Short-term sympathoadrenal inhibition augments the thermogenic response to β-adrenergic receptor stimulation

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Abstract

Sedentary behavior is associated with an attenuated thermogenic response to β-adrenergic receptor (β-AR) stimulation, an important regulator of energy expenditure (EE) in humans. Chronic stimulation of β-ARs, via heightened activity of the sympathoadrenal system, leads to diminished β-AR function. We have investigated the hypothesis that the thermogenic response of sedentary adults to β-AR stimulation will be increased during short-term sympathoadrenal inhibition. Using a randomly ordered, repeated measures study design, resting EE (REE; indirect calorimetry, ventilated hood technique) and the % increase in EE above REE (%ΔEE) during acute i.v. isoproterenol administration (nonselective β-AR agonist; 6, 12, and 24 ng/kg fat-free mass per min) were determined in 16 sedentary adults (nine females and seven males, 25±1 years, body mass index: 26·1±0·9 kg/m^2, maximal oxygen uptake: 40±2 ml/kg per min (mean±S.E.M.)) in the basal state and on the 6th day of transdermal clonidine administration (centrally acting α2-AR agonist; 0·2 mg/day). Relative to baseline, clonidine inhibited sympathoadrenal activity, as evidenced by decreased plasma norepinephrine concentration (1·04±0·13 vs 0·34±0·03 nmol/l; $P<0·001$), skeletal muscle sympathetic nerve activity (22·5±3·8 vs 8·5±1·9 bursts/min; $P=0·003$), and resting heart rate (63±2 vs 49±1 beats/min; $P<0·001$). Sympathoadrenal inhibition decreased REE (6510±243 vs 5857±218 kJ/day; $P<0·001$), increased respiratory exchange ratio (0·84±0·01 vs 0·86±0·01; $P=0·03$), and augmented the thermogenic response to β-AR stimulation (%ΔEE: 11±2, 16±2, and 24±2 vs 14±1, 20±2, and 31±2; $P=0·04$). These data demonstrate that in sedentary humans, short-term inhibition of sympathoadrenal activity increases the thermogenic response to β-AR stimulation, an important determinant of EE and hence energy balance.

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Introduction

Stimulation of β-adrenergic receptors (β-AR) by the sympathoadrenal system is an important physiological determinant of total daily energy expenditure (EE) and hence energy balance in humans (van Baak 2001). Evidence for this is overwhelming: during β-AR blockade, both resting metabolic rate (RMR) and the magnitude of increase in EE following energy intake (thermic effect of feeding) are decreased (Tappy *et al.* 1986, Welle *et al.* 1991, Bell *et al.* 2001, Monroe *et al.* 2001). Furthermore, the thermic effect of feeding is positively associated with the thermogenic response to β-AR stimulation (Stob *et al.* 2007*a*), and observations of weight gain in patients prescribed β-AR blockers are not uncommon (Sharma *et al.* 2001).

The sympathoadrenal system and β-ARs are also very important for cardiovascular regulation. Several studies have demonstrated that chronically high sympathoadrenal activity (such as observed with heart failure or aging) is associated with the downregulation of β-ARs (decreased receptor density), and inhibited inotropic and chronotropic responses to β-AR stimulation (White & Leenen 1994, White *et al.* 1994, Lohse *et al.* 1996, Liggett 2001); however, at least some of these indications of β-AR dysfunction/downregulation may be reversed during short-term sympathoadrenal inhibition (Nattel *et al.* 1979, Krukemyer *et al.* 1989, Madden *et al.* 2006). For example, short-term (14 days) administration of clonidine decreases sympathoadrenal activation, thus attenuating the magnitude of day-to-day β-AR stimulation, and augments the chronotropic response to acute β-AR stimulation (Madden *et al.* 2006). It is currently unknown whether decreasing sympathoadrenal activation with clonidine in humans will improve β-AR thermogenic function.

