner was analysed for energy intake and macronutrient content using a computerised version of McCance and Widdowson's (revised by Holland et al., 1993) food composition tables and elative energy intake (REI) was calculated for both exercise trials in EXP-1 as energy intake minus the energy cost of the exercise (Douglas et al., 1982).

Rating of perceived exertion (breathlessness and leg exertion) (Borg, 1982) and heart rate (HR) (Polar Sport Tester, Polar Electro Oy, Finland) were recorded every 10 min during the moderate exercise and the infusion interventions. For EXP 1, expired gas was collected in Douglas bags for 5 min at rest, and thereafter 1 min collections were obtained every 10 min during the moderate exercise interventions. Expired gases were analysed within 5 min of collection for [O_2] (Servomex 570A, East Sussex, UK) and [CO_2] (Servomex 1400 B4, East Sussex, UK), volume (dry gas meter, Harvard Apparatus Ltd., Hertfordshire, UK) and temperature (C6600 10-Channel Microprocessor, Comark, Hertfordshire, UK). Barometric pressure was measured using a standard mercury barometer. Oxygen uptake ($\dot{V}O_2$), carbon dioxide production ($\dot{V}CO_2$) and respiratory exchange ratio (RER, i.e. $\dot{V}O_2 / \dot{V}CO_2$) were subsequently evaluated and the percentages of fuel oxidation were determined. Energy

expenditure (kcal·min^{-1}) (Ravussin et al., 1985) and the rates of fat and carbohydrate oxidation (g·min^{-1}) (Alkahtani et al., 2014) were calculated by standard equations: Energy expenditure = {4.686 + [(RER - 0.707) / 0.293] x 0.361} x VO_2; Fat oxidation = (1.67 x VO_2) - (1.67 x VCO_2); Carbohydrate oxidation = (4.55 x VCO_2) - (3.21 x VO_2).

Blood treatment and analyses

Venous blood was collected into K_3EDTA vacutainers for the measurement of blood glucose, plasma free fatty acids (FFA) (colorimetric method, Boehringer Mannheim Biochemica, London, UK) and into clot activator vacutainers for serum leptin measurement. Duplicate aliquots (400 µl) of whole blood from the K_3EDTA tube were rapidly deproteinised in 800 µl of 0.3 mol·l^{-1}perchloric acid; following centrifugation the supernatant was used for the measurement of glucose (Maughan, 1982). Plasma supernatant was separated and plasma (500 µl) was mixed with 50 µl EGTA-glutathione and stored at -70°C for subsequent determination of adrenaline and noradrenaline (Forster, 1999). The remaining plasma was stored at -20°C and later used for the measurement of FFA (colorimetric method, Boehringer Mannheim Biochemica, London, UK). Blood collected into the clot activator vacutainer was allowed to clot for 10 min. Following centrifugation, the serum was stored at -70°C and subsequently analysed for leptin by radioimmunoassay.

Statistical analysis

Statistical analyses were carried out with IBM SPSS v22 for Windows. To assess the impact of interventions statistical analysis of the data was carried out using General Linear Model (GLM) with repeated measures followed by pairwise analysis with Bonferroni

adjustment. Results are presented as estimated marginal means ± SEM. Correlation analysis was also carried out between serum leptin concentrations and appetite measures (for each time point separately) and adiposity indices. Statistical significance was taken as $p < 0.05$.

Results

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

Profiles of hunger, desire to eat, prospective food consumption (PFC), fullness and satiety throughout each intervention in both experiments are shown in Figures 2a and 2b. In both EXP-1 and EXP-2, a main time effect was observed in all appetite-satiety measures and there were no significant differences on appetite/satiety measures between interventions.

In EXP 1: General Linear Model showed a significant time effect for huger ratings ($p = 0.003$), satiety, desire to eat, and for PFC ratings ($p = 0.002$). No differences were found over time in prospective food consumption or fullness ratings (Figure 2a). In EXP-2: there was a significant time effect for hunger, satiety, fullness, PFC and for the desire to eat ($p < 0.001$).

Self-selected food intake at dinner did not differ significantly between trials in either EXP-1 or EXP-2 (Table 2).

Effects on biochemical measures in both experiments

In EXP-1: There was no effects of intervention ($p = 0.6$) and time by intervention interaction ($p = 0.4$) for serum leptin. Significant differences were found in blood glucose and plasma FFA between the two moderate exercise interventions. Blood glucose concentrations were significantly higher and plasma FFA were significantly lower for 1h after the Moderate exercise plus α/β blocker intervention compared to Exercise plus placebo (Table 3).

