

Catecholamine Reuptake Inhibition Causes Weight Loss by Increasing Locomotor Activity and Thermogenesis

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Bupropion (BUP) is a dopamine (DA) and norepinephrine (NE) reuptake inhibitor that causes mild weight loss in obese adults. Subchronic (7 day) coadministration of selective DA and NE reuptake inhibitors also causes weight loss in mice. Because weight loss was not associated with decreased caloric intake, subchronic BUP might cause weight loss through increased energy expenditure. Acute studies demonstrate that BUP or DA + NE reuptake inhibitors cause transient hypophagia and increased locomotion; though the effects on temperature are inconsistent. Because subchronic DA + NE reuptake inhibition does not affect appetite, there is clearly a difference between the acute and subchronic effects of DA + NE reuptake inhibitors; however the effects of chronic (or subchronic) BUP on energy balance have never been directly studied in an animal model. Therefore, the acute and subchronic effects of BUP or selective DA and NE reuptake inhibitors on food intake, body weight, locomotor activity, and interscapular temperature were determined in mice. Generally, selective inhibition of DA reuptake (by GBR12783) increased activity while selective inhibition of NE reuptake (by nisoxetine, NIS) decreased activity and temperature. BUP increased activity and temperature but subchronic BUP did not significantly reduce body weight due to a compensatory increase in food intake. Subchronic DA + NE reuptake inhibitor coadministration mimicked the effect of BUP on activity and temperature, but caused weight loss because daily food intake was not increased. The results of this study suggest that the mild weight loss effect of BUP in humans may be due to increased locomotion or heat production. More importantly, inhibition of DA + NE reuptake (with GBR + NIS) increased energy expenditure without a compensatory increase in food intake, supporting a role for novel combination catecholamine reuptake inhibitors in pharmacotherapy for obesity.

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INTRODUCTION

Bupropion (BUP) is currently approved for the treatment of depression (Wellbutrin[®]) and as an aid in smoking cessation (Zyban[®]) (Ascher *et al*, 1995; Foley *et al*, 2006). Originally associated with a 'lack of weight gain', BUP was recently shown to cause mild (5%) weight loss in clinical trials with obese human subjects (Gadde and Xiong, 2007). Although BUP is undergoing further clinical studies as a pharmacotherapy for obesity, there are no controlled comprehensive animal studies that assess how chronic BUP affects energy balance.

Like many antidepressants and the only centrally acting, FDA-approved long-term obesity treatment, sibutramine (Meridia[®]), BUP inhibits biogenic amine plasma membrane reuptake transporters (Ascher *et al*, 1995; Stahl *et al*, 2004; Kaplan, 2005). By selectively inhibiting the reuptake transporters for dopamine (DA) and norepinephrine (NE), BUP impinges on the primary means for clearance of

extracellular catecholamines (Hoffman *et al*, 1998). Through blockade of the DA and NE transporters, BUP causes acute increases in interstitial catecholamine concentrations and increased feedback inhibition of presynaptic neurons (Ascher *et al*, 1995; Dong and Blier, 2001).

BUP reduces food intake when administered acutely to rodents (Zarrindast and Hosseini-Nia, 1988; Billes and Cowley, 2007). Because acute coadministration of selective DA + NE reuptake inhibitors produces an additive inhibitory effect on food intake in mice, both catecholamines probably contribute to the acute hypophagic effect of BUP. Both DA and NE also appear to contribute to the weight loss effect of BUP that has been documented in humans, as subchronic (7 day) coadministration of DA + NE reuptake inhibitors also causes weight loss in mice (Billes and Cowley, 2007). Interestingly, weight loss caused by subchronic DA + NE reuptake inhibition in mice occurred in the absence of a reduction in food intake, which is also similar to the observation that chronic BUP administration does not affect caloric intake in humans (Griffith *et al*, 1983; Harto-Truax *et al*, 1983). This suggests that catecholamine reuptake inhibition may cause weight loss by increasing energy expenditure.

Current data in rodents indicate that acute BUP may increase energy expenditure by increasing temperature

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(Liu *et al*, 2002, 2004). Because reports on the acute effects of BUP on temperature are inconsistent, further research is needed to determine if increased thermogenesis might also contribute to increased energy expenditure by BUP (Zarrindast and Abolfathi-Araghi, 1992; Liu *et al*, 2002; Hasegawa *et al*, 2005). We and others have shown that acute peripheral BUP also dose dependently stimulates locomotor activity in rodents, an effect that is consistent with inhibition of the DA transporter (Soroko *et al*, 1977; Cooper *et al*, 1980; Nielsen *et al*, 1986; Zarrindast and Hosseini-Nia, 1988; Vassout *et al*, 1993; Redolat *et al*, 2005; Mitchell *et al*, 2006).

Obesity is generally regarded as a chronic disease requiring continuous intervention to maintain low body weight (Appolinario *et al*, 2004; Ioannides-Demos *et al*, 2005). Chronic (5–21 days) treatment with drugs like BUP can affect receptor expression, intracellular signaling mechanisms, and transporter expression and activity (Frazer and Benmansour, 2002). Thus, chronic BUP treatment can result in behavioral tolerance and/or sensitization such that the acute effects of BUP on energy balance may differ from BUP's chronic effects. Studies that address how chronic DA + NE reuptake inhibition affect energy intake and expenditure are necessary to determine how long-term drug administration affects energy balance.

There is a conspicuous absence of data on the comprehensive effects of chronic BUP treatment on energy balance in animal models. It is also unclear how DA and NE contribute to the possible metabolic effects of BUP, as no studies have examined how chronic administration of selective DA or NE reuptake inhibitors affects energy expenditure. Studies comparing the effects of BUP with that of selective DA or NE reuptake inhibitors on multiple measures of energy balance (such as food intake, locomotor activity, thermogenesis, and body weight) are necessary for a complete analysis of the effects of drugs like BUP on energy balance. The purpose of the present study was to examine the acute and chronic effects of DA + NE reuptake inhibition on energy balance in mice, particularly to shed light on the mechanism of weight loss by drugs like BUP. To this aim, we first investigated the effects of acute administration of BUP or selective DA and NE reuptake inhibitors on locomotor activity and interscapular temperature. We then investigated the effects of subchronic (7 day) BUP or selective DA and NE reuptake inhibitors on locomotor activity, interscapular temperature, daily food intake, and daily body weight.

MATERIALS AND METHODS

Animal Care and Housing

All animal procedures were approved by the Oregon National Primate Research Center Institutional Animal Care and Use Committee. Adult male C57Bl/6J mice (Jackson Labs, Bar Harbor, ME) were individually housed under a 12 h light/dark cycle and constant temperature ($22 \pm 1^\circ\text{C}$). Food and water were available *ad libitum*, unless specified otherwise. Mice were maintained on standard chow (Purina Lab Chow, no. 5001). All surgical procedures were performed under isoflurane anesthesia using aseptic surgical procedure.