Sedentary adult humans demonstrate attenuated thermogenic responses to β-AR stimulation compared with habitual exercisers (Bell *et al.* 2006*a*, Stob *et al.* 2007*a*,*b*). A sedentary lifestyle is associated with a variety of factors, such as increased total and visceral fat mass (Booth *et al.* 2008, Church 2009), and elevated circulating concentrations of insulin and leptin (Bell *et al.* 2001, Jones *et al.* 2004), which are in turn associated with increased day-to-day sympathoadrenal activation (Jones *et al.* 1997, Monroe *et al.* 2000, Alvarez *et al.* 2002).

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Chronic pharmacological stimulation of β-ARs (14 days of terbutaline sulfate administration) leads to a diminished thermogenic response to acute i.v. β-AR stimulation (Scheidegger *et al.* 1984). Together, these observations imply that in sedentary adults, increased sympathoadrenal activation (Grassi *et al.* 1994, Roveda *et al.* 2003) leads to downregulation/dysfunction of β-ARs. Accordingly, the purpose of this study was to investigate the hypothesis that short-term inhibition of the sympathoadrenal system would augment the thermogenic response to β-AR stimulation in sedentary adult humans.

Subjects and methods

We studied 16 adult males and females (18–39 years). All were considered sedentary, in that none had performed any type of regularly scheduled exercise (i.e. <3 times/week) during the previous 2 years, and compared with age-adjusted US population normative values, all were at or below the 60th percentile for maximal oxygen uptake ($VO_{2\ max}$), a measure of the upper limit of aerobic capacity (Franklin *et al.* 2000). Subjects were nonsmokers and not medicated. The nature, purpose, and risks of the study were explained to each subject before written informed consent was obtained. The experimental protocol conformed to the standards set by the Declaration of Helsinki, and was approved by the Institutional Review Board at Colorado State University.

Experimental protocol

Following routine screening procedures (graded exercise test, 12-lead electrocardiogram, health history questionnaire, and measures of body composition), subjects were studied during two randomly ordered mornings separated by 7–30 days. During both of these mornings, RMR and skeletal muscle sympathetic nerve activity (MSNA) were determined simultaneously, followed by the thermogenic response to i.v. β-AR stimulation. These measures were performed in the basal state and on the 6th day of transdermal clonidine administration (Catapres-TTS; 0·2 mg/day). Clonidine is a blood pressure medication. Its mechanism of action is via prejunctional stimulation of α-2-ARs, including those located in the locus coeruleus, resulting in centrally mediated peripheral sympathoadrenal inhibition, as reflected by decreased norepinephrine release (Schwartz *et al.* 1990*a*,*b*) and attenuated MSNA (Muzi *et al.* 1992, Furlan *et al.* 2006). 0·2 mg/day is typical of a regular clinical dose for the treatment of hypertension (Prisant 2002, Ross *et al.* 2002). The affinity of clonidine for β-ARs is negligible (Zawilska *et al.* 2000). The plasma half-life of clonidine is 12·7±7 h.

Experimental procedures

Subjects reported to the laboratory between 0600 and 0800 h following a 12-h fast, 24-h abstention from vigorous exercise, 12-h abstention from caffeine, and 2-h abstention from water (confirmed verbally by each subject). On arrival, they were instrumented for determination of beat-by-beat heart rate (3-lead electrocardiogram) and blood pressure (automated physiological monitor: Cardiocap 5, GE Datex-Ohmeda, Madison, WI, USA), and an i.v. catheter was inserted into an antecubital or dorsal hand vein. The catheter was kept patent via a saline drip. Subjects were studied under quiet resting conditions in the semi-recumbent position. Measurements were performed in a dimly lit room at a comfortable temperature (~23 °C).

Efferent multiunit postganglionic MSNA was measured from the peroneal nerve via standard microneurography procedures as previously described (Bell *et al.* 2003, 2004). Briefly, two tungsten microelectrodes (FHC, Inc., Bowdoin, ME, USA) were inserted into the lower limb distal to the knee: one impaled the peroneal nerve, while the other served as a reference electrode and was placed nearby, but not in the nerve. Neural activity was amplified (×8000), filtered (700–2000 Hz), full-wave rectified, and integrated (time constant 0·1 s; Nerve Traffic Analyzer, model 662c-3, University of Iowa Bioengineering). Neurograms were considered acceptable as recordings of efferent MSNA according to previously published criteria (Wallin & Fagius 1988, Ng *et al.* 1994), and were analyzed offline by a single investigator (C B) who was naïve to the treatment condition during which the recordings were made. MSNA was expressed as bursts of integrated activity per minute over 10 min of continuous recording during simultaneous measurement of RMR.