In EXP-2: There was no significant difference on serum leptin concentrations and blood glucose concentrations between the adrenaline and the saline infusions or over time, throughout the trials ($p > 0.05$). Plasma concentrations of FFA were significantly higher immediately after the adrenaline infusion compared to saline infusion (FFA $p = 0.032$). In addition, plasma NA concentrations showed a borderline significant difference between treatments (Table 4).

Baseline serum leptin concentrations correlated significantly with body mass index (BMI ($kg{\cdot}m^{-2}$), fat mass (FM (%) and waist circumference (BMI $r = 0.78$, $p = 0.01$, FM $r = 0.63$, $p= 0.04$, Waist $r = 0.71$ $p = 0.02$). No significant associations were found between serum leptin concentrations and appetite-satiety measures at any time point in the two experiments ($p > 0.05$).

Physiological responses to treadmill walking and to adrenaline infusion

HR, perceived breathlessness and leg-tiredness during the moderate exercise and the infusion interventions are show in Table 5; there was no significant difference in HR between trials in either EXP-1 or EXP-2 (Table 5). The average energy expenditure (EE) of participants was 136 kcal (± 30) and 128 (± 40) in exercise plus placebo and exercise plus α/β blocker respectively; the EE was not significantly different between exercise trails. In both EXP-1 and EXP-2, oxygen uptake ($\dot{V}O_2$), carbon dioxide production ($\dot{V}CO_2$), respiratory exchange ratio (RER) and fuel oxidation rates were not significantly different between trials (Table 6).

Discussion

In the current study, we examined the effects of exogenous adrenaline and α-/β-adrenoceptor blockade in combination with moderate exercise on serum leptin concentration,

appetite/satiety sensations and food intake in obese women. It was envisaged that this approach would allow us to identify whether adrenergic stimulation mediates the central effect of leptin on appetite regulation. The novel result of the current study is that moderate manipulation of adrenergic activity via adrenaline infusion or α/β-adrenoceptor blockade using 100 mg labetalol during moderate intensity exercise was not found to affect post-exercise appetite/satiety sensations and subsequent energy intake in obese women.

Previous studies have shown impaired catecholamine responses to physical exercise in obese individuals (Salvadori et al. 2003). In the current study, plasma noradrenaline concentration increased to 2.3nmol·l^{-}at the end of the adrenaline infusion[1] (only borderline significance was found though), typical of the suppressed levels found during exercise in obesity; substantial variation was reported in noradrenaline concentration during intense or exhaustive exercise in obese, young individuals (from 4.28 to 5.9 nmol·l^{-1}) (Zouhal et al. 2013). HR tended to increase towards the end of the adrenaline infusion (82b.min^{-1}) at similar levels with previous adrenaline infusion studies in obese women (Walsh et al. 1998) but we did not observe significant differences; plasma FFA reached concentrations of 1.09mmol·l^{-1}, which is indicative of adrenaline-stimulated lipolysis (Webber et al. 1994). We were not able to determine post adrenaline infusion values of circulating adrenaline concentrations due to unresolved peaks co-eluting with adrenaline. However, the plasma FFA profiles would be consistent with responses to plasma adrenaline concentrations above 0.6nmol·l^{-1} (~0.8nmol·l^{-1} during 20 min of 12.5ng per kg IBW per minute adrenaline infusion), a level that would stimulate lipolysis (Webber et al. 1994).

Catecholamines have long been implicated in appetite regulation as clinical appetite suppressants in obese patients (Lean and Finer, 2006) and it is demonstrated that they exert regulatory effects upon the expression of mRNA leptin and circulating leptin concentrations

(Ricci and Fried, 1999). The current study, is the first study though to investigate the role of short-term increases in adrenergic activity in the acute appetite response following exercise in humans. It was observed that 20min of adrenaline infusion did not affect acute appetite or serum leptin concentration and leptin concentrations did not also change after 20min of moderate intensity exercise. This is in agreement with others that found decreases in leptin only after prolonged moderate intensity exercise in trained men (Zaccaria et al. 2013) and overweight women (Tiryaki-Sonmez et al., 2013) or a delayed leptin reduction in active individuals within a 24h timeframe post-exercise (King et al., 2015). Notably, exercise-induced noradrenaline increase, but not other biochemical factors (i.e. cortisol or FFA), was suggested to account for the reduction in post-exercise circulating leptin (Zaccaria et al. 2013). However these studies did either not measure subsequent effects on appetite/satiety feelings post exercise or found no compensatory appetite response (King et al., 2015). As the exercise-induced appetite regulatory response, both hormonal and behavioural, might diverge in the presence of obesity (Heden et al. 2013) whether there is interplay between adrenergic activity, leptin response and appetite expression after exercise remains to be clarified utilising different modes of exercise in individuals with different body weights.

