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Effects of beacon administration on energy expenditure and substrate utilisation in Psammomys obesus (Israeli sand rats)

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OBJECTIVE: To investigate whether beacon administration affects substrate utilisation, physical activity levels or energy expenditure in Psammomys obesus.

DESIGN: Pairs of age- and sex-matched Psammomys obesus were randomly assigned to either beacon-treated (15 µg/day for 7 days (i.c.v.)) or control (i.c.v. saline) groups.

MEASUREMENTS: Indirect calorimetry on day 0 and day 7 to measure oxygen consumption and carbon dioxide production, which were used to calculate fat oxidation, carbohydrate oxidation and total energy expenditure. Physical activity in the calorimeter was measured using an infrared beam system. Food intake and body weight were measured daily.

RESULTS: The administration of beacon significantly increased body weight compared to saline-treated control animals. This body weight gain was primarily due to increased body fat content. Average daily food intake tended to be higher in beacon-treated Psammomys obesus, but no effect of beacon administration on substrate oxidation, activity or energy expenditure was detected.

CONCLUSION: The effects of beacon on body weight are due to increased food intake, with no detectable effect on nutrient partitioning, physical activity or energy expenditure.


Keywords: beacon; Psammomys obesus; calorimetry; physical activity; energy expenditure; adipose tissue

Introduction

The beacon gene was recently discovered by differential display polymerase chain reaction of hypothalamic RNA from lean vs obese Psammomys obesus,¹ a polygenic animal model of obesity and type 2 diabetes.²–⁴ Although commonly referred to as the Israeli sand rat, Psammomys obesus is in fact a gerbil.⁵ In their native desert habitat, Psammomys obesus remain lean and healthy. However, under laboratory conditions when fed standard rodent chow diet, a proportion of the animals remain lean and healthy while others develop a range of metabolic disturbances including obesity, insulin resistance and type 2 diabetes.²–⁵

Beacon, which is overexpressed in the hypothalamus of obese animals, encodes a highly conserved 73 amino acid protein. Expression of beacon in the hypothalamus was correlated with body fat content in Psammomys obesus, and immunohistochemical studies localised beacon expression to the retrochiasmatic area of the hypothalamus, a region that is thought to be involved in the regulation of energy balance.¹

The human beacon gene is located on chromosome 19 and consists of five exons, while the Psammomys obesus beacon gene comprises four exons. Sequencing of the entire beacon gene (2194 nt) in 35 human subjects (15 obese, 20 lean) and 43 animals failed to reveal any common polymorphic loci associated with obesity.¹

Intracerebroventricular (i.c.v.) administration of beacon for 7 days increased food intake and body weight gain in a dose-dependent manner in Psammomys obesus, and resulted in a 2-fold increase in the hypothalamic expression of
neuropeptide (NPY). Co-administration of beacon and NPY for 7 days resulted in a profound increase in food intake and body weight that was significantly greater than expected given the results of separate administration of NPY and beacon. Therefore it was suggested that part of beacon’s actions are mediated through the NPY pathway, but beacon also acts independent of NPY to increase food intake and body weight.

It was suggested that beacon is a new neuropeptide involved in the control of energy balance in the hypothalamus. However, it is not known whether beacon administration affects physical activity or total energy expenditure in addition to its effects on food intake. The aim of this study was to investigate the effects of i.c.v. beacon administration in Psammomys obesus on substrate oxidation, physical activity and energy expenditure.

Methods

Experimental animals
A colony of Psammomys obesus is maintained at Deakin University, Geelong, Australia. Breeding pairs are fed ad libitum a diet of lucerne and chow. Experimental animals were weaned at 4 weeks of age and given a diet of standard laboratory chow, from which 12% of energy was derived from fat, 63% from carbohydrate and 25% from protein (Barastoc, Pakenham, Australia). Animals were housed in a temperature-controlled room (22 ± 1°C) with a 12–12 h light–dark cycle (light 0600–1800).

Male Psammomys obesus were classified at 18 weeks of age according to their blood glucose and plasma insulin concentrations as previously described. Whole blood glucose was measured using an enzymatic glucose analyzer (Model 27, Yellow Springs Instruments, Ohio). Plasma insulin concentrations were determined using a double-antibody solid phase radioimmunoassay (Phadeseph, Kabi Pharmacia Diagnostics, Sweden). Only normoglycemic (< 8 mmol/l) and normoinsulinemic (< 150 μU/l) animals were included in this study.