RMR was measured over 45 min. The first 15 min were considered a habituation/relaxation period, thus the RMR measurement corresponded to data collected during minutes 15–45, and the MSNA data during minutes 15–25. VO_2 and carbon dioxide production (VCO_2) were averaged each minute for 30 min using a custom built ventilated hood indirect calorimetry system (Nighthawk Design, Boulder, CO, USA) that utilized a respiratory mass spectrometer (Perkin Elmer MGA 1100, MA Tech Services, St Louis, MO, USA) and an ultrasonic flow sensor (ndd Medizintechnik AG, Zürich, Switzerland). The system was calibrated daily with precision mixed gases (Airgas, Denver, CO, USA). EE was calculated using the Weir formula (Weir 1949). In our laboratory, the measurement of RMR has a coefficient of variation (CV) of 3·3% and a test retest r^2 of 0·93 (Newsom *et al.* 2008).

Immediately following determination of RMR and MSNA, the thermogenic response to β-AR stimulation was assessed during continuous and incremental i.v. administration of the nonselective β-AR agonist, isoproterenol (6, 12, and 24 ng/kg fat-free mass (FFM) per min), as previously described (Bell *et al.* 2006*a*,*b*, Stob *et al.* 2007*a*,*b*, Richards *et al.* 2010). Each dose was administered over 30 min. EE was calculated from the average of the final 25 min of each 30 min collection. Steady state was confirmed during each of these 25-min periods if the change in VO_2 and VCO_2 between the first and last minute was ≤5%. The thermogenic response to β-AR stimulation was quantified as the percentage increase in EE above RMR (%ΔEE).

Venous blood samples (20 ml preserved with K_3 ethylendiaminetetraacetic acid plus 5 ml preserved with ethylene glycol tetraacetic acid/glutathione), collected in chilled tubes during the measurement of RMR, were immediately placed on ice and centrifuged within 60 min of collection to isolate plasma. Plasma samples were stored at −80 °C until analysis. Plasma catecholamine concentrations were analyzed in duplicate using HPLC (CV within: 5·2%; CV between: 5·4%). ELISAs were used to measure, in duplicate, plasma concentrations of adiponectin (CV within: 4·1%; CV between: 7·9%), pigment epithelium-derived factor (PEDF; CV within: 5·6%; CV between: 6·3%), and insulin (CV within: 5·2%; CV between: 8·1%; all assays purchased from Millipore Corporation, Billerica, MA, USA), and nonesterified fatty acids (NEFA; CV within: 1·9%; CV between: 4·9%; Wako Diagnostics, Richmond, VA, USA). Blood glucose concentration was determined via an automated analyzer (CV within: 1·2%; CV between: 4·6%; YSI 2300 STST Plus, YSI Incorporated, Yellow Springs, OH, USA).

Fat mass and FFM were measured using dual-energy X-ray absorptiometry (Lunar Radiation Corp., Madison, WI, USA software version 4.1). $VO_{2\ max}$ was determined with a metabolic cart (Parvo Medics, Sandy, UT, USA) during incremental treadmill exercise as previously described (Bell *et al.* 2005). Briefly, subjects walked/ran on a treadmill at an increasing grade until three of the following criteria were satisfied: volitional exhaustion (defined as an inability to continue), a heart rate within 10 beats/min of their age-related maximum (Tanaka *et al.* 2001), a plateau in the VO_2–work rate relation, and a rating of perceived exertion ≥19 (Borg 1982).

Control group

In order to determine the day-to-day variability in the primary outcome variables, RMR and the thermogenic response to β-AR stimulation were determined on two different mornings, separated by 7–30 days in a control group (six males and three females, age: 28±2 years, body mass index: 25·3±1·2 kg/m^2, $VO_{2\ max}$: 42±5 ml/kg per min).

