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

Fotini Tsofliou1,2*, Yannis P. Pitsiladis3,4, Jose Lara5, Marios Hadjicharalambous3,6, Ian A. Macdonald7, Mike A, Wallace8 & Mike E.J. Lean1

1Human Nutrition, School of Medicine, University of Glasgow
2Department of Rehabilitation and Sport Sciences, Faculty of Health and Social Sciences, Bournemouth University, United Kingdom,
3College of Medical Veterinary and Life Science, Institute of Cardiovascular & Medical Sciences, University of Glasgow, United Kingdom
4Centre for Sport and Exercise Science and Medicine (CESAME), University of Brighton, Eastbourne United Kingdom,
5Department of Applied Sciences, Faculty of Health and Life Sciences, Northumbria University, United Kingdom
6Human Performance Laboratory, Department of Life & Health Sciences, School of Sciences and Engineering, University of Nicosia, Cyprus
7School of Life Sciences, University of Nottingham Medical School, Queen's Medical Centre, United Kingdom
8University Department of Pathological Biochemistry, Glasgow Royal Infirmary, Castle St, Glasgow G4 0SF, United Kingdom (deceased)

Corresponding Author:
Fotini Tsofliou, PhD
Faculty of Health and Social Sciences
Department of Rehabilitation and Sport Sciences
Bournemouth University
Christchurch Road, Bournemouth BH1 3LT
Telephone: 01202 961583
Email: ftsofliou@bournemouth.ac.uk

1
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 $\alpha/\beta$ 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 $\alpha/\beta$ 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 $\alpha/\beta$ 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
associated with impaired appetite control, this implies that other factors may influence the anorexic effects of leptin.

Several studies have demonstrated the acute regulation of circulating leptin turnover by adrenergic agents and catecholamine (Keller et al., 2005; Scriba et al., 2000) and the role of endogenous catecholamine in the hypothalamic paraventricular nucleus (PVN) has been related to eating or satiety (Wellman, 2000). Activation for example, of $\alpha_2$-adrenoceptors in the PVN enhances eating, whereas activation of $\alpha_1$-adrenoceptors inhibits eating (Wellman et al., 1993). Moreover, an acute effect of elevated adrenaline levels on enhanced leptin transport into the brain through activation of predominantly $\alpha_1$-adrenoceptors was found in rats (Banks et al., 2001). A link between obesity, inactivity and raised circulating leptin concentrations has been clearly demonstrated (Chu et al., 2001), which suggests that high circulating leptin concentrations are ineffective in regulating appetite and body mass when physically inactive. Studies in lean and obese rats suggested that acute and chronic exercise improved the antiorexigenic action of leptin, as well as hypothalamic leptin signalling (Krawczewski et al., 2011; Ropelle et al., 2010).

We also reported an association between circulating leptin and appetite suppression in obese individuals, but only following an acute bout of moderate-intensity exercise (Tsofliou et al., 2003). These studies support a role of exercise in mediating the action of leptin on appetite regulation in the short term. As even light exercise is known to produce a marked stress-response in sedentary individuals (Salvadori et al., 2003), the increase in catecholamines that normally accompanies such a response might be responsible for the coupling of leptin and appetite. Adrenaline may facilitate leptin transport into the brain through stimulation of $\alpha$-adrenoceptors located at the blood side of the blood brain barrier (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 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
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 $\alpha/\beta$-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 $\alpha_1$, $\beta_1$- and $\beta_2$-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 $\alpha/\beta$-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 relative 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 $\text{[O}_2\text{]}$ (Servomex 570A, East Sussex, UK) and $\text{[CO}_2\text{]}$ (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.
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 = \(4.686 + \left[\frac{(RER - 0.707)}{0.293}\right] \times 0.361\) × VO₂; Fat oxidation = \((1.67 \times VO₂) - (1.67 \times VCO₂)\); Carbohydrate oxidation = \((4.55 \times VCO₂) - (3.21 \times VO₂)\).

