Thermogenic responsiveness to β-adrenergic stimulation is augmented in exercising *versus* sedentary adults: role of oxidative stress

Christopher Bell, Nicole R. Stob and Douglas R. Seals

Department of Integrative Physiology, University of Colorado, Boulder, CO 80309-0354, USA

β-Adrenergic receptor (β-AR) modulation of resting and postprandial energy expenditure (EE) is augmented in regularly exercising compared with sedentary adults, but the underlying physiological mechanisms are unknown. Differences in thermogenic responsiveness to β-AR stimulation, perhaps secondary to reactive oxygen species (ROS) bioactivity, may be involved. To determine habitual exercise-related differences in β-AR thermogenic responsiveness and the possible influence of ROS, we measured the percentage increase in EE (ΔEE%; indirect calorimetry, ventilated hood method) above resting EE in response to non-specific β-AR stimulation (intravenous isoproterenol (isoprenaline): 6, 12 and 24 ng (kg fat-free mass)$^{-1}$ min^{-1}) in 25 sedentary (11 males; 51 ± 4 years; body mass index 25.0 ± 0.8 kg m^{-2}, maximal oxygen uptake 29 ± 1 ml kg^{-1} min^{-1} (mean ± S.E.M.)) and 14 habitually aerobic exercising (9 males, 46 ± 6 years, 23.1 ± 0.7 kg m^{-2}, 44 ± 3 ml kg^{-1} min^{-1}) healthy adults under normal (control) conditions and during acute intravenous administration of a potent antioxidant, ascorbic acid (vitamin C; 0.04 g (kg fat-free mass)$^{-1}$). ΔEE% was greater ($P = 0.02$) in the habitually exercising (8.6 ± 1.2, 12.9 ± 1.2, 20.0 ± 1.4) *versus* sedentary (6.3 ± 0.7, 10.4 ± 0.8, 16.0 ± 1.0) adults. Ascorbic acid increased ($P = 0.01$) ΔEE% only in the sedentary adults (to 9.5 ± 0.9, 12.4 ± 0.7, 18.5 ± 0.8), abolishing baseline group differences. ΔEE% was not related to the amount of body fat, sex, or any other baseline characteristic. Thermogenic responsiveness to β-AR stimulation is augmented in habitually exercising adults. The mechanism is ascorbic acid dependent, suggesting that it may be linked to decreased ROS bioactivity. Our findings advance a novel mechanism by which habitual physical activity may modulate EE in humans, with potential implications for energy balance and body weight control.

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Corresponding author C. Bell: Department of Health and Exercise Science, 205E Moby-B Complex/1582 Campus Delivery, Colorado State University, Fort Collins, CO 80521-1582, USA. Email: cbell@cahs.colostate.edu

In humans, stimulation of β-adrenergic receptors (β-AR) by the sympathoadrenal system is an important physiological determinant of resting metabolic rate (RMR) (Lamont *et al.* 1989; Welle *et al.* 1991; Bell *et al.* 2001, 2004; Monroe *et al.* 2001) and the postprandial increase in energy expenditure (EE) associated with energy intake (thermic effect of feeding) (Schwartz *et al.* 1988; Jones *et al.* 2004). β-AR thermogenesis is also a critical physiological determinant of total daily energy expenditure and resistance to obesity in knockout mice triply null for β-ARs (Bachman *et al.* 2002) β-AR thermogenesis is particularly important during high-fat feeding (Bachman *et al.* 2002).

Adult humans who regularly perform aerobic endurance exercise often demonstrate lower body fat and greater body composition-normalized RMR and thermic effect of feeding compared with their sedentary peers (Van Pelt *et al.* 1997, 1998; Bell *et al.* 2001; Jones *et al.* 2004). However, sympathoadrenal system activity at rest and in response to acute energy intake is not augmented in exercise-trained compared with sedentary adults (Bell *et al.* 2001; Jones *et al.* 2004). This suggests that thermogenic responsiveness to β-AR stimulation is increased in regularly exercising humans, but at present no data are available on this issue.