Furthermore, research in physical exercise and appetite regulation has shown that single bouts of exercise might suppress the orexigenic ghrelin while simultaneously elevating anorexigenic signals peptide YY (PYY), glucagon-like peptide-1 (GLP-1), cholecystokinin (CCK) and pancreatic polypeptide (PP) (Zouhal et al.2019). These observations have been reported mainly in lean, physically active males while evidence in females and particularly in individuals with obesity is sparse and contradictory. It is also suggested that exercise training in women with obesity might influence the regulation of food intake via improved leptin sensitivity (Martins et al., 2013). New evidence from animal studies indicates that

leptin might enhance the effects of gut satiety hormones highlighting the importance of interactions among the feeding-related hormones which probably lead into an integrated anorectic signal (Akieda-Asai et al., 2014). Future studies need to measure leptin in conjunction with the other appetite-regulating peptides (acylated ghrelin, PYY, GLP-1, CCK and PP) to enable a better understanding of how exercise-induced responses to appetite-regulating hormones might differ in obesity (Dorling et al., 2018).

With regard to the effect of adrenaline infusion on acute appetite control in obese women, previous studies reported reduced circulating leptin concentrations after 60min of adrenaline infusion (0.010μg/kg fat free mass/min) suggesting that a decrease in obesity-related leptinemia could stimulate a compensatory appetite response but this was not assessed (Couillard et al., 2002). The lack of any significant adrenaline-induced decrease in serum leptin concentrations in the present study may be due to the shorter period of adrenaline infusion compared to previous studies which found reduced circulating leptin levels after infusions of 60 to 180min (Couillard et al., 2002). Secondly, the large variability in leptin response to adrenaline previously observed in human obesity, i.e low- and high-leptin responders, could account for the present unchanged leptin concentrations during adrenaline infusion and could indicate a potential heterogeneity in leptin sensitivity among obese individuals (Couillard et al., 2002). It is possible that adrenaline-induced changes in leptin could induce changes in appetite/satiety sensations and food intake in the short-term, but additional work is necessary to understand the complexity of this physiological mechanism, the timeframe of its action and whether there are differences in regulation of appetite and food intake between low- and high-leptin responders to adrenaline.

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

placebo in healthy males (Beachen et al., 2002). However, few evidence has indicated a dose-dependent reduction in post-exercise HR at 1 and 2h (Tham et al. 1993).

The present findings suggest that combined α/β-adrenergic blockade during moderate-intensity exercise does not influence appetite/satiety sensations or subsequent food intake following exercise in obese women. The changes in blood glucose and plasma FFA suggest that the 100mg of α/β adrenergic blocker were sufficient to induce β-adrenergic blockade. Labetalol was chosen as a safe and well understood α/β blocker, however, it has greater affinity for β- than α-adrenoceptors (MacCarthy et al., 1983). For this reason, any conclusions with respect to α- adrenoceptor blockade should be drawn with caution. Labetalol decreased circulating FFA and increased glucose concentrations, which indicate inhibition of catecholamine-stimulated lipolysis and confirm the primarily β-adrenoceptor blockade. There is no simple way to know if α-blockade was adequate. There is evidence which attributes the anorexigenic effect of catecholamines to α-adrenoceptors in the brain (Wellman et al. 1993). It is this effect that a popular class of antiobesity drugs exploit to reduce eating behaviour (e.g. sibutramine) by blocking noradrenaline (NA) reuptake through activation of brain α_1-adrenoceptor receptors (Lean, 2001).

Study limitations

The monitoring period of appetite response was relatively brief in our study. According to recent findings changes in appetite hormones could emerge over the following 24 hrs (King et al., 2015). Determining the energy intake response might also require multiple *ad libitum* meals, rather than single feeding episodes (Deighton et al., 2014). In our study, all women were premenopausal but menstrual cycle was not controlled for in the study design to account for the perceived confounding effect of the menstrual cycle on appetite sensations, appetite-

regulating hormones and energy intake (Brennan et al. 2009). However, we did not find any differences in appetite responses and energy intake between the interventions which could have been confounded by cyclical changes in sex hormones in our women.