Blood treatment and analyses

Venous blood was collected into K₃EDTA 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 K₃EDTA 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 correction.
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 hunger 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 $\alpha/\beta$ 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 $\text{kg.m}^{-2}$), 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 ($\pm 30$) and 128 ($\pm 40$) in exercise plus placebo and exercise plus $\alpha/\beta$ blocker respectively; the EE was not significantly different between exercise trials. 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 $\alpha$-/\$\beta\$-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 $\alpha/\beta$-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$^{-1}$ 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 60 min of adrenaline infusion (0.010 µg/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 180 min (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
evidence for a difference in energy intake (EI) 1h after the moderate exercise with placebo (average 813kcal) compared to α/β-adrenergic blockade (average kcal 900) ($p = 0.2$). When the relative EI (REI) was additionally calculated for the exercise trials, no difference in REI incurred between exercise with placebo (677 kcal) and exercise with α/β-adrenergic blockade (772 kcal). Previous data from walking studies reported no compensatory response in absolute EI in lean and obese individuals and either no changes in relative EI or a significant decrease when the median energy deficit of exercise was around 335kcal (Schubert et al., 2013). The present findings indicate that α/β-adrenergic blockade was not able to induce a different appetite response to exercise with placebo and did not trigger a compensatory response in EI and appetite sensations after an acute exercise-induced energy deficit. These findings however were derived from a small sample and require further verification.

In the present study, labetalol 100mg resulted in a lower plasma FFA concentration immediately after and 1h after moderate exercise (0.49nmol.l$^{-1}$, 0.59nmol.l$^{-1}$ respectively) compared to placebo (0.74nmol.l$^{-1}$, 0.73nmol.l$^{-1}$ respectively) possibly by blocking the β-receptor mediated lipolysis (Ladage et al., 2013). The α/β-adrenergic blockade also induced a significant increase in post-exercise blood glucose concentration (4.9mmol.l$^{-1}$) compared to placebo (4.5mmol.l$^{-1}$). These results are supported by earlier studies (Hartling, 1980). However, they are disputed by recent reports suggesting that β-blockers differ in terms of their mechanism of action and their effects on glucose and lipid metabolism with respect to their molecular pharmacological mechanisms (Ladage et al., 2013); and particularly, nonvasodilating β-blockers are associated with even a worsening of glycemic and lipidic control at rest (Fonseca, 2010). With regard to α-blockade, 100mg labetalol, did not produce significant differences in resting and post-exercise HR. This is in line with previous studies 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 $\alpha/\beta$-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 $\alpha/\beta$ adrenergic blocker were sufficient to induce $\beta$-adrenergic blockade. Labetalol was chosen as a safe and well understood $\alpha/\beta$ blocker, however, it has greater affinity for $\beta$- than $\alpha$-adrenoceptors (MacCarthy et al., 1983). For this reason, any conclusions with respect to $\alpha$- 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 $\beta$-adrenoceptor blockade. There is no simple way to know if $\alpha$-blockade was adequate. There is evidence which attributes the anorexigenic effect of catecholamines to $\alpha$-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 $\alpha_1$-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 $\alpha$-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 $\alpha$-adrenergic stimulation on leptin-mediated appetite sensitivity after exercise should be investigated.

Acknowledgements

We thank the study participants for their dedication and effort.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Author Contributions: FT, YPP and MEJL conceived and designed the studies, oversaw its implementation and contributed to the writing of the manuscript. MH supported acquisition of data and contributed to the revision of the manuscript. AMW, JL and IAM
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.

**References**

Akieda-Asai, S., Poleni, P.E., and Date, Y. 2014. Coinjection of CCK and leptin reduces food
intake via increased CART/TRH and reduced AMPK phosphorylation in the
hypothalamus. Am J Physiol Endocrinol Metab. 306(11): E1284-E1291.

Alkahtani, S. 2014. Comparing fat oxidation in an exercise test with moderate-intensity
interval training. J Sports Sci Med. 13: 51–58.

Banks, W.A. 2001. Enhanced leptin transport across the blood-brain barrier by α1 adrenergic
agents. Brain Res. 899(1-2): 209-17.

Banks, W.A., and Farrell, C.L. 2003. Impaired transport of leptin across the blood-brain
barrier in obesity is acquired and reversible. Am. J. Physiol. Endocrinol. Metab. 285(1): E10-5.

Beachen, E.A., Muldoon, M.F., Matthews, K.A., and Manuck, S.B. 2002. Effects of
Hemoconcentration and Sympathetic Activation on Serum Lipid Responses to Brief
Mental Stress. Psychosom. Med. 64(4): 587-594.

Bellisle, F., and Dalix, A.M. 2001. Cognitive restraint can be offset by destruction, leading to
increased meal intake in women. Am. J. Clin. Nutr. 74(2): 197-200.

