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NICOTINE TREATMENT DECREASES FOOD INTAKE AND BODY WEIGHT VIA A LEPTIN INDEPENDENT PATHWAY IN *PSAMMOMYS OBESUS*

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Running head: Nicotine induced weight loss in *Psammomys obesus*

Key Words: Nicotine, Israeli sand rat, leptin, obesity

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Abstract

It has previously been reported that leptin may be involved in nicotine’s ability to reduce body weight. Our aim was to investigate whether the anorexic action of nicotine is related to the actions of leptin by utilizing lean leptin-sensitive and obese leptin-resistant *Psammomys obesus* (*P. obesus*). Lean and obese *P. obesus* were assigned to receive nicotine sulphate at 6, 9 or 12mg day\(^{-1}\) or saline (control) for nine days (*n* = 6-10 in each group) administered using mini-osmotic pumps. Food intake, body weight, plasma leptin concentrations, plasma insulin and blood glucose were measured at baseline and throughout the study period. Nicotine treatment reduced food intake by up to 40% in lean and obese *P. obesus*. Plasma leptin levels fell significantly only in lean nicotine treated animals, whereas no changes were observed in obese nicotine treated animals. However, both lean and obese nicotine treated animals had similar reductions in body weight. Our results show that nicotine has dramatic effects on food intake and body weight, however these changes appear to be independent of the leptin signaling mechanisms.
Introduction

Numerous studies have demonstrated an inverse relationship between tobacco smoking and body weight (1-6). This action of smoking appears to be nicotine mediated as indicated by Hajek et al. (7) who demonstrated significantly lower weight gain in individuals using nicotine chewing gum for 12 months compared to alternative non-nicotine treatments.

Although not fully understood, several mechanisms have been proposed to account for the actions of nicotine in lowering body weight. Nicotine binding sites have been demonstrated in appetite-regulating regions of the hypothalamus (8) suggesting that centrally mediated actions contribute to increased energy expenditure during nicotine treatment (9). Recently Yoshida et al. (6) demonstrated that nicotine treatment also appeared to act peripherally by increasing mRNA and protein content of uncoupling protein 1 in both brown and white adipose tissue.

These actions of nicotine on both hypothalamic appetite centres and peripheral metabolism suggest a possible link between nicotine and the anorexic hormone, leptin. Leptin is known to be an important regulator of appetite, peripheral energy expenditure and fuel homeostasis (10, 11). Leptin is primarily secreted by adipocytes in proportion to adipose tissue mass, such that leptin levels are higher in obese compared to lean subjects, indicating leptin resistance (12). An epidemiological study demonstrated a link between the actions of nicotine on body weight and leptin, suggesting that smoking via nicotinic mechanisms may modify the sensitivity of leptin receptors (13).

*P. obesus* are desert rodents which when fed a standard laboratory diet a proportion of the animals remain lean while others develop varying degrees of obesity and diabetes (14) similar to
that observed in susceptible human populations (15). We have previously demonstrated that in *P. obesus* exogenous leptin infusion reduced bodyweight in lean animals (16), whereas obese animals were unaffected by leptin infusion suggesting a disruption in the leptin signaling pathway. We propose that if the actions of nicotine on weight loss are inter-connected with leptin signaling, diminished sensitivity to nicotine might be expected in obese *P. obesus*. To examine this relationship plasma leptin levels were measured in both lean leptin-sensitive and obese leptin-resistant *P. obesus* during mini-osmotic pump nicotine delivery.
Methods and Materials

Animals.

Mixed sex *P. obesus* were maintained on a 12 hr light-dark cycle at 21±1°C and fed *ad libitum* standard rat chow (Barastoc, Pakenham, Australia). One week prior to the study commencement, baseline measurements were taken including food intake, body weight, blood glucose, plasma insulin and plasma leptin concentrations (n=66). Using these baseline parameters 16 week old *P. obesus* were selected and divided into two groups, lean (n=37) and obese/insulin resistant (n=29). Within each group, animals were randomly subdivided to receive nicotine sulphate at 6, 9 or 12mg day⁻¹ or saline (sham controls) for nine days via mini-osmotic pumps (Alzet model 2002, CA) implanted subcutaneously between the scapulae under light anesthetization (sodium pentobarbitol, 60mg/kg). Animals were allowed to recover for 48 hours before daily bodyweight and food intake measurements were monitored. During the study period blood was taken for glucose, insulin and leptin measurements. All experimental procedures were performed according to institutional (Deakin University Animal Ethics Committee) guidelines.

