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Elevated hypothalamic beacon gene expression in Psammomys obesus prone to develop obesity and type 2 diabetes

K Walder1*, E Ziv2, R Kalman2, K Whitecross1, E Shafrir2, P Zimmet3 and GR Collier1

1Metabolic Research Unit, School of Health Sciences, Deakin University, Waurn Ponds, Victoria, Australia; 2Diabetes Research Unit, Hadassah University Hospital, Jerusalem, Israel; and 3International Diabetes Institute, Caulfield, Victoria, Australia

OBJECTIVE: To investigate hypothalamic beacon gene expression at various developmental stages in genetically selected diabetes-resistant and diabetes-prone Psammomys obesus. In addition, effects of dietary energy composition on beacon gene expression were investigated in diabetes-prone P. obesus.

METHODS: Hypothalamic beacon gene expression was measured using Taqman® fluorogenic PCR in 4-, 8- and 16-week-old animals from each genetically selected line.

RESULTS: Expression of beacon was elevated in the diabetes-prone compared with diabetes-resistant P. obesus at 4 weeks of age despite no difference in body weight between the groups. At 8 weeks of age, hypothalamic beacon gene expression was elevated in diabetes-prone animals fed a high-energy diet, and was correlated with serum insulin concentration.

CONCLUSION: P. obesus with a genetic predisposition for the development of obesity and type 2 diabetes have elevated hypothalamic beacon gene expression at an early age. Overexpression of beacon may contribute to the development of obesity and insulin resistance in these animals.


Keywords: beacon gene; Psammomys obesus; obesity; hypothalamus; gene expression

Introduction

Psammomys obesus (the Israeli sand rat) is a polygenic animal model of obesity and type 2 diabetes.1–3 A number of metabolic abnormalities have previously been demonstrated in obese, diabetic P. obesus including muscle and liver insulin resistance,4,5 impaired insulin receptor function,6 low levels of muscle GLUT4 and overexpression of several protein kinase C isoforms in skeletal muscle.7 In outbred colonies of P. obesus fed ad libitum a diet of standard rodent laboratory chow, approximately half of the animals develop obesity of varying degrees, while about one-third develop insulin resistance and type 2 diabetes.1–3

*Correspondence: K Walder, Metabolic Research Unit, School of Health Sciences, Deakin University, Piddons Road, Waurn Ponds, Victoria 3217, Australia.
E-mail walder@deakin.edu.au
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We have previously conducted studies to identify differentially expressed genes in the hypothalamus of lean and obese 18-week-old P. obesus from our outbred colony at Deakin University, Australia. Recently, the beacon gene, which encodes a highly conserved 73 amino acid protein, was shown to be overexpressed in the hypothalamus of obese, outbred P. obesus relative to their lean, outbred littermates.8 Expression of beacon in the hypothalamus was correlated with body fat content in P. obesus. Furthermore, immunohistochemical studies localized beacon expression to the retrochiasmatic area of the hypothalamus, a region that is thought to be involved in the regulation of energy balance.8

Intracerebroventricular (i.c.v.) administration of beacon for 7 days increased food intake and body weight gain in a dose-dependent manner in P. obesus, and resulted in a 2-fold increase in the hypothalamic expression of 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.8 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. Using indirect calorimetry, it was shown that i.c.v. beacon administration did not affect energy expenditure, physical activity or substrate utilization in P. obesus, suggesting that the effects of beacon administration on body weight gain were predominantly due to its effect on food intake. Thus beacon is a new neuropeptide involved in the control of energy balance in the hypothalamus, and further studies to characterize the physiological role of beacon in P. obesus are required.

Through assortative mating, two genetically defined lines of P. obesus have been developed at Hadassah University Hospital, Israel, that differ in their utilization of dietary energy. The two lines are known as diabetes-prone (DP) and diabetes-resistant (DR), and are described in detail elsewhere. In the DP line, obesity and type 2 diabetes develop rapidly (1–2 weeks) when the animals are fed a relatively high-energy diet, but are delayed or prevented by a low-energy diet. The DP line is characterized by hyperglycemia, hyperinsulinemia, hypercholesterolemia, hypertriglyceridemia and obesity. The animals are not hyperphagic on the high-energy diet, and obesity and diabetes are thought to result from increased metabolic efficiency.

The aim of this study was to investigate the hypothalamic expression of the beacon gene at various ages in P. obesus with different genetic propensity for development of obesity and type 2 diabetes. The effect of dietary energy composition on beacon gene expression was also assessed in DP P. obesus.

**Methods**

**Experimental animals**

*Psammomys obesus* were raised in a colony at the Animal Farm of the Hebrew University Hadassah Medical School. Animals from both the genetically selected DR and DP lines were included in this study. All animals were housed individually and had *ad libitum* access to food and water. All DR animals were fed high-energy (HE) chow diet supplying digestible energy of 3.1 kcal/g and comprised of 22.4% protein, 2.1% fat, 66.6% carbohydrate (of which 80.5% was digestible) and 6.9% ash. DP animals received either the HE diet described above or low-energy (LE) diet supplying 2.4 kcal/g and comprised 16.7% protein, 3.1% fat, 70.0% carbohydrate (of which 64.0% was digestible) and 10.2% ash. All experimental procedures were authorized by the Hebrew University Institutional Animal Care Committee.