Brennan, I.M., Feltrin, K.L., Nair, N.S., et al. 2009. Effects of the phases of the menstrual
cycle on gastric emptying, glycemia, plasma GLP-1 and insulin, and energy intake in
healthy lean women. Am J Physiol Gastrointest Liver Physiol. 297(3): G602-G610.

Centers for Disease Control (CDC). 2007. National Health and Nutrition Examination Survey
(NHANES): Anthropometry procedures manual.

Chu, N.F., Stampfer, M.J., Spiegelman, D., Rifai, N., Hotamisligil, G.S., and Rimm, E.B. 2001. Dietary and lifestyle factors in relation to plasma leptin concentrations among normal weight and overweight men. Int. J. Obes. Relat. Metab. Disord. 25(1): 106-14.

Considine, R.V., Sinha, M.K., Heiman, M.L., Kriauciunas, A, Stephens, T.W., Nyce, M.R., et al. 1996. Serum immunoreactive-leptin concentrations in normal-weight and obese humans. N. Engl. J. Med. 334(5): 292-5.

Couillard, C., Mauriege, P., Prud'homme, D., Nadeau, A., Tremblay, A., Bouchard, C., et al. 2002. Plasma leptin response to an epinephrine infusion in lean and obese women. Obes. Res. 10(01): 6-13.

Deighton, K., and Stensel, D.J. 2014. Creating an acute energy deficit without stimulating compensatory increases in appetite: is there an optimal exercise protocol? Proc. Nutr. Soc. 73(2): 352-8.

Dorling, J., Broom, D.R., Burns, S.F., et al. 2018. Acute and Chronic Effects of Exercise on Appetite, Energy Intake, and Appetite-Related Hormones: The Modulating Effect of Adiposity, Sex, and Habitual Physical Activity. Nutrients. 10(9):1140.

Douglas, J.A., King, J.A., McFarlane, E., Baker, L., Bradley, C., Crouch, N., et al. 2015. Appetite, appetite hormone and energy intake responses to two consecutive days of aerobic exercise in healthy young men. Appetite. 92: 57-65.

Farooqi, I.S., O'Rahilly, S. 2009. Leptin: a pivotal regulator of human energy homeostasis. Am. J. Clin. Nutr. 2009, 89,(3): 980S-4S.

Fonseca V.A. 2010. Effects of β-blockers on glucose and lipid metabolism, Curr. Med. Res. Opin. 26(3): 615-629.
Forster, C.D., and Macdonald, I.A. 1999. The assay of the catecholamine content of small volumes of human plasma. Biomed. Chromatogr. 13(3): 209–15.

Gustafson, A.B., Farrell, P.A., and Kalkhoff, R.K. 1990. Impaired plasma catecholamine response to submaximal treadmill exercise in obese women. Metabolism. 39(4): 410-7.

Hartling, O.J., Svendsen, T.L., Trap-Jensen, J. 1980. Haemodynamic and metabolic effects of combined adrenergic alpha- and beta-receptor blockade with labetalol in the exercising human forearm. Eur J Clin Invest. 10:431-5.

Heden, T.D., Liu, Y., Park, Y., Dellsperger, K.C., and Kanaley, J.A. 2013. Acute aerobic exercise differentially alters acylated ghrelin and perceived fullness in normal-weight and obese individuals. J. Appl. Physiol. 115(5): 680-7.

Holland, B., Welch, A.A., Unwin, I.D., Buss, D.H., Paul, A.A., and Southgate, D.A.T. 1993. McCance and Widdowson's The Composition of Foods. Fifth Edition. The Royal Society of Chemistry & Ministry of Agriculture, Fisheries and Food, Cambridge, Goodfellow & Egan Phototypesetting Ltd; 1991.

Horner, K.M., Byrne, N.M., and King, N.A. 2014. Reproducibility of subjective appetite ratings and ad libitum test meal energy intake in overweight and obese males. Appetite. 81: 116-122.

Keller, P., Keller, C., Steensberg, A., Robinson, L.E., and Pedersen, B.K. 2005. Leptin gene expression and systemic levels in healthy men: effect of exercise, carbohydrate, interleukin-6, and epinephrine. J. Appl. Physiol. (1985). 98(5): 1805-12.