Drugs

Drugs were prepared fresh on day of use. For intraperitoneal (i.p.) administration, BUP (Sigma, St Louis, MO) and nisoxetine (NIS; Tocris, Ellisville, MO) were dissolved in sterile nonpyrogenic 0.9% NaCl. GBR12783 (GBR; Tocris) and GBR + NIS were dissolved in 10% dimethyl sulfoxide and saline. All acutely administered drugs were given i.p. in a volume of 0.1 ± 0.02 ml (according to body weight). Control mice received vehicle in a corresponding volume. For subchronic administration (via Alzet[®] minipumps), NIS was dissolved in 10% dimethyl sulfoxide and sterile nonpyrogenic saline. BUP and GBR were dissolved in 50% dimethyl sulfoxide and 50% sterile H₂O. Previously published data were used to determine drug doses that would have a moderate effect on energy balance (Billes and Cowley, 2007).

Telemetric Transponder Implantation and Locomotor Activity and Temperature Measurement

Remote biotelemetry was performed using precalibrated sensitive transmitters (PDT-4000 G2 E-Mitter[®] sensors, Mini Mitter Company, Sun River, OR). Under isoflurane anesthesia, E-Mitters were implanted beneath the interscapular brown adipose tissue (IBAT) pad between the scapulae, and wounds were closed with sutures. Mice were allowed 1-week recovery before studies commenced. Signals emitted by the E-Mitter transmitters were detected by a receiver positioned underneath the animal's home cage and converted into activity counts (arbitrary units) by VitalView[®] software (Mini Mitter) (Harkin *et al*, 2002). Locomotor activity counts are a relative measure of gross motor activity. For all experiments, activity counts and interscapular temperature measurements were taken every 6 min.

Acute Drug Administration Studies

Mice were habituated to the behavioral testing paradigm by daily i.p. injection of 0.1 ml sterile saline and 16 h (overnight) fasting every third day for at least 2 weeks. Then mice were implanted with interscapular PDT-4000 E-Mitter sensors, allowed to recover, and habituated for 2 weeks before beginning feeding studies. All mice were assigned to treatment groups balanced for body weight. On the morning of testing, 16 h fasted mice received i.p. injection of freshly prepared drug or vehicle, were returned to their cage, and given six pre-weighed food pellets. As a positive control for drug efficacy, food was weighed at 1, 2, and 4 h post injection.

Subchronic Drug Administration Studies

Because chronic drug administration studies usually encompass 2–3 weeks of drug treatment, we have termed our abbreviated 7-day chronic study as subchronic. We were limited to 7 days by drug solubility; extending the study further would require changing minipumps after each week of treatment, potentially confounding results. Because of these technical complications, we chose to conduct a week-long subchronic study. Mice were implanted with

interscapular PDT-4000 G2 E-Mitter[®] sensors and allowed to recover for 1 week. They were then divided into weight-matched groups and implanted with subcutaneous Alzet mini-osmotic pumps (Durect Corporation, Cupertino, CA) that delivered drug for 7 days. Osmotic pumps were implanted via a small incision in the skin 1 cm caudal to the scapulae and slightly lateral to the vertebrae. The incision was closed with wound clips and mice were monitored daily. Mice in the subchronic BUP study were implanted with one minipump (model no. 2001) that contained either BUP (10 mg/kg/h) or vehicle. To minimize possible drug interactions, mice in the GBR + NIS coadministration study received two minipumps that contained either NIS (1.5 mg/kg/h) or vehicle (model no. 1007D) and GBR (2.5 mg/kg/h) or vehicle (model no. 2001). Food intake (corrected for spillage) and body weights were recorded daily at 1100 hours. The average initial body weight for animals in subchronic BUP studies was 27.0 g. The average initial body weight for animals in subchronic GBR + NIS studies was 24.5 g.

Statistical Analysis

Data sets were analyzed using Prism Software (Graph-Pad Software, San Diego, CA). For locomotor activity, area under the curve (AUC) was calculated by trapezoid analysis. For acute studies, one-way analysis of variance (ANOVA) and Bonferroni's multiple comparison post-tests were used to determine significant differences in AUC locomotor activity, average interscapular temperature, and food intake between vehicle- and drug-treated animals at different post-injection time intervals. For studies in Figures 1 and 2, vehicle-treated animals were pooled within their respective experiments. For subchronic drug administration studies, average daily locomotor activity was calculated as the average of AUC (counts/12 h) for either light or dark phase on days 2–7. Average interscapular temperature was calculated as the average temperature during either the light or dark phase for days 2–7. Student's *t*-test was used to determine significant differences in average activity or temperature between vehicle- and BUP-treated animals in either the light or dark phase. One-way ANOVA followed by Bonferroni's multiple comparison post-tests was used to determine significant differences in average light- or dark-phase activity or temperature between vehicle-, NIS-, GBR-, or GBR + NIS-treated animals. Two-way ANOVA followed by Bonferroni's post-tests was used to compare differences in average daily AUC locomotor activity, average temperature, daily food intake, and body weight between vehicle- and drug-treated animals across time (days 1–7). Data are expressed as mean and the standard error of the mean (SEM) except where noted. *P*-values of <0.05, <0.01, and <0.001 were considered significant.

RESULTS

Acute BUP Caused Mild Transient Hypothermia Followed by Mild Hyperthermia

We have previously shown that acute BUP causes a dose-dependent and transient increase in locomotor activity in mice that is concurrent with a transient inhibitory effect on

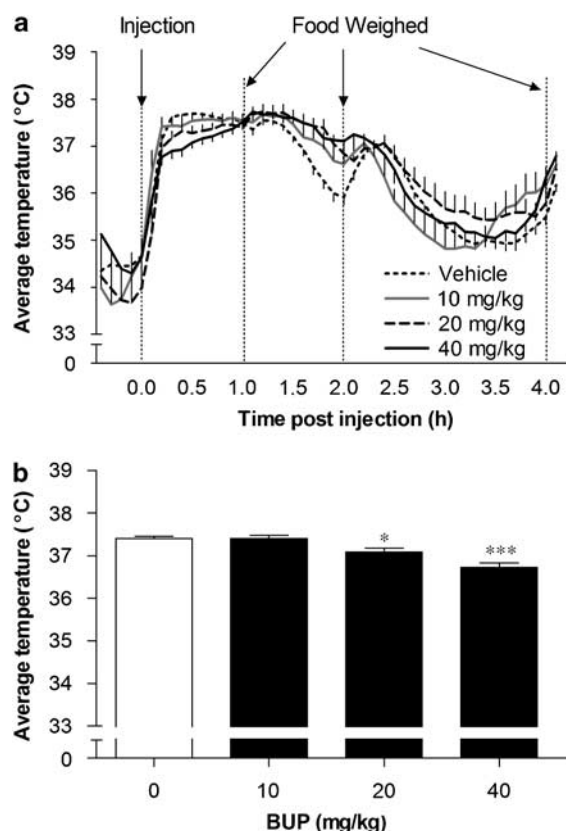


Figure 1 Effect of acute bupropion (BUP) on interscapular temperature. Overnight food-deprived mice were treated with BUP (10, 20, or 40 mg/kg i.p.) or vehicle and food was returned to the cage. (a) Time course for interscapular temperature for BUP-treated mice and control. (b) BUP (20 and 40 mg/kg) significantly reduced 1 h temperature compared to vehicle (**P* < 0.05, ****P* < 0.001 for treatment group vs control).