Conclusions

In conclusion, neither inhibition of exercise-induced adrenergic activity by combined alpha/beta adrenergic blockade, nor moderate increases in adrenergic activity induced by intravenous adrenaline infusion, significantly affected acute appetite ratings or ad-libitum intake in obese premenopausal women. Testing with a more potent α-blockade may be necessary to trigger a detectable effect and elucidate the role of adrenergic activity in exercise-induced anorexia. In this way we could conclude with complete confidence that the observed anorexic effect of exercise on appetite in obese women is not mediated by increased adrenergic activity. Finally, to definitively exclude sympathetic system involvement in exercise-related appetite regulation, the effects of more selective α-adrenergic stimulation on leptin-mediated appetite sensitivity after exercise should be investigated.

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contributed in data analysis. FT wrote the first draft of the manuscript. All authors contributed to the interpretation of data and approved the final manuscript.

Conflicting Interests

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 ($kg \cdot m^{-2}$)	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.

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).

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

						p-Value		
	Interventions	**(-60 min)**	**(0 min)**	**(20 min)**	**(80 min)**	**Time**	**Intervention**	**Intervention x Time**
Serum leptin ($ng \cdot ml^{-1}$)	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 ($mmol \cdot l^{-1}$)	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 ($mmol \cdot l^{-1}$)	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) $p = 0.005$).

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

						p-Value		
	Interventions	**(-60 min)**	**(0 min)**	**(20 min)**	**(80 min)**	**Time**	**Intervention**	**Intervention x Time**
Serum leptin ($ng \cdot ml^{-1}$)	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 ($mmol \cdot l^{-1}$)	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 ($mmol \cdot l^{-1}$)	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 ($nmol \cdot l^{-1}$)	Adrenaline infusion	-	0.17 ± 0.26	-	-			
	Saline infusion	-	0.16± 0.20	-	-			
Plasma NA ($nmol \cdot l^{-1}$)	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.

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*			

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

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 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

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

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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 objectives	2a	Scientific background and explanation of rationale	2
	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 generation	8a	Method used to generate the random allocation sequence	3
	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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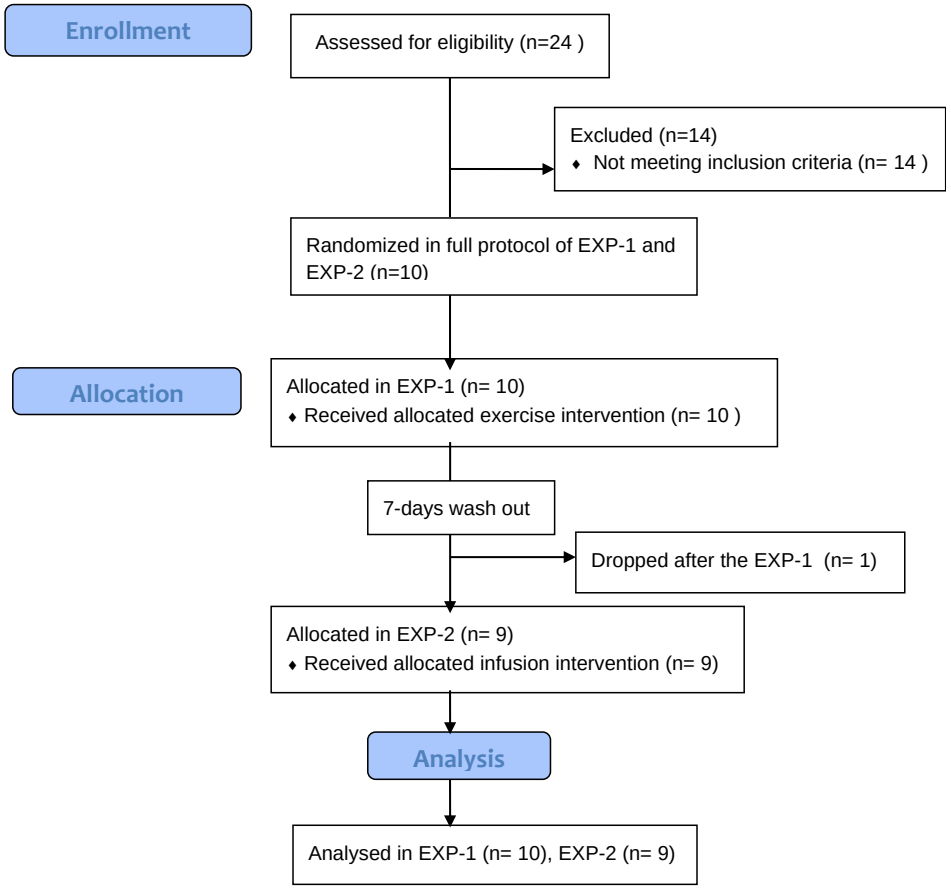
Figure S1. Participant flow diagram.



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