King, J.A., Garnham, J.O., Jackson, A.P., Kelly, B.M., Xenophonotos, S., and Nimmo, M.A. 2015. Appetite-regulatory hormone responses on the day following a prolonged bout of moderate-intensity exercise. Physiol. Behav. 15: 23-31.
Krawczewski, Carhuatanta, K.A., Demuro, G., Tschöp, M.H., Pfluger, P.T., Benoit, S.C., and Obici S. 2011. Voluntary exercise improves high-fat diet-induced leptin resistance independent of adiposity. Endocrinology. 152(7): 2655-64.

Ladage, D., Schwinger, R.H.G., and Brixius, K. 2013. Cardio-Selective Beta-Blocker: Pharmacological Evidence and Their Influence on Exercise Capacity. Cardiovasc. Ther. 31(2): 76–83.

Lara, J., Taylor, M.A., and Macdonald, I.A. 2010. Is ad libitum energy intake in overweight subjects reproducible in laboratory studies using the preload paradigm? Eur J Clin Nutr. 64(9): 1028-1031.

Lean, M., and Finer, N. 2006. ABC of obesity. Management: part II-drugs. Br Med J. 14:794-7.

Lean, M.E., Han, T.S., and Deurenberg, P. 1996. Predicting body composition by densitometry from simple anthropometric measurements. Am. J. Clin. Nutr. 63(1):4-14.

Lean, M.E., and Malkova, D. 2016. Altered gut and adipose tissue hormones in overweight and obese individuals: cause or consequence? Int. J. Obes. 40(4):622-32.

Lean, M.E.J. 2001. How does sibutramine work? Int J Obes Relat Metab Disord. 25: S8-11.

MacCarthy, E.P., and Bloomfield, S.S. 1983. Labetalol: a review of its pharmacology, pharmacokinetics, clinical uses and adverse effects. Pharmacotherapy. 3(4): 193-219.

Martins, C., Kulseng, B., Rehfeld, J.F., King, N.A., and Blundell, J.E. 2013. Effect of chronic exercise on appetite control in overweight and obese individuals. Med Sci Sports Exerc. 45(5):805-812.

Martins, C., Morgan, L., and Truby, H. 2008. A review of the effects of exercise on appetite regulation: an obesity perspective. Int. J. Obes. 32(9):1337-47.
Maughan, R.J. 1982. A simple, rapid method for determination of glucose, lactate, pyruvate, alanine, 3-hydroxybutyrate and acetoacetate in a single 2 µl blood sample. Clinica Chemica Acta. 122(2): 231-40.

McLoughlin, P., Popham, P., Linton, R.A., Bruce, R.C., and Band, D.M. 1992. Use of arterialized venous blood sampling during incremental exercise tests. J. Appl. Physiol. 73(3):937-40.

Morris, D.L, and Rui, L. 2009. Recent advances in understanding leptin signaling and leptin resistance. Am. J. Physiol. Endocrinol. Metab. 297(6): E1247-59.

Ng, M., Fleming, T., Robinson, M., Thomson, B., Graetz, N., Margono, C., et al. 2014. Global, regional, and national prevalence of overweight and obesity in children and adults during 1980-2013: a systematic analysis for the Global Burden of Disease Study 2013. Lancet. 384(9945):766-81.

Ravussin, E., Schutz, Y., Acheson, K., Dusmet, M., Bourquin, L., and Jequier, E. 1985 Short-term, mixed-diet overfeeding in man: No evidence of “luxuskonsumtion”. Am J Physiol. 249: E470–477.

Ricci, M.R., Fried, S.K. Isoproterenol decreases leptin expression in adipose tissue of obese humans. Obes Res 1999;7(3):233-40.

Ropelle, E.R., Flores, M.B., Cintra, D.E., Rocha, G.Z., Pauli, J.R., Morari, J., et al. 2010. IL-6 and IL-10 anti-inflammatory activity links exercise to hypothalamic insulin and leptin sensitivity through IKKbeta and ER stress inhibition. PLoS Biol. 8(8): e1000465.

Salvadori, A., Fanari, P., Giacomotti, E., Palmulli, P., Bolla, G., Tovaglieri, I., et al. 2003. Kinetics of catecholamines and potassium, and heart rate during exercise testing in obese subjects. Heart rate regulation in obese during exercise. Eur. J. Nutr. 42(4): 181-7.
Schubert, M.M., Desbrow, B., Sabapathy, S., and Leveritt, M. 2013. Acute exercise and subsequent energy intake. A meta-analysis. Appetite. 63: 92-104.