food intake up to 1 h post injection (Billes and Cowley, 2007). The time course for average interscapular temperature following 10, 20, and 40 mg/kg BUP is shown in Figure 1a (*n* = 8, animals/treatment group; *n* = 22, vehicle-treated animals). BUP had a significant effect on average interscapular temperature during the 60 min following injection ($F(3, 446) = 16.40$, $P < 0.001$). BUP (20 and 40 mg/kg) significantly decreased average interscapular temperature compared to control (Figure 1b). However, between 1 and 2 h post injection, 10, 20, and 40 mg/kg BUP prevented the decrease in average interscapular temperature in control animals by 0.4, 0.6, and 0.64 °C ($P < 0.01$), respectively ($F(3, 446) = 21.96$, $P < 0.001$). Only 20 mg/kg increased average temperature 2–4 h post injection by 0.3 °C ($F(3, 916) = 5.22$, $P = 0.0014$; 20 mg/kg, $P < 0.01$).

Acute Coadministration of Selective DA and NE Reuptake Inhibitors Increased Activity and Caused Acute Mild Hypothermia Followed by Hyperthermia

As has been previously reported, NIS (4 mg/kg), GBR (7.5 mg/kg), and NIS (4 mg/kg) + GBR (7.5 mg/kg) all significantly reduced food intake at 1 h post injection ($F(3, 32) = 17.53$, $P < 0.001$), but not at any later time points (data not shown). There was a significant effect of treatment on locomotor activity at 1 h post injection ($F(3, 32) = 10.29$,

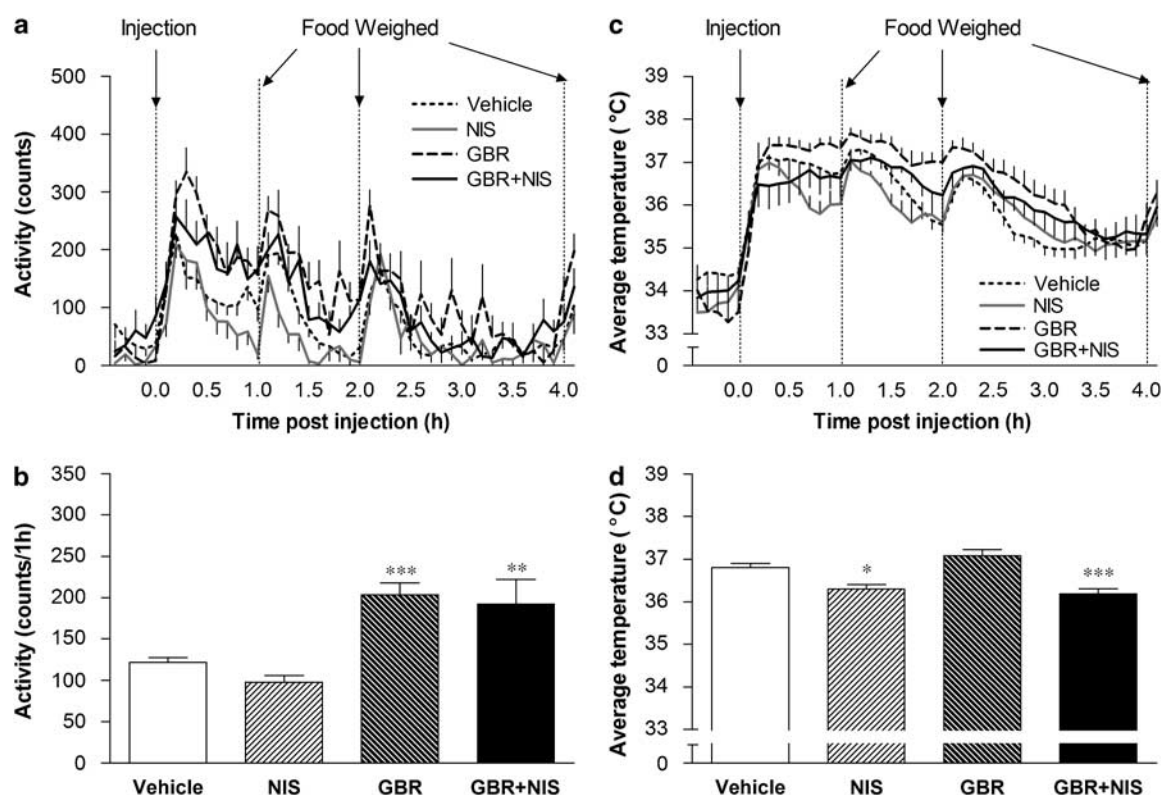


Figure 2 Effect of acute inhibition of DA and NE reuptake on locomotor activity and interscapular temperature. Overnight food-deprived mice were treated with nisoxetine (NIS, 4 mg/kg), GBR 12783 (GBR, 7.5 mg/kg), NIS (4 mg/kg) + GBR (7.5 mg/kg) or vehicle and food was returned to the cage. (a) Time course for activity following drug or vehicle injection. (b) AUC for locomotor activity from 0 to 1 h post injection. (c) Time course for interscapular temperature. (d) Average interscapular temperature from 0 to 1 h post injection for all treatment groups (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ for treatment group vs control).

$P < 0.001$; $n = 5-7$, animals/treatment group; $n = 18$, vehicle-treated animals). Inhibition of DA reuptake with GBR and GBR + NIS significantly increased activity at 1 h post injection (Figure 2a and b). GBR also significantly increased locomotor activity from 1 to 2 h post injection ($F(3, 32) = 10.24$, $P < 0.001$) (GBR; $P < 0.01$ data not shown). There was no significant effect of any treatment on locomotor activity from 2 to 4 h post injection.