One mechanism that could contribute to habitual exercise-associated differences in thermogenic responsiveness to β-AR stimulation is the bioactivity of reactive oxygen species (ROS). ROS or free radicals are any atom, molecule or compound that has one or more unpaired electrons (Knight, 2000). ROS, the most important of which biologically is the superoxide anion, are important signalling molecules and can modulate β-AR responsiveness (Kaneko *et al.* 1991; Vatner *et al.* 1993; Persad *et al.* 1997, 1998*a*,*b*, 1999; Nishizawa *et al.*

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2004). In patients with normal left ventricular function, dobutamine (a β_1-AR agonist)-stimulated increases in left ventricular contractility at rest are augmented in the presence of ascorbic acid (vitamin C) (Mak & Newton, 2001, 2004), a potent non-enzymatic antioxidant that scavenges superoxide anions when administered at supra-physiological concentrations (Frei *et al.* 1989; Jackson *et al.* 1998). There is some evidence that habitual aerobic endurance exercise may be associated with reduced ROS bioavailability or 'oxidative stress', defined as a state in which ROS formation exceeds antioxidant defences (Adams *et al.* 2005; Kojda & Hambrecht, 2005). However, indirect systemic plasma markers of oxidative stress are relatively insensitive and do not always reflect differences in ROS bioactivity between groups of healthy humans, including exercising and sedentary adults (Eskurza *et al.* 2004*b*). As such, inhibition of ROS bioavailability via antioxidant administration provides more direct insight into the modulatory effects of ROS on physiological function.

Accordingly, in the present investigation we hypothesized that: (1) adult humans performing regular aerobic endurance exercise would demonstrate greater increases in metabolic rate in response to β-AR stimulation than their sedentary peers; and (2) these differences would be explained, at least in part, by corresponding differences in ROS modulation of β-AR responsiveness. To determine this, EE was established at rest and in response to β-AR stimulation with isoproterenol, a non-selective β-AR agonist, during infusion of ascorbic acid or saline (placebo) in groups of healthy habitually exercising and sedentary adults. We predicted that habitually exercising adults would demonstrate greater increases in metabolic rate in response to β-AR stimulation during placebo, but not ascorbic acid infusion, compared with sedentary adults.

Methods

Experimental subjects

We studied 39 healthy adult male and females (18–74 years): 25 sedentary and 14 habitual exercisers. Sedentary subjects had performed no regular, formalized physical exercise during the previous 2 years and were in the lower 50th percentile for maximal oxygen uptake ($\dot{V}_{O_2max}$) (Franklin *et al.* 2000). Habitually exercising subjects had regularly performed a minimum of 40 min of vigorous physical exercise $\geq$ 4 days per week for the previous 2 years, and were in the upper 10th percentile for $\dot{V}_{O_2max}$. All subjects were healthy as assessed by medical history, and fasting plasma glucose and insulin concentrations. In addition, older subjects ($\geq$ 35 years) underwent a physical examination with resting electrocardiogram (ECG) as well as ECG and blood pressure assessments during graded treadmill exercise to exhaustion. Subjects were non-smokers and were not regularly taking any medication or vitamin/antioxidant supplements. The nature, purpose and risks of the study were explained to each subject before written consent was obtained. The experimental protocol conformed to the standards set by the *Declaration of Helsinki* and was approved by the Human Research Committee at the University of Colorado at Boulder.