Assessment of body fat content in Psammomys obesus was made by surgically removing and weighing selected major adipose tissue depots from animals after sacrifice. These fat depots included the perirenal, supracapicular, mesenteric and intramuscular (from between the heads of gastrocnemius).

i.c.v. Administration of beacon
Stainless steel outer guide cannulae (23 gauge; Terumo, MD) were implanted into the right lateral ventricle using a Kopf stereotaxic instrument. Animals were anesthetized using phenobarbital (Rhone Merieux, Australia) then the head cleaned and shaved before washing with a peroxide solution. The animal was placed into the stereotaxic instrument and the head held in place with two ear bars and an incisor bar. An incision was made along the midline of the head and the cannula placed in the lateral ventricle (1 mm posterior to the bregma, 2 mm lateral to the midline and 2 mm ventral to the surface). The cannula was held in place with self-cure dental acrylic (De Trey GmbH, Germany). Correct positioning of the cannula was confirmed by a positive dopsonic response to angiotensin II administration (5 μg; Sigma, St Louis, MO, USA). All animals were allowed to recover for 1 week before an Alzet pump (model 7001) was implanted subcutaneously and connected to the i.c.v. cannula using 2 cm of PVC tubing (o.d. 1.50 × i.d. 0.50 mm; Critchley Electrical Products, Auburn, Australia) at day 0. The pumps delivered a 33 amino acid synthetic beacon fragment, previously shown to increase food intake and body weight in Psammomys obesus, at a dose of 15 μg/day (n = 10) for a total of 7 days with saline as a diluent. Control animals matched for age, sex, body weight, blood glucose and plasma insulin concentration received an i.c.v. infusion of saline only via model 7001 Alzet pump (n = 10). After implantation of the pump, daily measurements of food intake and body weight were recorded. All animals were housed individually and allowed free access to standard laboratory chow and water.

Indirect calorimetry
On day 0 and day 7, the animals were placed in an indirect calorimeter (Oxymax, Columbus Instruments, Ohio) for a period of 24 h. Oxygen consumption and carbon dioxide production were measured and used to calculate fat oxidation, carbohydrate oxidation, respiratory quotient and energy expenditure as previously described. Physical activity within the calorimeter was measured using an OptoVarimax Mini-Infrared Animal Activity Monitor System (Columbus Instruments, Ohio).

Statistical analysis
All data are expressed as mean ± s.e.m. Comparisons between groups were made by paired or independent sample t-test as appropriate. Homogeneity of variance was assessed using Levene’s test. Differences were considered significant at P < 0.05.

Results
The baseline characteristics of the animals used in this study are shown in Table 1. There was no difference in body weight, blood glucose or plasma insulin concentration between the two groups.

Beacon administration i.c.v. for 7 days resulted in significant body weight gain in Psammomys obesus. Body weight was significantly increased from day 3 of treatment through to day 7 compared to day 0 (P < 0.05, paired sample t-test). In contrast, there was no significant change in body weight in control animals treated with equivalent volume of saline i.c.v. (Figure 1). At the completion of the study, beacon-treated animals had gained 7.7 ± 2.0 g, compared with 2.1 ± 1.8 g in controls (P < 0.05, independent samples t-test).
Table 1 Baseline characteristics of *Psammomys obesus* used in this study (mean±s.e.m.)

<table>
<thead>
<tr>
<th></th>
<th>Beacon-treated</th>
<th>Control</th>
<th>P-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>10</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>192±6</td>
<td>187±4</td>
<td>0.528</td>
</tr>
<tr>
<td>Blood glucose (mmol/l)</td>
<td>4.2±0.2</td>
<td>4.4±0.2</td>
<td>0.600</td>
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<tr>
<td>Plasma insulin (mU/l)</td>
<td>54±13</td>
<td>51±9</td>
<td>0.833</td>
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*Independent samples t-test.

The increase in body weight following beacon treatment was largely due to increased body fat content (Table 2). Beacon-treated *Psammomys obesus* had a significant increase in white adipose tissue mass compared with saline-treated control animals (*P* = 0.04). The weights of several fat depots were significantly increased in the beacon-treated group, including the perirenal (*P* = 0.045) and mesenteric (*P* = 0.035) fat pads (Table 2). There was no evidence to suggest a difference in the weights of key organs or muscles after beacon treatment.

Average daily food intake tended to be increased in the beacon-treated animals compared to the control group (64.7±4.7 vs 53.6±2.2 mg/g body weight, *P* = 0.055). Over the 7-day study period, the beacon-treated animals consumed an average of 12.6g more than the control animals, which corresponds to a 21% increase in food intake. Average daily food intake was significantly correlated with the change in body weight throughout the study (*r* = 0.529, *P* = 0.014).

Indirect calorimetry revealed no apparent effect of beacon administration on substrate oxidation, activity or total energy expenditure (Figures 2 and 3). At baseline, there was no difference between the two groups for measures of substrate oxidation or energy expenditure, and there was no difference between days 0 and 7 within either group for carbohydrate oxidation, fat oxidation, respiratory quotient (RQ), activity or total energy expenditure. In addition, there was no difference in feed efficiency (intake/weight gain) between the beacon-treated and control groups (*P* = 0.50).