There was a significant effect of treatment on average interscapular temperature at 1 h post injection ($F(3, 356) = 9.40$, $P < 0.001$), 2 h post injection ($F(3, 356) = 21.64$, $P < 0.001$), and 4 h post injection ($F(3, 716) = 15.84$, $P < 0.001$). Inhibition of NE reuptake by both NIS and GBR + NIS significantly decreased average interscapular temperature from control between 0 and 1 h post injection (Figure 2d). However, between 1 and 2 h post injection, only NIS-treated animals were hypothermic by 0.36°C compared to control animals ($P < 0.05$; data not shown). Between 1–2 and 2–4 h post injection, DA reuptake inhibition by GBR or GBR + NIS increased average temperature by 0.76 and 0.44°C at 2 h, and 0.64 and 0.5°C at 4 h ($P < 0.001$; data not shown).

Subchronic DA + NE Reuptake Inhibition by BUP Increased Activity and Temperature

Average hourly light- and dark (shaded)-phase activity from day 3 is presented in Figure 3a to illustrate the effect

of subchronic BUP infusion on daily locomotor activity. To better separate the acute and chronic effects of BUP, average daily locomotor activity or interscapular temperature was calculated as the average of days 2–7. On days 2–7, BUP significantly increased average light- ($t(64) = 3.99$, $P = 0.0002$) and dark-phase activity ($t(64) = 2.41$, $P = 0.019$) (Figure 3b). On day 1 of infusion, BUP (10 mg/kg/h; $n = 5$) caused an acute threefold increase in light-phase activity compared to vehicle ($n = 4$) (Figure 3c). BUP had a significant main effect on locomotor activity during the light phase ($F(1, 42) = 20.46$, $P < 0.0001$) (Figure 3c) but not the dark phase (Figure 3d). Overall, BUP treatment caused a 20% increase in cumulative locomotor activity at day 7 ($t(22) = 9.419$, $P < 0.0001$) (data not shown).

Figure 3e is a representative trace of average hourly temperature of subchronic vehicle- or BUP-treated animals on day 3 of infusion. Compared to vehicle, subchronic BUP administration significantly increased average interscapular temperature during both the light phase by $0.63 \pm 0.03^{\circ}\text{C}$ compared to control ($t(64) = 6.12$, $P < 0.0001$) and dark phase by $0.19 \pm 0.04^{\circ}\text{C}$ compared to control ($t(64) = 4.56$, $P < 0.0001$) (Figure 3f). BUP significantly increased interscapular temperature on days 1, 2, and 3, and had a significant main effect on interscapular temperature during the light phase ($F(1, 42) = 17.35$, $P = 0.0042$) (Figure 3g) but not the dark phase (Figure 3h). BUP infusion did not significantly affect body weight, although there was a trend toward 2–3% weight loss compared to control (Figure 4a).

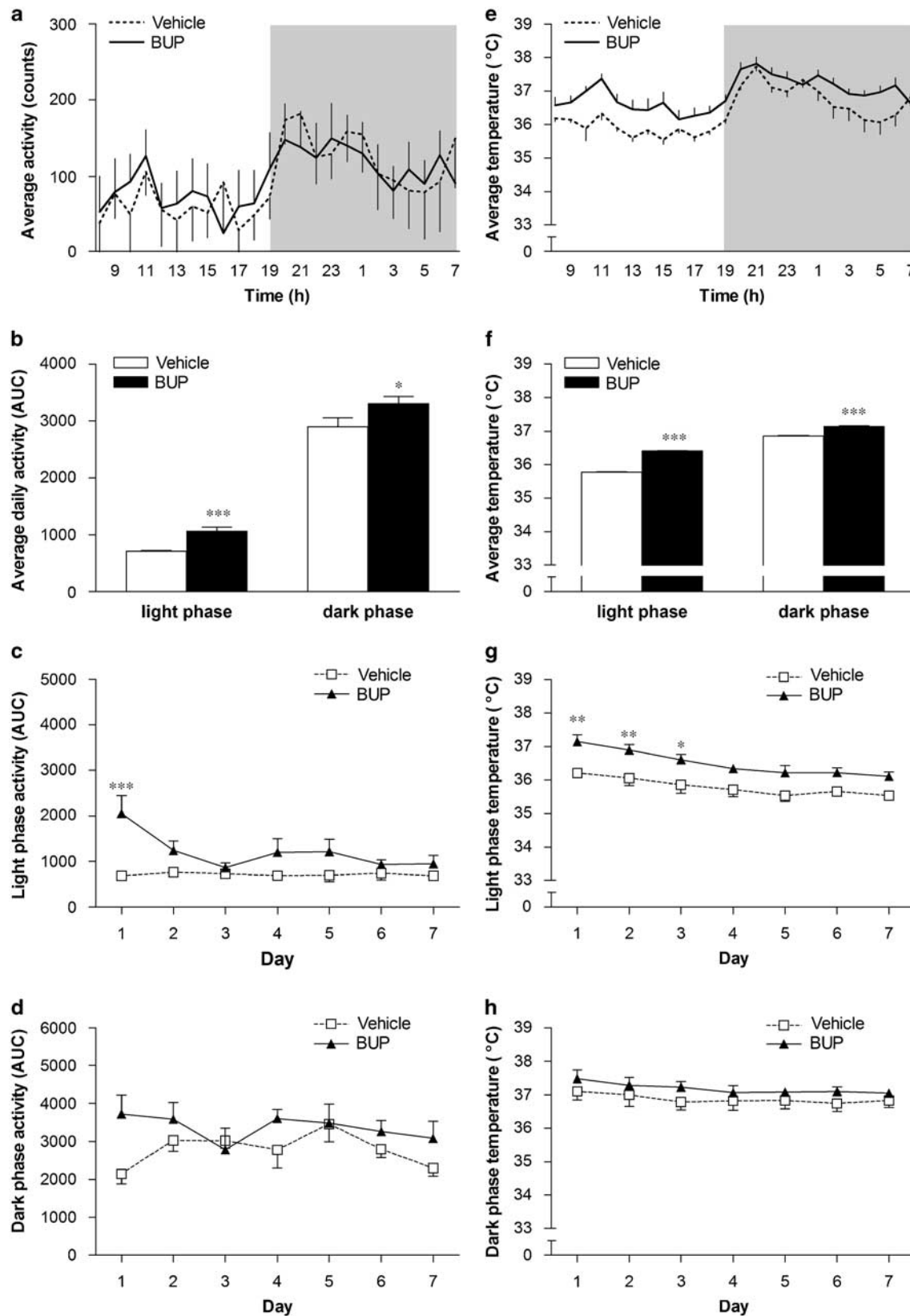


Figure 3 Subchronic bupropion (BUP) infusion increases locomotor activity and interscapular temperature. Adult mice were implanted with mini-osmotic pumps delivering either vehicle or BUP (10 mg/kg/h) for 7 days. (a) Representative trace of hourly activity (taken from day 3) for vehicle- and BUP-treated animals. (b) Average of area under the curve (AUC) values for locomotor activity from days 2 to 7 during the 12 h light and 12 h dark phases. (c) AUC of light-phase locomotor activity. (d) AUC of dark-phase locomotor activity. (e) Representative trace of hourly interscapular temperature (taken from day 3) for BUP- or vehicle-treated animals. (f) Average temperature for the 12 h light and 12 h dark phase from days 2 to 7 of infusion. (g) Average daily light-phase temperature. (h) Average daily dark-phase temperature (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ for treatment group vs control).