Experimental procedures

All measurements were made at the University of Colorado at Boulder General Clinical Research Center (GCRC) during two mornings, each after a 12 h fast, 2 h abstention from water, and 24 h abstention from vigorous physical exercise. Subjects were studied under quiet resting conditions in a semi-recumbent position. Measurements were performed between 07.00 and 09.00 h in a dimly lit room at a comfortable temperature (~23°C). The increase in energy expenditure above RMR in response to intravenous administration of the non-selective β-AR agonist isoproterenol (6, 12 and 24 ng (kg fat-free mass (FFM))$^{-1}$ min^{-1}) was measured during: (1) continuous intravenous ascorbic acid administration (American Regent Laboratories Inc., NY, USA; 0.04 g (kg FFM)$^{-1}$ dissolved in 100 ml of saline); and (2) continuous saline infusion (Fig. 1). The order of co-infusions (saline *versus* ascorbic acid) was randomized and administered in a single blind fashion. The rationale for the doses

Habituation	Baseline RMR	Δ % Energy Expenditure during Isoproterenol (ng (kg FFM)$^{-1}$ min^{-1})		
		6	12	24
0 - 15 min	15 - 45 min	0 - 30 min	0 - 30 min	0 - 30 min
	Ascorbic acid (0.04 g (kg FFM)$^{-1}$) and/or saline administration			

Figure 1. Schematic representation of protocol
The increase in energy expenditure above resting metabolic rate (RMR) in response to intravenous administration of the non-selective β-AR agonist isoproterenol (6, 12 and 24 ng (kg fat-free mass (FFM))$^{-1}$ min^{-1}) was measured during: (1) continuous intravenous ascorbic acid administration (0.04 g (kg FFM)$^{-1}$ dissolved in 100 ml of saline); and (2) continuous saline infusion. The order of co-infusions (saline *versus* ascorbic acid) was randomized and administered in a single blind fashion.

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and method of ascorbic acid administration has been previously described (Bell *et al.* 2003, 2005; Eskurza *et al.* 2004*a*,*b*), and we have established previously that this dose acutely decreases oxidative stress in adult humans (Bell *et al.* 2003, 2005). Subjects were instrumented for measurement of heart rate (ECG) and blood pressure. A catheter was placed in an antecubital vein and was kept patent with heparin. After a 30 min rest period following instrumentation baseline RMR was measured, as previously described (Bell *et al.* 2001; Monroe *et al.* 2001). The first 15 min were considered an habituation period after which oxygen consumption and carbon dioxide production were averaged each minute for 30 min using a ventilated hood indirect calorimetry system (DeltaTrac II Metabolic Monitor, SensorMedics Corp., Yorba Linda, CA, USA). This measurement was then repeated over three consecutive 30 min periods during incremental infusion of isoproterenol. Energy expenditure was calculated from the average of the final 25 min of each 30 min collection using the Weir formula (Weir, 1949). Steady state was confirmed during each of these 25 min periods by comparing oxygen consumption and carbon dioxide production during the 1st and last minute.

Dietary intake of vitamin C, together with macro- and micronutrients, were estimated from food diaries maintained for three consecutive days (two weekdays and one weekend day). Subjects kept accurate and complete diet records and were provided with diet scales (Scaleman, Target Corporation, Minneapolis, MN, USA) to weigh all food. A GCRC registered dietician subsequently analysed all of the food diaries using standard computer-assisted procedures (ESHA Research, The Food Processor, version 7.6, Salem, OR, USA).

Fat mass and FFM were measured using dual-energy X-ray absorptiometry (DXA-IQ; Lunar Radiation corp., Madison, WI, USA, software version 4.1). Maximal oxygen uptake was determined via incremental treadmill exercise as previously described (Bell *et al.* 2005).

Statistical analysis

RMR was adjusted for differences in FFM (relation between RMR and FFM: $r = 0.84$, $P < 0.001$). Two-way (saline *versus* ascorbic acid) analysis of variance (ANOVA) with repeated measures on one factor (relative change in energy expenditure above RMR across three doses of isoproterenol) was used to examine differences among sedentary adults and habitual exercisers. Thermogenic responsiveness to β-AR stimulation was not adjusted for differences in FFM because the doses of isoproterenol were normalized for individual FFM. Multiple comparisons of factor means were performed using the Neuman-Keuls test. Relations between variables of interest were determined by simple correlation analysis.