Biochemical Analyses.

Blood was collected from the tail vein in heparinized microtubes and blood glucose determined using an automated enzymatic analyzer (2300 Stat Plus, Yellow Springs Instruments, Ohio). Plasma leptin levels were measured by radioimmunoassay using a multi-species primary antibody (Linco, St Charles, MO). Plasma insulin levels were determined by radioimmunoassay using a human primary antibody (Phadeseph, Kabi Pharmacia Diagnostics, Sweden).
Statistical analysis

All results are expressed as means ± SEM. Paired sample $t$ test were used to evaluate differences between the start and end of the study while unpaired t-tests were used for comparisons between lean and obese animals within each respective group. One-way analysis of variance (ANOVA) followed by LSD post-hoc analysis was performed. In all instances, $p<0.05$ was considered significant. Analyses were performed using SPSS statistical software (version 10.0 for Windows, Chicago, IL).
Results

Effects of nicotine on Body Weight, Food Intake and Metabolic Characteristics

In comparison to lean *P. obesus*, obese animals had increased plasma insulin and leptin levels at the beginning of the study (Table 1). After 9 days of nicotine infusion, the lean 6mg day\(^{-1}\) group showed no change in food intake from baseline levels, whereas food intake was significantly reduced in the lean 9 and 12mg day\(^{-1}\) groups by 26 and 46% respectively (Table 1), demonstrating a dose dependent reduction in food intake with increasing nicotine dose. Nicotine treated obese animals showed relatively consistent reductions in food intake ranging between 30 and 38% (Table 1). No significant differences in food intake were observed in saline treated lean or obese controls (Table 1). Bodyweight was significantly reduced for all lean and obese nicotine treated groups (Table 1, Fig 1A) ranging from 5 to 10%. Control lean and obese *P. obesus* showed no significant changes in body weight during the study period (Table 1, Fig 1A).

Plasma leptin concentrations were significantly reduced by up to 50% in the lean 9 and 12mg day\(^{-1}\) nicotine treated *P. obesus*, whereas no effect of nicotine treatment was evident in obese animals (Table 1, Fig 1B). Plasma insulin levels decreased in most nicotine treated groups, being most marked in the obese hyperinsulinemic animals where 3-fold reductions were observed. Blood glucose levels remained unchanged throughout the study period (Table 1).
Discussion

This study demonstrated that continuous nicotine infusion significantly lowered body weight and food intake in both lean and obese *P. obesus*. These changes occurred in the presence of significantly reduced plasma leptin concentrations in lean *P. obesus*, whereas in obese animals leptin levels remained unchanged. Previously we have demonstrated that high levels of exogenous leptin were successful only in reducing food intake and body weight in lean leptin sensitive animals without affect in obese animals, indicating severe leptin resistance (17). Hodge et al (13) demonstrated a link between the actions of nicotine on body weight and leptin, suggesting that smoking via nicotinic mechanisms may modify the sensitivity of leptin receptors. If the actions of nicotine on food intake and weight loss are inter-connected with leptin signaling, we would expect obese leptin-resistant animals to have no or a diminished response to nicotine in relation to reducing food intake and body weight compared to lean leptin-sensitive animals. Therefore, the effectiveness of nicotine to lower body weight in both lean and leptin-resistant obese *P. obesus* demonstrates that functional leptin signaling is not required for the suppression of body weight by nicotine.

The reductions in food intake and body weight seen in *P. obesus* in response to continuous nicotine infusion are consistent with previous literature in rodents (1-6). However, *P. obesus* required close to double the infusion dose to achieve a comparable degree of weight loss. Preliminary investigation using lower doses of nicotine (5mg·kg⁻¹·day⁻¹) failed to suppress body weight. Interestingly in lean *P. obesus* receiving the lowest nicotine dose (6mg day⁻¹), food intake remained constant although body weight decreased. This may suggest an increase in metabolic rate without a lowered food intake or a possible transient reduction in food intake as observed previously with low-dose nicotine treatment (4).
Obese *P. obesus*, consistent with our previous studies (17) had circulating leptin concentrations which were significantly higher than lean animals. Following nicotine treatment, the loss of body weight and reduction in food intake was not accompanied by any alteration in circulating leptin levels in these obese animals. In contrast, lean *P. obesus* treated with 9 and 12mg day\(^{-1}\) nicotine doses demonstrated significantly lowered plasma leptin concentrations. From our study it is unclear why decreases in body weight were accompanied by reductions in leptin in lean animals only. It is possible that this is the result of significant falls in insulin in the lean animals, as insulin is known to stimulate the release of leptin (18). In obese animals, although insulin levels were also significantly reduced, these animals remained obese and insulin resistant thereby possibly maintaining higher leptin concentrations. Further, it would have be interesting to examine alterations in body fatness with nicotine treatment in both lean and obese animals and determine if there was any dysregulation in leptin production from various fat depots.