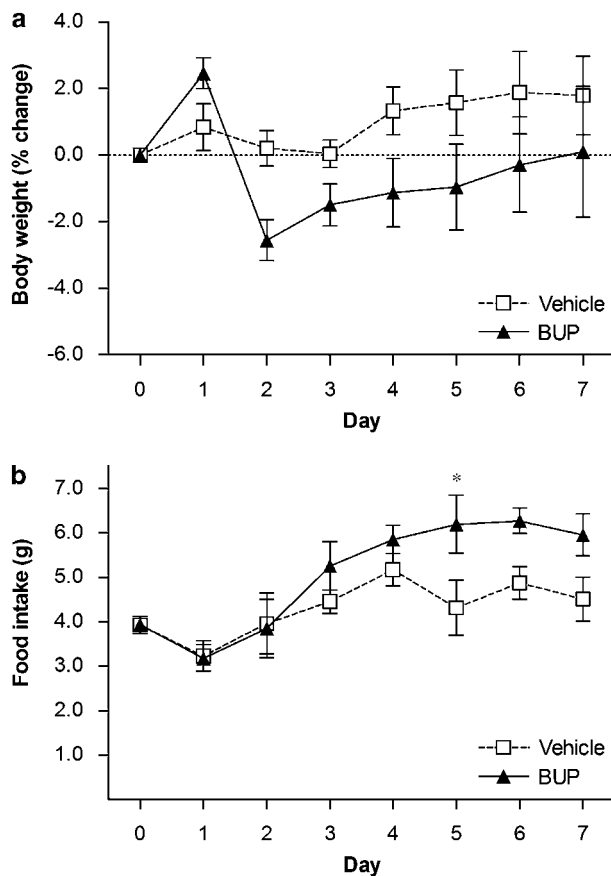


Figure 4 Effect of subchronic bupropion (BUP) administration on daily body weight and food intake. Adult mice received either subchronic BUP (10 mg/kg/h) or vehicle infusion via mini-osmotic pump. (a) Effect of BUP on daily body weight. (b) Effect of BUP on daily food intake (* $P < 0.05$ for treatment group vs control).

There was no main effect of BUP on daily food intake, however there was a significant increase in food intake in BUP-treated animals on day 5 (Figure 4b). Cumulative food intake at day 7 was increased by 17% in BUP-treated animals ($t(52) = 3.14$, $P = 0.0028$) (data not shown).

Subchronic Coadministration of Selective DA and NE Reuptake Inhibitors Increased Activity and Temperature and Caused Weight Loss

Figure 5a is a representative trace of the effect of subchronic selective DA and NE reuptake inhibitors on locomotor activity on day 3 of infusion. During days 2–7 of infusion, there was a significant effect of treatment on average locomotor activity during the light ($F(3, 119) = 12.71$, $P < 0.0001$) and dark phases ($F(3, 119) = 18.26$, $P < 0.0001$) (Figure 5b). Post-tests revealed that GBR (2.5 mg/kg/h; $n = 3$ –4) and NIS (1.5 mg/kg/h) + GBR (2.5 mg/kg/h; $n = 4$)

significantly increased average locomotor activity from vehicle ($n = 5$) during the light phase. During the dark phase, NIS (1.5 mg/kg/h; $n = 5$) significantly decreased average locomotor activity and GBR significantly increased average locomotor activity. There was a significant main effect of treatment during the light phase on days 1–7 ($F(3, 78) = 11.16$, $P < 0.0001$) (Figure 5c). On day 1, GBR caused a fivefold increase in locomotor activity and GBR + NIS caused a threefold increase in locomotor activity compared to vehicle-treated animals during the light phase. Both treatments also doubled activity on day 2 in the light phase. In the dark phase, there was a significant main effect of treatment ($F(3, 78) = 9.527$, $P < 0.01$); GBR and GBR + NIS increased locomotor activity on day 1 only (Figure 5d). Overall, NIS treatment caused a 29% decrease in cumulative locomotor activity at day 7 and GBR and GBR + NIS caused a 58 and 28% increase in locomotor activity, respectively ($F(3, 13) = 11.29$, $P < 0.0006$) (data not shown).

Figure 5e is a representative trace of average interscapular temperature on day 3 of infusion. There was a significant effect of treatment on average interscapular temperature during the light ($F(3, 119) = 11.26$, $P < 0.0001$) and dark phase ($F(3, 119) = 11.04$, $P < 0.0001$) on days 2–7 of infusion (Figure 5f). During the light phase, NIS significantly decreased average interscapular temperature while GBR and GBR + NIS significantly increased average temperature. GBR + NIS caused a $0.38 \pm 0.04^\circ\text{C}$ increase in average light-phase temperature. NIS and GBR significantly decreased average interscapular temperature during the dark phase while GBR + NIS had no effect (Figure 5f). There was a significant main effect of treatment on interscapular temperature during the light phase on days 1–7 ($F(3, 78) = 25.55$, $P < 0.0001$) (Figure 5g). On days 1, 4, and 5, NIS significantly decreased temperature. GBR + NIS significantly increased light-phase temperature on days 2 and 3. There was no effect of treatment during the dark phase on days 1–7 (Figure 5h).

Only coadministration of catecholamine reuptake inhibitors significantly affected body weight; GBR + NIS significantly decreased body weight on days 4–6 of infusion (Figure 6a). There was also a significant effect of treatment on food intake ($F(3, 13) = 3.47$, $P = 0.048$) (Figure 6b). NIS significantly decreased food intake only on day 1 of infusion and GBR significantly increased food intake on day 4 of infusion. Although not statistically significant, GBR caused a 7% decrease in food intake. GBR + NIS and NIS treatment significantly affected cumulative food intake at day 7, causing a 10 and 19% decrease in food intake from control, respectively ($F(3, 13) = 5.00$, $P < 0.05$) (data not shown).