Table 1. Selected subject characteristics

	Sedentary	Habitual exercisers
Sex (M/F)	11/14	9/5
Age (years)	51 ± 4	46 ± 6
Height (m)	1.69 ± 0.02	1.72 ± 0.03
Mass (kg)	72.1 ± 3.2	68.4 ± 2.9
BMI (kg m^{-2})	25.0 ± 0.8	23.1 ± 0.7
Waist-to-hip ratio	0.84 ± 0.02	0.82 ± 0.02
Percentage body fat	31.4 ± 2.1	21.3 ± 2.2*
Fat mass (kg)	22.9 ± 2.0	14.4 ± 1.4*
Fat-free mass (kg)	49.1 ± 2.4	54.3 ± 3.2
$\dot{V}_{O_2max}$ (ml kg^{-1} min^{-1})	29.3 ± 1.5	43.8 ± 2.6*
RMR (kJ day^{-1})	5503 ± 206	5870 ± 253
Heart rate (beats min^{-1})	61 ± 1	53 ± 1*
Blood pressure (mmHg)	120/68 ± 2/1	112*/65 ± 1/2
Insulin (pmol l^{-1})	52.6 ± 9.0	39.5 ± 8.3
Glucose (mmol l^{-1})	5.1 ± 0.1	4.8 ± 0.1
Cholesterol (mmol l^{-1})	5.1 ± 0.2	5.0 ± 0.2
HDL-C (mmol l^{-1})	1.5 ± 0.1	1.6 ± 0.8
Triglyceride (mmol l^{-1})	1.1 ± 0.1	0.9 ± 0.1
LDL-C (mmol l^{-1})	3.1 ± 0.2	3.0 ± 0.2

Data: mean ± S.E.M. BMI, body mass index; $\dot{V}_{O_2max}$, maximal oxygen uptake; RMR, resting metabolic rate (adjusted for fat-free mass); HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol. *Different to sedentary ($P = 0.01$).

The level of statistical significance was set at $P < 0.05$. Data are expressed as mean ± S.E.M.

Results

The exercising adults had lower percentage body fat, fat mass, resting heart rate and systolic blood pressure, and greater maximal oxygen uptake than the sedentary subjects ($P = 0.01$), whereas age, body mass, FFM, RMR adjusted for FFM, and plasma concentrations of glucose, insulin and lipids were not different between groups (Table 1). Mean total daily energy intake (sedentary: 8528 ± 705 *versus* exercisers: 10 180 ± 1097 kJ day^{-1}), and percentage carbohydrate (52 ± 2 *versus* 51 ± 2%), fat (29 ± 2 *versus* 28 ± 2%), protein (16 ± 1 *versus* 16 ± 1%) and vitamin C intakes did not differ between groups ($P > 0.05$). Absolute RMR was greater in the exercising (5949 ± 253 kJ day^{-1}) than sedentary (5317 ± 206 kJ day^{-1}) subjects ($P = 0.05$).

Augmented thermogenic responsiveness to β-AR stimulation in habitual exercisers

EE increased above RMR in response to incremental β-AR stimulation in both sedentary and exercising adults. However, the magnitude of increase was greater ($P = 0.02$) in the exercising adults indicating augmented β-AR-stimulated thermogenesis (Fig. 2*A*).

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Ascorbic acid increases β-AR thermogenesis only in sedentary adults

Administration of ascorbic acid did not affect baseline FFM-adjusted RMR in either sedentary (from 5503 ± 206 to 5430 ± 193 kJ day^{-1}) or exercising adults (from 5870 ± 253 to 5665 ± 234 kJ day^{-1}) ($P = 0.72$). β-AR-stimulated thermogenesis was increased ($P = 0.01$) in sedentary adults during administration of ascorbic acid (Fig. 2*B*). In contrast, ascorbic acid had no effect on β-AR-stimulated thermogenesis in the exercising adults ($P = 0.93$; Fig. 2*C*).

Ascorbic acid abrogates habitual exercise-associated differences in β-AR thermogenesis

During ascorbic acid administration the baseline (saline control) difference in β-AR thermogenesis between sedentary adults and habitual exercisers was abolished ($P = 0.86$; Fig. 2*D*).