Statistical analysis

This was a controlled, randomly ordered, repeated measures study. Accordingly, the influence of transdermal clonidine administration on selected baseline characteristics (e.g. heart rate, blood pressure, catecholamines, etc.) was examined via one-way repeated measures ANOVA. Two-way ANOVA with repeated measures was used to examine differences in %ΔEE during incremental β-AR stimulation measured during the basal state and during transdermal clonidine administration. Multiple comparisons of factor means were performed using the Newman–Keuls test. The level of statistical significance was set at $P<0·05$. Data are expressed as mean±S.E.M., unless otherwise stated.

Results

Subject characteristics

Selected subject characteristics are presented in Table 1. Briefly, subjects demonstrated physiological attributes typical of young sedentary adults. That is, on average, they were slightly overweight (based on body mass index), of low to average aerobic capacity (based on $VO_{2\ max}$), but were otherwise healthy (i.e. normotensive and normoglycemic).

Clonidine inhibits sympathoadrenal activation

Relative to the basal state, 6 days of transdermal clonidine administration decreased resting heart rate (mean change: −16±2 beats/min), systolic (−7±2 mmHg) and diastolic (−8±1 mmHg) blood pressure, MSNA (−13·8±4·1 bursts/min; $n=11$), and plasma norepinephrine concentration (−0·70±0·12 nmol/l; all variables $P<0·01$; Table 2). The S.D. of the R-to-R interval, a crude indicator of heart rate variability, was increased but the magnitude of this increase did not attain statistical significance ($P=0·07$). Similarly, the decrease in plasma epinephrine concentration also did not attain statistical significance ($P=0·11$).

Thermogenic/metabolic effects of short-term sympathoadrenal inhibition

Sympathoadrenal inhibition decreased RMR in every subject (mean response: −653±75 kJ/day; ∼10%; Table 2). Furthermore, respiratory exchange ratio (a crude indicator of substrate utilization) was increased, suggesting a greater reliance on carbohydrate oxidation for energy. Body mass was also increased slightly (0·8±0·3 kg), albeit significantly. Circulating concentrations of NEFA were decreased, and blood glucose concentration was increased, but neither change attained statistical significance (Table 2; both $P>0·07$). Plasma insulin, adiponectin, and PEDF concentrations were unchanged. The thermogenic effect of β-AR stimulation was appreciably

Table 1 Selected physiological characteristics. Data: mean±S.D.

Variable	Mean±S.D.
Sex (M/F)	7/9
Age (years)	25±4
Body mass (kg)	75·2±12·0
Body mass index (kg/m^2)	26·1±3·6
%Body fat	27·7±10·0
Fat mass (kg)	20·1±8·8
Fat-free mass (kg)	53·9±11·2
Blood pressure (mmHg)	112/68±4/4
Fasting glucose (mmol/l)	4·90±0·72
Maximal oxygen uptake (ml/kg per min)	40±8
Maximal respiratory exchange ratio	1·14±0·08
Maximal heart rate (beats/min)	194±12

Table 2 Influence of transdermal clonidine (0·2 mg/day over 6 days) on selected fasting metabolic and cardiovascular parameters. Data: mean±S.D.

	Basal	Clonidine	*P* value
Heart rate (beats/min)	63±8	49±4	<0·0001
S.D. of R-to-R interval (ms)	91±36	113±56	0·07
Blood pressure (mmHg)	112/68±4/4	105/59±12/8	0·03/<0·0001
Epinephrine (nmol/l)	0·26±0·20	0·23±0·12	0·11
Norepinephrine (nmol/l)	1·04±0·52	0·34±0·12	<0·0001
MSNA (bursts/min)	22·5±15·2	8·5±7·6	0·003
Body mass (kg)	75·2±12·0	76·0±12·4	0·01
RMR (kJ/day)	6510±972	5857±872	<0·0001
Respiratory exchange ratio	0·84±0·04	0·86±0·04	0·03
Glucose (mmol/l)	4·90±0·72	5·12±0·64	0·07
Insulin (pmol/l)	32·9±0·68	29·9±15·2	0·41
NEFA (mmol/l)	0·49±0·24	0·40±0·20	0·099
Adiponectin (μg/ml)	7·82±3·16	7·77±2·92	0·89
PEDF (μg/ml)	4·43±1·6	3·80±1·12	0·15