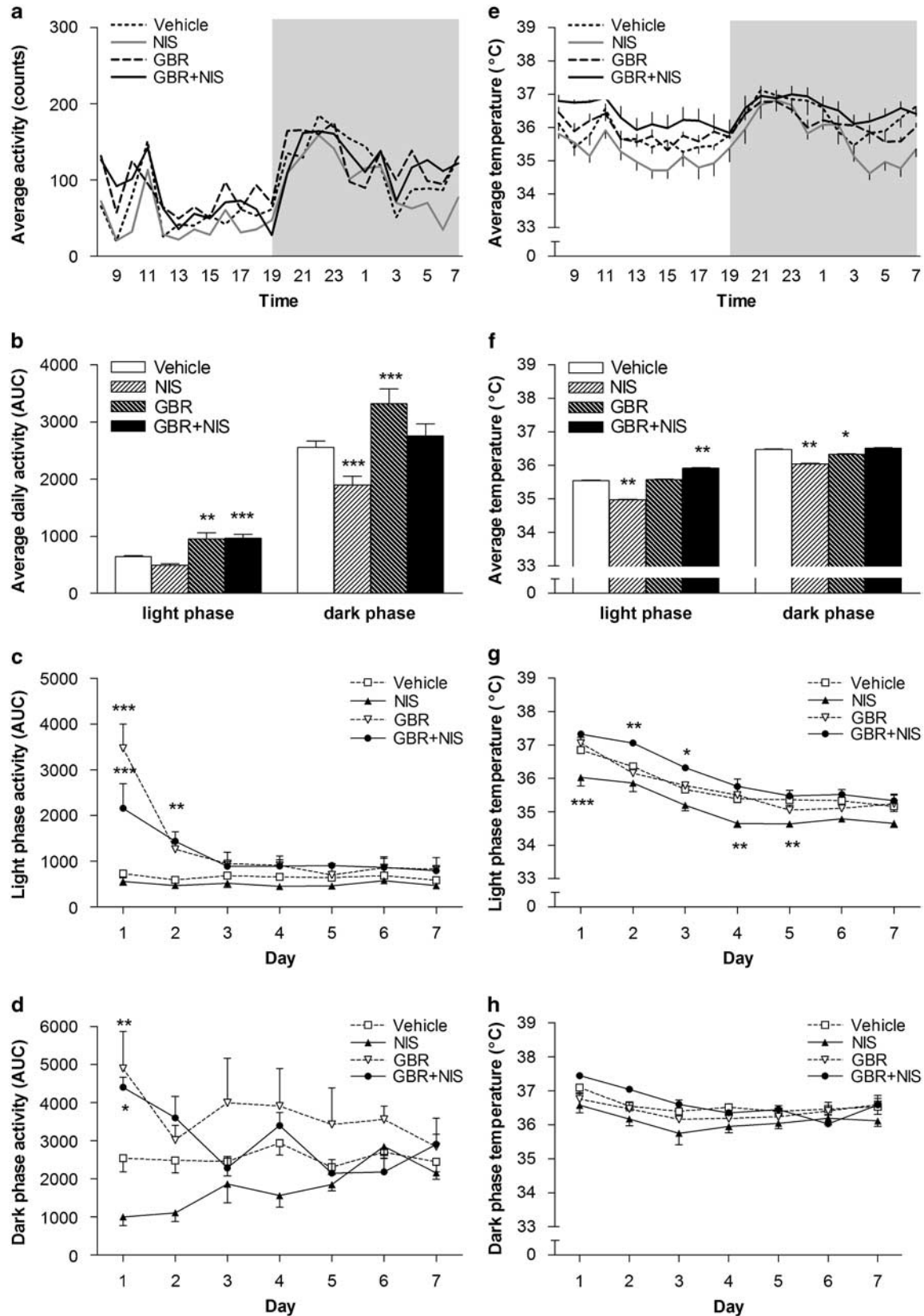
DISCUSSION

The present study demonstrates that subchronic (7 day) combined DA + NE reuptake inhibition causes weight loss

Figure 5 Effect of subchronic inhibition of DA and NE reuptake on locomotor activity and interscapular temperature. Adult male mice received vehicle or nisoxetine (NIS, 1.5 mg/kg/h) and vehicle or GBR12783 (GBR, 2.5 mg/kg/h) infusion mini-osmotic pump for 7 days. (a) Representative trace of hourly locomotor activity (taken from day 3) during subchronic infusion of NIS, GBR, GBR + NIS, and vehicle-treated animals. SEM not included for clarity. (b) Average area under the curve (AUC) values for locomotor activity from days 2 to 7 of infusion during the 12 h light and 12 h dark phases. (c) AUC of light-phase locomotor activity. (d) AUC of dark-phase locomotor activity. (e) Representative hourly interscapular temperature (taken from day 3) in NIS, GBR, GBR + NIS, and vehicle-treated animals. (f) Average temperature for the 12 h light and 12 h dark phases on days 2–7 of infusion. (g) Average light-phase temperature. (h) Average dark-phase temperature (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ for treatment group vs control).

by increasing locomotor activity and interscapular temperature and not by inhibiting food intake. Combined catecholamine reuptake inhibition with either BUP or

GBR+NIS increased both activity and temperature, but BUP did not cause significant weight loss owing to a compensatory increase in food intake. Because the effect of



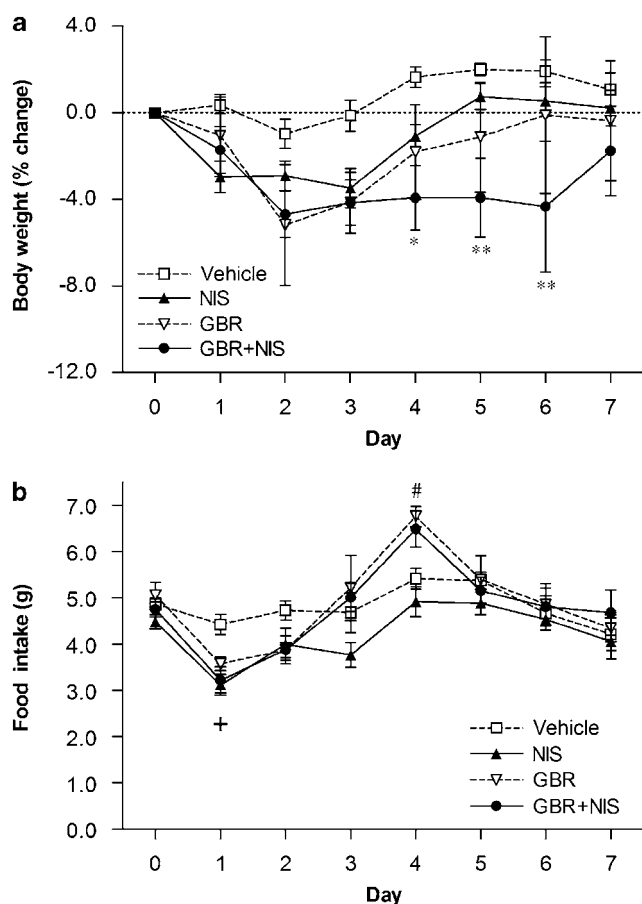


Figure 6 Effect of subchronic DA and NE reuptake inhibition on daily body weight and food intake. Adult mice received subchronic infusion of either nisoxetine (NIS, 1.5 mg/kg/h), GBR12783 (GBR, 2.5 mg/kg/h), NIS (1.5 mg/kg/h) + GBR (2.5 mg/kg/h), or vehicle. (a) Effect of NIS, GBR, GBR + NIS, or vehicle on daily body weight. (b) Effect of NIS, GBR, GBR + NIS, or vehicle on daily food intake (* $P < 0.05$, ** $P < 0.01$ for treatment group vs control; + $P < 0.05$ for NIS-treated animals vs control; # $P < 0.05$ for GBR-treated animals vs control).