Correlates of β-AR thermogenesis

There were no main effects of sex or sex–exercise–ascorbic acid interactions on β-AR thermogenesis ($P > 0.05$). Thus, correcting the groups for sex composition did not alter the significant main effect of habitual exercise status. Augmentation of β-AR thermogenesis with ascorbic acid was inversely related to baseline β-AR-stimulated thermogenesis ($r = -0.66$, $P < 0.001$), but not with any other baseline function or any subject characteristic including fasting insulin and glucose concentrations, or body-composition-related descriptor (Table 1).

Other responses

Respiratory exchange ratio was 0.82–0.84 at baseline and was not significantly affected by isoproterenol dose, habitual exercise status or ascorbic acid administration (interaction $P > 0.05$). Heart rate increased above baseline resting levels by up to ~30 beats min^{-1} in both groups in response to isoproterenol, whereas arterial blood pressure was not consistently affected in either group. There were no significant interactions between isoproterenol dose, habitual exercise status and ascorbic acid administration for heart rate or blood pressure (all $P > 0.05$).

Discussion

Our results demonstrate for the first time that thermogenic responsiveness to β-AR stimulation is greater in

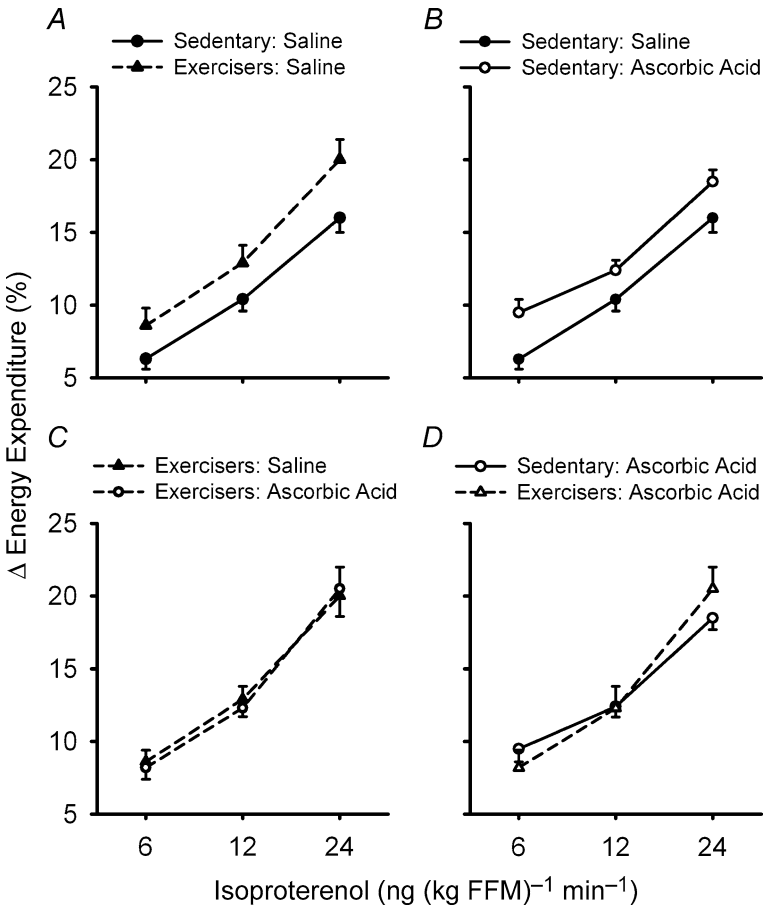

Figure 2. The increase in energy expenditure above resting metabolic rate in response to incremental β-adrenergic receptor stimulation (isoproterenol) in habitually exercising and sedentary adults, with and without co-administration of ascorbic acid
A, the magnitude of increase in energy expenditure above resting metabolic rate (RMR) in response to incremental β-adrenergic receptor (β-AR) stimulation with isoproterenol is greater in habitually exercising *versus* sedentary adults ($P = 0.02$). *B*, β-AR thermogenesis is augmented by acute ascorbic acid administration (0.04 g (kg fat-free mass (FFM))$^{-1}$) in sedentary adults ($P = 0.01$). *C*, β-AR thermogenesis is unaffected by acute ascorbic acid administration in habitual exercisers ($P = 0.93$). *D*, β-AR thermogenesis is not different between sedentary adults and habitual exercisers during acute administration of ascorbic acid ($P = 0.86$).