<table>
<thead>
<tr>
<th></th>
<th>Beacon-treated</th>
<th>Control</th>
<th>P-value*</th>
</tr>
</thead>
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<tr>
<td>White adipose tissue</td>
<td>39.9±6.9</td>
<td>22.9±2.4</td>
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<tr>
<td>Perirenal fat</td>
<td>8.8±1.6</td>
<td>5.1±0.5</td>
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<tr>
<td>Mesenteric fat</td>
<td>12.2±2.4</td>
<td>6.3±0.8</td>
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<tr>
<td>Supraspinal fat</td>
<td>12.0±2.6</td>
<td>6.2±1.0</td>
<td>0.060</td>
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<tr>
<td>Intramuscular fat</td>
<td>3.4±0.4</td>
<td>2.7±0.2</td>
<td>0.123</td>
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<tr>
<td>Liver</td>
<td>33.4±1.5</td>
<td>34.0±1.5</td>
<td>0.803</td>
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<tr>
<td>Heart</td>
<td>3.1±0.2</td>
<td>3.1±0.1</td>
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<tr>
<td>Kidney</td>
<td>5.5±0.1</td>
<td>5.3±0.2</td>
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<tr>
<td>Pancreas</td>
<td>1.3±0.1</td>
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<tr>
<td>Spleen</td>
<td>0.92±0.09</td>
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<td>Soleus</td>
<td>0.32±0.01</td>
<td>0.34±0.02</td>
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<tr>
<td>Gastrocnemius</td>
<td>4.7±0.2</td>
<td>4.8±0.2</td>
<td>0.633</td>
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<td>Plantaris</td>
<td>1.5±0.1</td>
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<td>0.511</td>
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<tr>
<td>Ext. dig. longus</td>
<td>2.3±0.1</td>
<td>2.4±0.1</td>
<td>0.494</td>
</tr>
<tr>
<td>Leg muscle mass</td>
<td>17.5±0.5</td>
<td>18.2±0.5</td>
<td>0.366</td>
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</table>

*Independent samples t-test.

**Discussion**

Administration i.c.v. of beacon causes an increase in body weight that is primarily due to accumulation of body fat in the major adipose depots. The body weight gain appears to be due to increased food intake rather than altered substrate

**Figure 1** Effects of i.c.v. beacon administration on body weight gain in *Psammomys obesus* (mean±s.e.m.). *P* < 0.05 compared with day 0.

**Figure 2** Effects of i.c.v. beacon administration on substrate oxidation in *Psammomys obesus* (mean±s.e.m.).

**Figure 3** Effects of i.c.v. beacon administration on respiratory quotient in *Psammomys obesus* (mean±s.e.m.).
oxidation or reduced physical activity and/or energy expenditure. Therefore beacon can be characterized as an orexigenic peptide that acts to promote the accumulation of body fat.

I.C.V. Administration of beacon to *Psammomys obesus* resulted in significant body weight gain. The beacon-treated animals gained an average of 5.6 g more body weight than control animals, similar to what we found in a previous study. In this study we showed that the body weight gain was attributable to accumulation of body fat in the major adipose depots, as beacon-treated animals had significantly increased white adipose tissue mass ($P = 0.038$, Table 2). The mass of the fat pads excised from beacon-treated animals was 3.4 g greater than that of control animals, which accounts for 61% of the 5.6 g relative increase in body weight. There was no difference in the weight of major organs or leg muscles between the beacon-treated and control animals, suggesting that beacon did not affect overall growth or cause a hypertrophic response in tissues other than adipose tissue.

The effects of beacon on energy balance were not due to alterations in physical activity or energy expenditure (Figure 3). Similarly, we found no evidence that beacon has any effect on carbohydrate or fat oxidation rates, or feed efficiency, demonstrating that beacon had no overall effect on energy partitioning and substrate utilisation (Figure 2). These results suggest that the effects of beacon are due to changes in energy intake. The food intake of the beacon-treated group showed a consistent tendency to be higher than the control group ($P = 0.055$), and in a previous study i.c.v. beacon administration at a higher dose (30 pg/day) did result in a significant increase in food intake. The increase in food intake in this study, an average of 1.8 g/day, would account for 12.6 g/week. The difference in body weight gain between the two groups was only 5.6 g, therefore the increase in body weight could easily be accounted for by elevated food intake. In addition, the average daily food intake of the animals was correlated with the change in body weight during the study, suggesting that these two variables are closely related. Considering this in conjunction with the indirect calorimetry data, we contend that the effects of beacon on body weight gain are due, at least in part, to increased food intake.

The finding that beacon regulates food intake without an accompanying change in energy expenditure makes it somewhat unusual compared to other hypothalamic peptides known to affect energy balance. For example, leptin acts on the hypothalamus to decrease food intake and increase energy expenditure, and NPY increases food intake and decreases energy expenditure. I.C.V. MCH increases food intake in the short term (1–5 days), without change in body weight, and MCH-deficient mice had increased metabolic rate, suggesting that, like NPY, MCH stimulates food intake and reduces energy expenditure. Orexin I.C.V. also increased food intake and energy expenditure, however the role of orexins in the regulation of energy balance is questionable as recent studies have shown they are involved in control of sleep/wakefulness states and narcolepsy.

All of the peptides listed above represent potential pathways for the development of pharmacological treatments of obesity. The relative 'purity' of beacon's actions on food intake may be an advantage in the development of new therapeutic approaches for obesity and type 2 diabetes. For example, the combination of an inhibitor of beacon with a mild exercise program represents a potential obesity treatment, and also an avenue for both the treatment and prevention of type 2 diabetes. Conversely, manipulation of pathways that also alter energy expenditure may have undesirable effects, such as those recently described for the orexin pathway.

**Acknowledgements**

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**References**