Schulz, K., Altman, D., and Moher, D. 2010. CONSORT. Statement: updated guidelines for reporting parallel group randomized trials. BMJ, 340.

Scriba, D., Aprath-Husmann, I., Blum, W.F., and Hauner, H. 2000. Catecholamines suppress leptin release from in vitro differentiated subcutaneous human adipocytes in primary culture via beta1- and beta2-adrenergic receptors. Eur. J. Endocrinol. 143(3): 439-445.

Shook, R.P., Hand, G.A., Drenowatz, C., Hebert, J.R., Paluch, A.E., Blundell, J.E., et al. 2015. Low levels of physical activity are associated with dysregulation of energy intake and fat mass gain over 1 year. Am. J. Clin. Nutr. 102(6): 1332-8.

Stubbs, R.J., Hughes, D.A., Johnstone, A.M., Rowley, E., Reid, C., Elia, et al. 2000. The use of visual analogue scales to assess motivation to eat in human subjects: a review of their reliability and validity with an evaluation of new hand-held computerized systems for temporal tracking of appetite ratings. Br. J. Nutr. 84(4): 405-15.

Tham, T.C., McKaigue, J.P., Guy, S., Shanks, R.G., and Riddell, J.G. 1993. The dose dependency of the alpha-adrenoceptor antagonist and beta-adrenoceptor partial agonist activity of dilevalol and labetalol in man. Br. J. Clin. Pharmacol. 36(3): 251-6.

Tiryaki-Sonmez, G., Ozen, S., Bugdayci, G., Karli, U., Ozen, G., Cogalgil, S., et al. 2013. Effect of exercise on appetite-regulating hormones in overweight women. Biol. Sport. 30(2): 75-80.

Tsouliou, F., Pitsiladis, Y.P., Malkova, D., Wallace, A.M., and Lean, M.E. 2003. Moderate physical activity permits acute coupling between serum leptin and appetite-satiety measures in obese women. Int. J. Obes. Relat. Metab. Disord. 27(11): 1332-9.

Walsh, K.M., Adams, C., Sinclair, A., Leen, E., and Lean, M.E. 1998. Influences on
adrenaline-induced thermogenesis in obese women and relationship to cardiovascular
responses. Clin. Sci. 94(2): 121-7.

Webber, J., Taylor, J., Greathead, H., Dawson, J., Buttery, P.J., and Macdonald, I.A. 1994. A
comparison of the thermogenic, metabolic and haemodynamic responses to infused
adrenaline in lean and obese subjects. Int. J. Ob. Relat. Metab. Dis. 18(11): 17-24.

Wellman, P.J. 2000. Norepinephrine and the control of food intake. Nutrition.16: 837-42.

Wellman, P.J., Davies, B.T., Morien, A., and McMahon, L. 1993. Modulation of feeding by
hypothalamic paraventricular nucleus alpha 1- and alpha 2-adrenergic receptors. Life
Sci. 53(9): 669-79.

Westerterp, K.R. 2010. Physical activity, food intake, and body weight regulation: insights
from doubly labeled water studies. Nutr. Rev. 68(3): 148-54.

Zaccaria, M., Ermolao, A., Brugin, E., and Bergamin, M. 2013. Plasma leptin and energy
expenditure during prolonged, moderate intensity, treadmill exercise. J. Endocrinol.
Invest. 36(6): 396-401.

Zouhal, H., Lemoine-Morel, S., Mathieu, M.E., Casazza, G.A., and Jabbour, G. 2013.
Catecholamines and obesity: effects of exercise and training. Sports Med. 43(7): 591-
600.