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adults who regularly perform aerobic exercise compared with their healthy, but sedentary peers. We also show that β-AR-stimulated thermogenesis is augmented by ascorbic acid administration in sedentary adults, but not those who exercise, thus abolishing baseline differences. Overall these observations support the idea that the state of regular aerobic endurance exercise is associated with augmented thermogenic responsiveness to β-AR activation in humans, and that the mechanism may involve differences in ascorbic acid-sensitive ROS bioactivity.

Greater thermogenic responsiveness to β-AR stimulation in adults who exercise

The mechanisms underlying our novel observation of greater thermogenic responsiveness to β-AR stimulation in habitually exercising compared with sedentary healthy adults are unknown, but could involve differences in β-AR density, agonist affinity, and/or post-receptor signalling. To our knowledge no information is available on the effects of habitual exercise status on the increase in energy expenditure during β-AR stimulation. The present findings are, however, directionally consistent with reports of improved β-AR-mediated lipolytic responses in adipose tissue (tissue biopsies and microdialysis) following aerobic exercise training (De Glisezinski *et al.* 1998; Stich *et al.* 1999). Thus, information is needed on the cellular and molecular mechanisms responsible for the augmented thermogenic responsiveness to β-AR stimulation in habitually exercising adults.

Aside from potential differences in β-AR signalling, another key determinant of the augmented thermogenic responsiveness to β-AR stimulation in habitually exercising adults is mitochondrial function. It is well established that relative to sedentary adults habitual exercisers have greater mitochrondrial density, oxidative enzyme activities, and capacity for ATP production (Hawley, 2002). Accordingly, it is feasible that the augmented thermogenic responsiveness to β-AR stimulation in habitually exercising adults is independent of β-AR properties *per se*. Rather it is the superior 'metabolic machinery' of habitual exercisers that distinguishes their thermogenic response to β-AR stimulation from sedentary adults.

In contrast to metabolic responsiveness, in the present study the increases in heart rate to isoproterenol were similar in the sedentary and exercising subjects. However, this cannot be interpreted as indicating a selective influence of habitual exercise status on metabolic responsiveness to β-AR activation. Cardiovascular responsiveness to β-AR stimulation cannot be assessed under the conditions of the present study because the haemodynamic effects of systemic isoproterenol administration activate baroreflexes that, in turn, actively buffer heart rate via cardiac autonomic adjustments (Christou *et al.* 2003). This effect can mask group differences in cardiovascular responsiveness to isoproterenol that are apparent under conditions of ganglionic blockade, that blocks baroreflex signalling (White & Leenen, 1994).

Modulation of the thermogenic response to β-AR stimulation by ascorbic acid

We have established (Bell *et al.* 2003; Eskurza *et al.* 2004*a*,*b*) that the dose of ascorbic acid infused in the present study results in supraphysiological plasma concentrations known to scavenge superoxide anions (Frei *et al.* 1989; Jackson *et al.* 1998), and acutely reduces systemic markers of oxidative stress including plasma concentrations of oxidized low-density lipoprotein and isoprostanes (Bell *et al.* 2003, 2005). We have also demonstrated that this ascorbic acid infusion reverses vascular endothelial dysfunction associated with the sedentary state in older adults, while not affecting the preserved endothelial function observed in older exercising adults (Eskurza *et al.* 2004*b*). Considered along with the fact that ROS bioactivity/oxidative stress modulates cardiac responsiveness to β-AR stimulation (Mak & Newton, 2001, 2004), the restoration of thermogenic responsiveness to isoproterenol by ascorbic acid in the sedentary subjects in the present study is consistent with the concept that increased ROS bioavailability or action contributes to the lower baseline β-AR responsiveness seen in sedentary adults. These observations also suggest, in turn, that the lack of augmentation of β-AR-stimulated thermogenesis by ascorbic acid in the habitually exercising subjects may reflect tonically decreased ROS-related effects, as has been postulated previously (Adams *et al.* 2005; Kojda & Hambrecht, 2005).