S.D. of R-to-R interval: calculated from ten continuous minutes of beat-by-beat electrocardiogram data. MSNA, skeletal muscle sympathetic nerve activity; RMR, resting metabolic rate; NEFA, nonesterified fatty acid; PEDF, pigment epithelium-derived factor.

increased (main effect of clonidine $P=0·049$; Fig. 1). Furthermore, the dose of isoproterenol required to increase EE 1050 kJ/day (~250 kcal/day) above RMR was lower ($P=0·049$) during clonidine administration (10·2±1·2 ng/kg-FFM per min) compared with basal responses (15·6±2·0).

Cardiovascular responses to β-AR stimulation following short-term sympathoadrenal inhibition

The chronotropic response to β-AR stimulation was substantially increased during sympathoadrenal inhibition (main effect of clonidine $P=0·0001$; clonidine–isoproterenol interaction $P=0·03$; Fig. 2). Additionally, the dose of isoproterenol required to increase heart rate 25 beats/min above rest was lower ($P=0·03$) during clonidine administration (10·0±0·86 ng/kg-FFM per min) compared with basal responses (14·9±0·86). Noteworthy, despite starting with a substantially lower resting heart rate during sympathoadrenal inhibition (Table 2), absolute heart rate was not different at the highest dose of β-AR stimulation (109±4 vs 107±3 beats/min).

Potential sex differences prior to and following sympathoadrenal inhibition

Although not a major focus of the current investigation, and hence not a consideration when performing *a priori* statistical power calculations, we have also investigated the potential for sex differences. Body mass index was not different ($P=0·96$) between men (26·0±1·3 kg/m^2) and women (26·1±1·3); however, men had lower % body fat (19·5±2·0 vs 34·1±2·5; $P<0·001$) and greater lean mass (65·1±1·8 vs 45·2±1·5 kg; $P<0·001$). Men also had lower fat mass than women (15·7±2·1 vs 23·5±3·1 kg), but this difference did not attain statistical significance ($P=0·07$). On average, the basal thermogenic responses to β-AR stimulation were similar (%ΔEE men: 10·1±1·5, 15·4±2·2, and 21·6±2·8; %ΔEE women: 12·2±2·5, 16·6±2·2, and 25·7±2·5), as were the responses during clonidine administration (%ΔEE men: 15·2±1·5, 19·5±2·9, and 29·2±2·7; %ΔEE women: 13·8±2·0, 19·8±1·8, and 32·2±3·0). Visual inspection of individual subject responses to β-AR stimulation in men and women collected in the basal condition and during sympathoadrenal inhibition (Fig. 3) suggests greater variability within the women's data, but the overall response (i.e. increased thermogenic responsiveness during clonidine administration) does not appear to be influenced by sex.

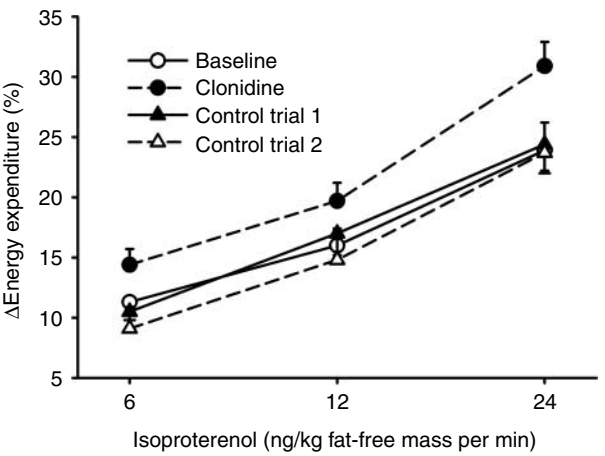

Figure 1 Relative to a control condition, short-term inhibition of the sympathoadrenal system (0·2 mg/day transdermal clonidine over 6 days) augments the thermogenic response to β-adrenergic receptor stimulation (main effect of clonidine: $P=0·04$; clonidine–isoproterenol interaction $P=0·07$). Data: mean±S.E.M. %ΔEE: the percentage increase in energy expenditure above resting metabolic rate.