combined selective DA + NE reuptake inhibitors on activity and temperature is different from the individual effects of these drugs, elevated energy expenditure is likely the result of an interaction between DA and NE systems. This study also provides new evidence that the weight loss observed with BUP treatment in obese humans is possibly the result of elevated energy expenditure by increased activity and thermogenesis caused by a combined dopaminergic and noradrenergic mechanism.

Body weight is determined by energy intake and energy expenditure. Energy expenditure can be further divided into basal metabolism, mechanical work (locomotor activity), and adaptive thermogenesis (Spiegelman and Flier, 2001). Thus, elevated locomotor activity and interscapular temperature probably caused increased energy expenditure. Pharmacological and genetic studies support this assertion. Elevated activity and temperature have already been demonstrated to account for increased energy expenditure caused by treatment with the weight loss drug sibutramine (Liu *et al*, 2002; Golozoubova *et al*, 2006). In mice on a high-fat diet, elevated activity and temperature prevent weight gain. However, mice lacking the melanocortin-4 receptor do

not increase activity and temperature in response to a high-fat diet and exhibit accelerated weight gain (Butler *et al*, 2001). This data set does not prove that the magnitude of the increase in interscapular temperature and locomotor activity regulates energy expenditure enough to produce changes in body weight. However, they provide a persuasive argument that this is the case. Changes in temperature and locomotor activity are in the appropriate qualitative direction, and in the case of BUP were sufficient to produce no net change in body weight, in spite of increased food intake. We cannot discount the formal possibility that increased interscapular temperature could be due to increased local blood flow, but the large increases in locomotor activity observed in this study would certainly cause an increase in energy use.

Remote biotelemetry allows for simultaneous and continuous measurement of locomotor activity and temperature in the animals' home cage, minimizing confounding variables and allowing for temporal data acquisition (Harkin *et al*, 2002). By using biotelemetry to measure mechanical work and adaptive thermogenesis in mice during both acute and chronic drug treatment, we were able to record the two types of energy expenditure that vary with energy state (Spiegelman and Flier, 2001).

Because E-Mitters were implanted underneath IBAT, the main organ for adaptive thermogenesis in small mammals, temperature fluctuations probably reflect activity of IBAT (Lowell and Spiegelman, 2000; Avram *et al*, 2005). The idea that BAT may be a viable target for obesity pharmacotherapy has already been proposed. Increasing BAT activity elevates metabolic rate and prevents diet-induced obesity in rodents, whereas genetic mutation and pharmacological inhibition of BAT can cause obesity (Spiegelman and Flier, 2001; Crowley *et al*, 2002). Because adaptive thermogenesis is believed to contribute to the decreased energy expenditure characteristic of obesity and caloric deficit (dieting), drugs that increase adaptive thermogenesis (by activating BAT) have been suggested as a possible means to achieve safe and sustained energy expenditure (Major *et al*, 2007).

Acute and chronically administered BUP has repeatedly been demonstrated to dose dependently increase locomotor activity in rodents with similar potency, regardless of species, age, or strain (Soroko *et al*, 1977; Nielsen *et al*, 1986; Zarrindast and Hosseini-Nia, 1988; Vassout *et al*, 1993; Redolat *et al*, 2005; Mitchell *et al*, 2006; Billes and Cowley, 2007). The current finding that BUP, DA, and DA + NE reuptake inhibition all transiently increased locomotor activity is consistent with the idea that inhibition of the DA transporter is sufficient to cause a significant short-term increase in locomotion and even reverse the decrease in activity caused by inhibition of NE reuptake. The transient increase in locomotor activity caused by subchronic BUP infusion is identical to the effect caused by subchronic infusion of various selective DA reuptake inhibitors (Izenwasser *et al*, 1999). A dopaminergic mechanism for increased locomotor activity by BUP is consistent with increased striatal DA concentrations in rats following acute BUP administration (Vassout *et al*, 1993), the failure of BUP to increase locomotor activity in rats with selective ablation of DA neurons (Cooper *et al*, 1980), and a recent report by Mitchell *et al* (2006) demonstrating that acute BUP

increases locomotor activity in NE transporter knockout mice. DA reuptake inhibition also accounts for increased locomotion by other mixed monoamine reuptake inhibitors including psychostimulants and the weight loss drug sibutramine (Missale *et al*, 1998; Izenwasser *et al*, 1999; Golozubova *et al*, 2006; Mitchell *et al*, 2006). Thus, the finding that BUP increases locomotor activity is widely supported.

Acute combined DA + NE reuptake inhibition, either with BUP or coadministration of selective DA + NE reuptake inhibitors, caused a brief decrease in interscapular temperature. The only other study examining the effects of BUP on temperature in mice reported that acute BUP causes rapid and pronounced hypothermia (Zarrindast and Abolfathi-Araghi, 1992). More recent studies in rats have demonstrated the opposite effect, instead showing that peripheral BUP causes a small increase in colonic and core temperature and also O₂ consumption (Liu *et al*, 2002, 2004; Hasegawa *et al*, 2005). Many variables may account for the conflicting acute effects of BUP on temperature. Factors such as species (mice *vs* rats), dose, route of administration, time of day, duration of temperature measurement, location of temperature measurement (core, peripheral, or IBAT), or ambient temperature could have influenced BUP's effects on temperature. Ambient temperature has been demonstrated to influence the effect of cocaine on body temperature (Lomax and Daniel, 1990). Similar to cocaine, BUP has little effect on temperature in humans in a thermoneutral environment, but may increase temperature in a warm environment due to a higher set point for hyperthermia and reduced compensatory heat loss (Griffith *et al*, 1983; Watson *et al*, 2005). Finally, because the current acute studies were performed in the light phase (when animals are less active and have lower body temperature) in fasted animals that were subsequently fed, these results may reflect an acute attenuation of the thermic effect of food by BUP, rather than a decrease in steady-state temperature (Lowell and Spiegelman, 2000). In fact, the mild but significant increase in temperature between 1 and 2 h post injection supports the larger collection of evidence that BUP, and perhaps its pharmacologically active metabolites, increase temperature (Ascher *et al*, 1995; Liu *et al*, 2002, 2004; Hasegawa *et al*, 2005).