Although we are unaware of any non-antioxidant properties that could explain our results, we recognize that it is possible that ascorbic acid influenced β-AR thermogenic responsiveness by another mechanism such as enhanced clearance/uptake of isoproterenol in sedentary but not habitually exercising subjects. We also cannot exclude a 'ceiling effect' whereby the higher baseline thermogenic responsiveness to β-AR stimulation in the exercising subjects prohibited further augmentation by ascorbic acid (or any other potential modulating factor). Finally, it is possible that the group differences in the effect of ascorbic acid infusion on β-AR stimulation of metabolic rate may have been influenced by differences in the plasma concentrations of ascorbic acid achieved. However, this is unlikely given that we have shown previously that sedentary and habitually exercising adults attain identical plasma concentrations of ascorbic acid during the same infusion as used in the present study (Eskurza *et al.* 2004*b*). Despite these caveats, however, taken together we believe that these observations reasonably support the idea

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that the smaller isoproterenol-mediated increase in EE in the sedentary state may be linked to oxidative stress in some or all tissues responsive to β-AR stimulation. The mechanisms by which acute ascorbic acid administration could exert an antioxidant effect and restore thermogenic responsiveness in sedentary adults remain to be discerned. Translocation of β-ARs to the cell membrane, increased agonist affinity for the receptor, enhanced G-protein activation of adenylyl cyclase, increased cyclic AMP production, and activation of downstream signalling are all possible redox-sensitive steps in the β-AR signalling cascade that could have been influenced by ascorbic acid (Kaneko *et al.* 1991; Vatner *et al.* 1993; Persad *et al.* 1997, 1998*a*,*b*, 1999; Nishizawa *et al.* 2004). Ascorbic acid may also act in mitochondria to reduce superoxide production and this, in turn, may improve electron transport chain activity, oxidative phosphorylation, and the ability of mitochondria to increase substrate metabolism in response to β-AR stimulation (Sharma & Mongan, 2001).

Physiological and clinical implications

The results of the present study have implications for EE under conditions of sympathoadrenal system β-AR activation. For example, acute energy intake is associated with significant postprandial sympathetic nervous system activation and release of adrenaline (epinephrine) from the adrenal medulla (Jones *et al.* 2004). Indeed, up to 30–40% of the thermic effect of food is thought to be mediated by sympathoadrenal activation and stimulation of β-AR thermogenesis (Tappy *et al.* 1986). Recently we have shown that the thermic effect of food is greater in habitually exercising compared with sedentary adults despite similar postprandial sympathetic activation and increases in circulating adrenaline (Jones *et al.* 2004). Thus, it is possible that greater thermogenic responsiveness to β-AR stimulation could contribute to the greater thermic effect of food observed in regularly exercising adults. It is unknown if the increased metabolic responsiveness to β-AR stimulation in habitually exercising adults modulates physical activity-related EE, particularly during types and intensities of exercise associated with marked, sustained sympathoadrenal system activation.

Summary and conclusions

In summary, our results demonstrate that thermogenic responsiveness to β-AR stimulation is augmented in adults who perform regular aerobic exercise compared with their sedentary peers. The mechanism is ascorbic acid dependent, suggesting that it may be linked to low tonic ROS bioactivity (oxidative stress) in the habitually exercising state. These findings advance a novel mechanism by which habitual aerobic endurance exercise may modulate EE in humans, with potential implications for energy balance and the regulation of body weight.

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