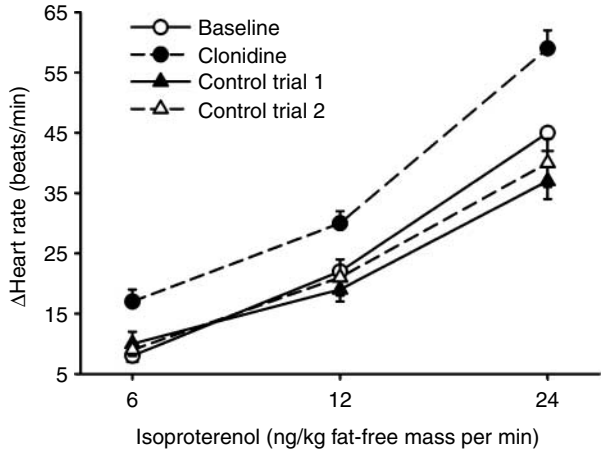

Figure 2 Relative to a control condition, short-term inhibition of the sympathoadrenal system (0·2 mg/day transdermal clonidine over 6 days) augments the chronotropic response to β-adrenergic receptor stimulation (main effect of clonidine $P=0·0001$; clonidine–isoproterenol interaction $P=0·03$). Data: mean ± S.E.M. ΔHR: the increase in heart rate above resting heart rate.

Time-control group responses

In the control subjects, relative to the influence of clonidine in the experimental group, there was no change in any of the primary outcome variables (all $P>0·05$): RMR (6452 ± 289 vs 6280 ± 281 kJ/day), body mass (74·0 ± 3·4 vs 74·1 ± 3·5 kg), %ΔEE during β-AR stimulation (10·4 ± 1·4, 17·0 ± 1·8, and 24·4 ± 2·3 vs 9·1 ± 1·8, 14·8 ± 2·6, and 23·7 ± 2·5), heart rate at rest (54 ± 4 vs 53 ± 4 beats/min) and during β-AR stimulation (63 ± 4, 73 ± 5, and 91 ± 5 vs 62 ± 4, 74 ± 5, and 93 ± 5 beats/min), and circulating concentrations of PEDF (4·7 ± 0·7 vs 4·6 ± 0·7 μg/ml), insulin (33·3 ± 8·3 vs 33·3 ± 4·9 pmol/l), glucose (4·24 ± 0·09 vs 4·25 ± 0·07 mmol/l), NEFA (0·45 ± 0·03 vs 0·42 ± 0·03 mmol/l), and adiponectin (8·71 ± 0·70 vs 8·73 ± 0·72 μg/ml).

Discussion

The novel finding of this investigation is that short-term central inhibition of the sympathoadrenal system augments the thermogenic response to β-AR stimulation. Our observation of decreased RMR and increased body mass during sympathoadrenal inhibition reinforces the notion that β-ARs are an important physiological regulator of EE and energy balance.

Several studies of humans have reported on the decrease in RMR and the thermic effect of feeding during administration of nonselective β-AR antagonists, such as propranolol or nadolol (Tappy *et al.* 1986, Welle *et al.* 1991, Bell *et al.* 2001, Monroe *et al.* 2001). Consistent with the majority of human studies, compared with wild-type mice, mice genetically modified such that they express none of the three β-AR subtypes demonstrate accelerated weight gain, despite no difference in energy intake (Bachman *et al.* 2002). Collectively, these observations speak to the important contribution of β-ARs to the control of EE, energy balance, and thus weight gain. Sedentary adult humans face an increased risk of weight/fat gain and consequently increased likelihood of developing a variety of metabolic and/or cardiovascular diseases, including diabetes and hypertension (Booth *et al.* 2008, Church 2009). Relative to their habitually exercising counterparts, sedentary humans demonstrate attenuated thermogenic responses to β-AR stimulation, as well as a smaller magnitude of decline in resting EE (REE) during β-AR blockade, indicative of decreased β-AR support of RMR (Bell *et al.* 2001, 2006*a*, Stob *et al.* 2007*a*,*b*). These observations may be partially mediated by decreased β-AR sensitivity, responsiveness, and/or density, that in turn may be partially explained by greater basal sympathetic activation, driven by several factors such as greater fat mass (Monroe *et al.* 2000) and higher circulating concentrations of insulin and leptin (Bell *et al.* 2001, Jones *et al.* 2004). Indeed, short-term (14 days) pharmacological stimulation of β-ARs (via terbutaline sulfate) leads to downregulation of β-ARs, and decreased thermogenic response to acute β-AR stimulation (Scheidegger *et al.* 1984). Similarly, in obese humans, a population in whom sympathoadrenal activation is chronically high (Grassi *et al.* 1995, Alvarez *et al.* 2002, Davy & Orr 2009), β-AR-mediated thermogenesis and lipid mobilization/oxidation is decreased (Blaak *et al.* 1994, 1995,