Zouhal, H., Sellami, M., Saeidi, A., et al. 2019. Effect of physical exercise and training on
gastrointestinal hormones in populations with different weight statuses. Nutr Rev.
77(7): 455-477.
| 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.
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.

| Interventions         | Time       | Serum leptin (ng.ml⁻¹) | Blood glucose (mmol.l⁻¹) | Plasma FFA (mmol.l⁻¹) |
|-----------------------|------------|------------------------|--------------------------|-----------------------|
|                       | (-60 min)  | (0 min)                | (20 min)                 | (80 min)              |
| 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           |
| Exercise plus placebo |            | 4.63 ± 0.16            | 4.53 ± 0.08              | 4.55 ± 0.09           | 4.52 ± 0.06           | *p = 0.659 | *p = 0.004 | *p = 0.028 |
| Exercise plus α/β blocker |            | 4.59 ± 0.16            | 4.83 ± 0.11              | 4.91 ± 0.07           | 4.89 ± 0.06           |
| 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.

|                     | Interventions        | (-60 min) | (0 min)  | (20 min) | (80 min) | 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       |
|                     | Saline infusion               | 65.80 ± 8.15 | 65.86 ± 8.07 | 65.31 ± 9.13 | 68.90 ± 7.73 | p = 0.688       |
| 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       |
|                     | Saline infusion               | 5.03 ± 0.27  | 4.72 ± 0.06  | 4.60 ± 0.05  | 4.575 ± 0.03  | p = 0.696       |
| 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       |
|                     | Saline infusion               | 0.56 ± 0.11  | 0.57 ± 0.13  | 0.65 ± 0.15  | 0.70 ± 0.10   | p = 0.025       |
| Plasma Adrenaline (nmol l\(^{-1}\)) | Adrenaline infusion        | -          | 0.17 ± 0.26  | -          | -          | p = 0.010       |
|                     | Saline infusion               | -          | 0.16± 0.20   | -          | -          | p = 0.063       |
| Plasma NA (nmol l\(^{-1}\)) | Adrenaline infusion        | -          | 1.59 ± 0.19  | 2.32±0.19   | -          | p = 0.060       |
|                     | Saline infusion               | -          | 1.49 ± 0.26  | 1.61±0.26   | -          | p = 0.060       |

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.
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 5. Heart rate, perceived breathlessness and leg-tiredness during exercise and infusion interventions in both experiments. |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| **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 \(\alpha/\beta\) 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)) | | | | | | | | | |
| Exercice 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 \(\alpha/\beta\) 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)) | | | | | | | | | |
| Exercice 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 \(\alpha/\beta\) 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* | | | |
**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 |
|------------------------------|-----------|----------------------|
| **VO₂ (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           |
| **VCO₂ (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.
### 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**       | 2a      | Scientific background and explanation of rationale                            | 2                   |
|                        | 2b      | Specific objectives or hypotheses                                             | 2                   |
| **Methods**            | 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:**     | 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                   |
|                        | 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
Blinding
   11a If done, who was blinded after assignment to interventions (for example, participants, care providers, those assessing outcomes) and how
   11b If relevant, description of the similarity of interventions
Statistical methods
   12a Statistical methods used to compare groups for primary and secondary outcomes
   12b Methods for additional analyses, such as subgroup analyses and adjusted analyses

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
   13b For each group, losses and exclusions after randomisation, together with reasons
Recruitment
   14a Dates defining the periods of recruitment and follow-up
   14b Why the trial ended or was stopped
Baseline data
   15 A table showing baseline demographic and clinical characteristics for each group
Numbers analysed
   16 For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups
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)
   17b For binary outcomes, presentation of both absolute and relative effect sizes is recommended
Ancillary analyses
   18 Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory
Harms
   19 All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)

Discussion
Limitations
   20 Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses
Generalisability
   21 Generalisability (external validity, applicability) of the trial findings
Interpretation
   22 Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence
| Other information |   |                                                                 |
|-------------------|---|-----------------------------------------------------------------|
| Registration      | 23| Registration number and name of trial registry                  |
| Protocol          | 24| Where the full trial protocol can be accessed, if available     |
| Funding           | 25| Sources of funding and other support (such as supply of drugs), role of funders |

|   |   |
|---|---|
|   | 3 |
|   | NA|
|   | 14|
**Figure S1.** Participant flow diagram.

- **Enrollment**
  - Assessed for eligibility (n=24)
  - Excluded (n=14)
    - Not meeting inclusion criteria (n=14)
  - Randomized in full protocol of EXP-1 and EXP-2 (n=10)

- **Allocation**
  - Allocated in EXP-1 (n=10)
    - Received allocated exercise intervention (n=10)
  - 7-days wash out
  - Dropped after the EXP-1 (n=1)
  - Allocated in EXP-2 (n=9)
    - Received allocated infusion intervention (n=9)

- **Analysis**
  - Analysed in EXP-1 (n=10), EXP-2 (n=9)