NE released from sympathetic nerve terminals activates β_3 -adrenoceptors on brown adipocytes in BAT and leads to lipolysis, increased activity of uncoupling protein-1 (UCP-1), and thermogenesis (Avram *et al*, 2005; Fan *et al*, 2005). By blocking NE reuptake in sympathetic nerve terminals, an NE reuptake inhibitor could increase β_3 -adrenoceptor activation, thereby increasing oxidative phosphorylation and the production of heat (Iversen, 1971). In rats, antagonism of the β_3 -adrenoceptor attenuates the increased O₂ consumption caused by BUP (Liu *et al*, 2004) and the weight loss drug sibutramine (Connoley *et al*, 1999), suggesting that BAT activation accounts for a significant proportion of the increased energy expenditure with these drugs. The current finding that selective NE reuptake inhibition decreased interscapular temperature emphasizes that additional dopaminergic (with BUP treatment) or serotonergic (with sibutramine treatment) input is important to maintain increased sympathetic tone and cause thermogenesis.

Because thermogenesis through BAT activation is a metabolic regulatory process that is controlled by the hypothalamus via descending sympathetic fibers (Lowell and Spiegelman, 2000), catecholamine reuptake inhibitors may act both centrally and peripherally to influence interscapular temperature (Wellman, 2005). BUP has been shown to increase DA and NE in hypothalamic nuclei that regulate body temperature such as the preoptic area and anterior hypothalamus (Hasegawa *et al*, 2005). Catecholamine reuptake inhibitors may also affect activity of cells in the hypothalamic melanocortin system, which regulates caloric intake and metabolic rate in response to energy availability (Fan *et al*, 2005; Ramos *et al*, 2005). For example, activation of the dopamine D2 receptor increases expression of anorexic pro-opiomelanocortin mRNA and decreases expression of orexigenic neuropeptide Y mRNA within the arcuate nucleus of the hypothalamus (Pelletier and Simard, 1991; Tong and Pelletier, 1992). By increasing extracellular DA, DA reuptake inhibitors could indirectly increase D2 receptor activation and affect activity of neurons in the melanocortin system.

Part of the efficacy of drugs like BUP is that they affect multiple systems, sometimes resulting in favorable drug interactions that could not be predicted based on the individual effects of selective DA and NE reuptake inhibitors (Kaplan, 2005). An example of a drug interaction that was not predicted is the effect of subchronic DA + NE reuptake coadministration on IBAT temperature. During the light phase, subchronic DA + NE reuptake inhibition caused an average 0.4°C increase in IBAT temperature, even though temperature was essentially unaffected by DA reuptake inhibition and decreased by NE reuptake inhibition. Although adult humans do not have defined peripheral BAT deposits, recent evidence shows that brown adipocytes may be dispersed within white adipose tissue deposits. Additional studies linking human obesity to β_3 -adrenoceptor and UCP-1 genetic polymorphisms suggest a role for BAT in energy expenditure in humans (Avram *et al*, 2005).

This study emphasizes that although acute studies may infer long-term drug effects, they do not necessarily predict a drug's chronic effects. Even though the chronic studies presented here were limited (by method of administration and drug solubility) to 1 week of treatment, they were sufficient to illustrate differences between the acute and subchronic effects of catecholamine reuptake inhibitors. Further studies assessing the effects of chronic (2–3 week) drug treatment could potentially offer a more comprehensive analysis of the chronic effects of catecholamine reuptake inhibitors on energy balance, but may be confounded by different methods of drug administration. Even daily peripheral BUP injection may not produce the same neuronal adaptations as chronic BUP infusion via minipump, which produces more stable drug and metabolite levels (Ferris and Beaman, 1983; Klimek *et al*, 1985; Ascher *et al*, 1995). Increasing evidence suggests that the cellular and behavioral effects of chronically administered catecholamine reuptake inhibitors and psychostimulants also depend on whether chronically administered drugs are delivered via daily bolus injection or continuous infusion (Ansah *et al*, 1996; Davidson *et al*, 2005). Because previous studies on the cellular effects of chronic BUP were conducted in rodents that received BUP orally or via daily

injection, further studies examining how chronic infusion of BUP or selective DA + NE reuptake inhibitors via osmotic minipump affects neuronal signaling are necessary to begin to elucidate the most probable mechanism through which catecholamine reuptake inhibition affects energy balance.

It has been established *in vitro* and *in vivo* that BUP inhibits both the DA and NE transporters (Nomikos *et al*, 1989; Ferris and Cooper, 1993; Wellman, 2005). We previously demonstrated that coadministration of selective DA + NE reuptake inhibitors produces similar effects on food intake and body weight as BUP (Billes and Cowley, 2007). We extend our previous finding by demonstrating that coadministration of DA + NE reuptake inhibitors mimics the effects of BUP on locomotor activity and interscapular temperature. This study also corroborates the modest effect of BUP on body weight in humans. More importantly, we illustrate the individual effects of selective DA or selective NE reuptake inhibition on activity and interscapular temperature and demonstrate that coadministration of selective DA + NE reuptake inhibitors increased activity and temperature and caused weight loss in lean mice. It remains to be determined whether catecholamine reuptake inhibition would have a greater effect on energy balance in an obese rodent model, as has been suggested by acute studies with BUP, NIS, and GBR (Billes and Cowley, 2007) and also sibutramine (Strack *et al*, 2002). The results of this study shed light on the mechanism through which catecholamine reuptake inhibitors cause weight loss and provide additional insight into the role of catecholamines in regulation of energy balance. The relevance for humans is not that combined catecholamine reuptake inhibitors may actually increase energy expenditure, but rather that they may prevent diet-induced decreases in adaptive thermogenesis, thereby facilitating weight loss.

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DISCLOSURE/CONFLICTS OF INTEREST

Michael A Cowley is chief scientific officer of, and owns stock in, Orexigen Therapeutics Inc. A company that is developing pharmaceutical approaches to treat obesity, and is developing combination therapies that include a formulation of bupropion. The work described in this manuscript was not supported by Orexigen Therapeutics Inc.

OHSU and Cowley have a significant financial interest in Orexigen Therapeutics Inc., a company that may have a commercial interest in the results of this research and technology. This potential conflict has been reviewed and managed by the OHSU Conflict of Interest in Research Committee and the Integrity Program Oversight Council.

Sonja K Billes has no conflicts of interest.

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