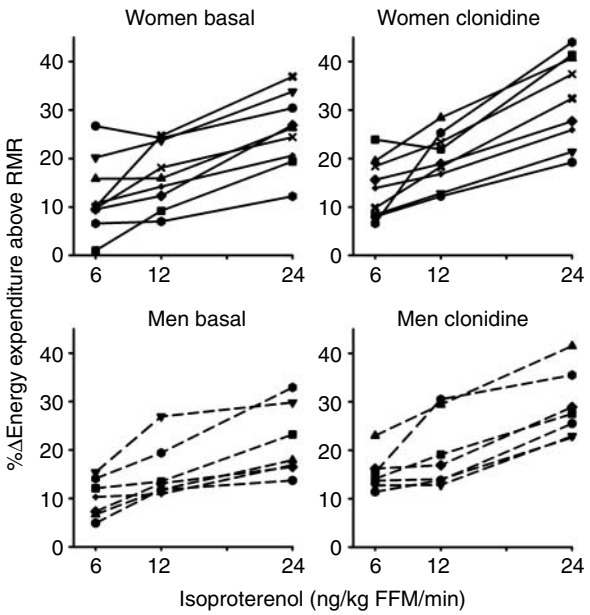

Figure 3 Individual thermogenic responses to beta-adrenergic receptor stimulation in men and women during the basal state and during short-term inhibition of the sympathoadrenal system (0·2 mg/day transdermal clonidine over 6 days). %ΔEnergy Expenditure Above RMR: the percentage increase in energy expenditure above resting metabolic rate.

Jocken *et al.* 2008). Data from the current investigation suggest that the β-ARs of sedentary adults may possess plasticity, i.e. they are not permanently dysfunctional. Future studies are warranted to identify potential strategies/interventions by which β-AR function might be improved. In this regard, regular endurance exercise (van Aggel-Leijssen *et al.* 2001) and/or administration of antioxidants (Mak & Newton 2001, 2004, Bell *et al.* 2006*a*) show promise.

Our observation of augmented thermogenic response to β-AR stimulation during sympathoadrenal inhibition may be due, in part, to increased β-AR density in metabolically active tissues. Chronically high sympathoadrenal activation and subsequent β-AR stimulation are associated with decreased β-AR density (White & Leenen 1994, White *et al.* 1994, Lohse *et al.* 1996, Liggett 2001). Conversely, clonidine administration has been shown to increase the density of β-ARs on peripheral blood mononuclear cells (Zoukos *et al.* 1993, 1994). Biopsy sampling of skeletal muscle and adipose tissue for assessment of β-AR density before and during clonidine administration would provide an opportunity for mechanistic insight.

Isoproterenol is a nonselective β-AR agonist; thus, we are unable to ascertain the influence of sympathoadrenal inhibition on specific β-AR subtypes. Given past (Lamont *et al.* 1997, Schiffelers *et al.* 2001, van Baak *et al.* 2002, Hoeks *et al.* 2003) and recent (Ishibashi & Seale 2010, Vegiopoulos *et al.* 2010) attention to the contribution of individual β-AR subtypes to the regulation of EE, then this would be an important question of considerable scientific and clinical interest for future studies.