As there is no evidence of a relationship between leptin and the anorexic actions of nicotine found in this study, the results suggest that the inhibitory effect of nicotine on food intake and body weight are most likely mediated via an alternative neural pathway. Nicotine is known to inhibit neuropeptide Y (NPY) (a potent stimulator of food intake) synthesis and increase dopamine and serotonin levels in the cortex (19) and lateral hypothalamus(4). Further, nicotine has also been shown to act in the paraventricular nucleus by indirectly activating corticotropin-releasing hormone, which is an inhibitor of food intake (20). Together, these neuropeptides most likely contribute to the anorexic effect of nicotine however further studies are required to elucidate the mechanism of nicotine’s effect on energy balance.
In summary, nicotine has dramatic effects on food intake and body weight in both lean leptin-sensitive and obese leptin-resistant *P. obesus*. Significant changes in plasma leptin concentrations occurred in lean nicotine treated animals only. These changes appear to be secondary to nicotine’s affect on food intake as both lean leptin-sensitive and obese leptin-resistant *P. obesus* had similar reductions in body weight. We can hypothesize that nicotine’s effects on food intake are mediated through the central nervous system by affecting a number of neurotransmitters involved in energy homeostasis and indirectly activating the sympathetic nervous system, although these actions appear to be independent of the leptin signaling pathway.
References


5. Winders SE, Wilkins DRd, Rushing PA, Dean JE. Effects of nicotine cycling on weight loss and regain in male rats. Pharmacol Biochem Behav 1993; **46(1)**: 209-213.


Legend to Figure

Figure 1: Percent change in body weight (A) and plasma leptin (B) from baseline measurements after 9 days of continuous nicotine infusion at 6, 9 and 12mg day\(^{-1}\). Lean *P. obesus* indicated in open bars, obese *P. obesus* in solid bars. Values are means ± SEM. Significantly different from 1 week run-in period, * p<0.05, # p<0.01.
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<td>(n=9)</td>
<td>(n=10)</td>
<td>(n=9)</td>
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<td>14.7 ± 1.1</td>
<td>11.6 ± 0.8</td>
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<td>End</td>
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<td>6.9 ± 2.1$^b$</td>
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<td>Obese</td>
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<td>9.8 ± 1.5$^b$</td>
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<tr>
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<td>Start</td>
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<td>168 ± 11</td>
<td>182 ± 11</td>
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<tr>
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<td>End</td>
<td>172 ± 10</td>
<td>159 ± 12$^b$</td>
<td>162 ± 9$^b$</td>
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<td>46 ± 11</td>
<td>42 ± 12$^b$</td>
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<td>Obese</td>
<td>301 ± 59$^a$</td>
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<td>502 ± 95$^a$</td>
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<td>101 ± 38$^b$</td>
<td>102 ± 19$^b$</td>
<td>167 ± 67$^b$</td>
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<td>4.1 ± 0.2</td>
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<td>4.6 ± 0.2</td>
<td>5.1 ± 0.5</td>
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<tr>
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<td>Obese</td>
<td>4.6 ± 0.3</td>
<td>4.5 ± 0.3</td>
<td>5.5 ± 0.5</td>
<td>5.0 ± 0.5</td>
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<td>4.3 ± 0.4</td>
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$^a$ Significantly different (p<0.05) from respective lean group at the start of study,

$^b$ Significantly different (p<0.05) from start of study.
Figure 1

A

% Change in Body Weight

Control 6 9 12

mg of nicotine per day

B

% Change in Plasma Leptin

Control 6 9 12

mg of nicotine per day

Lean

Obese