We also determined the influence of sympathoadrenal inhibition on several circulating factors that may be under sympathoadrenal control, and are known to have important prognostic value for various metabolic and/or cardiovascular diseases. Adiponectin is secreted from adipose tissue (Yamauchi *et al.* 2001), plays a regulatory role in insulin sensitivity and energy homeostasis (Yamauchi *et al.* 2001, Dridi & Taouis 2009), and may be, in part, regulated by the sympathoadrenal system (Fasshauer *et al.* 2001, Nowak *et al.* 2005, Lam *et al.* 2008). In the current investigation, following short-term sympathoadrenal inhibition with clonidine, adiponectin was unchanged. Possible explanations to account for the apparent discrepancy with previous studies (Nowak *et al.* 2005) likely relate to the duration and method of sympathoadrenal inhibition.

Another physiologically significant circulating endocrine factor measured in the current investigation was PEDF. PEDF is becoming widely recognized as an important determinant of oxidative stress (Zhang *et al.* 2008, Banumathi *et al.* 2010), inflammation and angiogenesis (Jenkins *et al.* 2007, Zhang *et al.* 2008), is inversely associated with insulin sensitivity and metabolic flexibility (Richards *et al.* 2010), is positively associated with characteristics of the metabolic syndrome (Yamagishi *et al.* 2006), and is predictive of future clinical events in patients with heart failure (Rychli *et al.* 2010). Contrary to animal and cell culture data (Lashbrook & Steinle 2005, Steinle *et al.* 2008), we have demonstrated that short-term inhibition of the sympathetic nervous system does not affect circulating concentrations of PEDF. Differences between our data and previous studies (Lashbrook & Steinle 2005, Steinle *et al.* 2008) may relate to species differences (adult humans versus female Sprague–Dawley rats), tissue differences (plasma versus cultured retinal pigment epithelial cells), method of sympathoadrenal inhibition (systemic pharmacology versus surgical sympathectomy), and/or duration of sympathoadrenal inhibition (6 days versus 6 weeks). Given the multiple positive associations in adult humans between fat mass and the metabolic syndrome (Klaus *et al.* 2009), and fat mass, metabolic syndrome and PEDF (Yamagishi *et al.* 2006, Crowe *et al.* 2009), identification of the biological processes responsible for the regulation of PEDF should be a high priority.

The current data, consistent with others (Mitchell *et al.* 2005, Sica & Grubbs 2005), show clonidine to be an effective intervention for lowering blood pressure; however, long-term use may result in weight gain (Morrison *et al.* 1990, Laurent & Safar 1992). Sympathoadrenal inhibition will lead to decreased β-AR stimulation, and hence decreased β-AR support of EE, creating a physiological environment favoring positive energy balance and weight gain (Spraul *et al.* 1993). After only 6 days of clonidine use, the research participants in the current study demonstrated small, but consistent and statistically significant weight gain (clonidine: $0{\cdot}8 \pm 0{\cdot}3$ kg versus control: $0{\cdot}1 \pm 0{\cdot}3$ kg). In addition to decreased EE, this weight gain may be attributable to fluid retention (Morrison *et al.* 1990, Laurent & Safar 1992), and perhaps also increased dietary intake (Leibowitz *et al.* 1993, Rieg & Aravich 1994, Delgado-Aros & Camilleri 2005).

We also report on the greater chronotropic response to β-AR stimulation during sympathoadrenal inhibition. This observation is supported by a previous investigation, in which isoproterenol administration elicited a greater increase in heart rate in young adults following 2 weeks of clonidine administration (Madden *et al.* 2006). One important caveat to both the current and previous investigation is that data were collected in the presence of an intact baroreflex. A definitive demonstration of augmented chronotropic responsiveness requires inhibition of the baroreflex, such as via ganglionic blockade (Christou & Seals 2008).

In summary, we have demonstrated that the thermogenic response to β-AR stimulation is augmented during short-term sympathetic inhibition. Given the important contribution of β-ARs to the control of EE, our data reinforce the idea that targeting β-AR function may be a useful strategy to improve metabolic regulation in adult humans.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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