At specific ages (4-, 8- or 16-weeks; n = 6 in each group), the animals were decapitated and tissues including the entire hypothalamus (30–60 mg of tissue per animal, varying according to the age and size of the animals) were removed immediately and frozen in liquid nitrogen. RNA was extracted using TriZol reagent (Life Technologies, Grand Island, USA) and reverse transcribed using SuperScript II (Life Technologies).

**Assays**

Blood samples were collected from the tail vein of all animals. Blood glucose was measured in tail vein samples (Glucose Elite, Bayer, USA) and serum insulin was determined by radioimmunassay using anti-human insulin antibodies (Sarin Biomedica, Saluggia, Italy) and human insulin standards as previously described.

**Gene expression**

The level of beacon gene expression in each hypothalamic cDNA sample was quantitated using Taqman PCR technology on an ABI Prism 7700 sequence detector. Cyclophilin was used as an endogenous control to standardize the amount of cDNA added to each reaction, and was not significantly different between any of the groups studied. Primer sequences were as follows: beacon gene forward, 5'-cga act ggc act cgt tgg aa-3'; beacon gene reverse, 5'-ggt ggg cca ggt gga gga a-3'; cyclophilin forward, 5'-ccc acc gtc ttc ttc gac a-3'; and cyclophilin reverse, 5'-cca ggg ctc aga gca cgg a-3'. Fluorogenic probe sequences were 5'-tgg taa taa agc ttc agg ttc atc cca tgg-3' for the beacon gene, and 5'-cgc gtc tcc ttc ggg ctt tgt gc-3' for the cyclophilin gene. The beacon and cyclophilin probes had the reporter dyes FAM and VIC, respectively, attached to the 5' end and both probes had the quencher dye TAMRA attached to the 3' end. PCR conditions were 50°C for 2 min, 95°C for 10 min followed by 40 cycles of 95°C for 15 s and 60°C for 1 min.

**Statistical analysis**

Data are expressed as mean ± s.e.m. The Kalmogorov–Smirnov test was used to check distribution of data. Serum glucose data were not normally distributed, so non-parametric analyses were used. Kruskal–Wallis or Mann–Whitney tests were used to test for differences between groups, and Spearman rank correlation was used to investigate associations between serum glucose and other linear variables. All other data were normally distributed. Differences between group means were assessed using ANOVA with post hoc LSD test or independent sample t-test where appropriate. Linear associations were investigated using Pearson correlation or linear regression modelling with beacon gene expression as the dependent variable and covariates as described in the text. All statistical tests were performed using SPSS version 9.0 (SPSS Inc., Chicago, IL, USA), with significance levels set at P < 0.05.

**Results and discussion**

At 4 weeks of age, there was no difference in body weight between the three groups of animals (P = 0.158, ANOVA;
Figure 1). *P. obesus* from the DP line fed the HE diet were hyperglycaemic (*P* = 0.003, Kruskal–Wallis test) and hyperinsulinaemic (*P* < 0.001, ANOVA) relative to the other two groups (DPLE and DRHE; Figure 1). *Beacon* gene expression was significantly different between the three groups of animals (*P* = 0.036, ANOVA). *Post hoc* LSD analysis showed overexpression of beacon in the hypothalamus of DPHE animals relative to the DRHE group (*P* = 0.012), while expression in DPLE animals was intermediate between these two groups (Figure 1). *Beacon* gene expression was higher in DP than DR *P. obesus* regardless of diet (*P* = 0.018, t-test). Hypothalamic expression of beacon in all 4-week-old animals was significantly correlated with both serum glucose (Spearman *r* = 0.484, *P* = 0.049) and insulin concentration (Pearson *r* = 0.487, *P* = 0.047).

These data provide evidence for a metabolic defect in the DP line of *P. obesus* that, in the presence of an HE diet, leads to insulin resistance (evidenced by marked hyperglycaemia and hyperinsulinaemia) by 4 weeks of age. This metabolic defect is also associated with elevated hypothalamic expression of beacon that was attenuated by feeding the animals an LE diet. Given previous findings demonstrating that beacon acts centrally to increase food intake and body weight gain,6 it is likely that overexpression of beacon in DPHE animals contributes to excessive body weight gain in these animals later in life. The linear relationships between *beacon* gene expression and glucose/insulin suggest that the severity of the metabolic defect is related to the levels of beacon expression. This in turn contributes to the accumulation of body fat and the worsening of insulin resistance in these animals.

By 8 weeks of age, DPHE animals were heavier than the DPLE group (*P* = 0.017; Figure 2). The DPHE group were hyperinsulinaemic relative to both DPLE (*P* = 0.004) and DRHE animals (*P* = 0.007), and were hyperglycaemic compared with the DRHE group (*P* = 0.020; Figure 2). Hypothalamic *beacon* gene expression was significantly elevated in the DPHE animals relative to both the DPLE (*P* = 0.004) and DRHE groups (*P* = 0.008; Figure 2). In all 8-week-old animals, beacon gene expression in the hypothalamus was highly correlated with serum insulin concentration (Pearson *r* = 0.806, *P* < 0.001).

These data show that, by 8 weeks of age, feeding of an LE diet can control the metabolic defect in DP animals. *Beacon* gene expression was normalized in the DPLE group, in which body weight, glucose and insulin were similar to the DR group. Conversely, in the obese, hyperglycaemic and hyperinsulinaemic DPHE group, *beacon* gene expression was significantly elevated. The linear relationship between *beacon* gene expression and insulin suggests a link between the

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Figure 1 Characteristics of 4-week-old *Pammomys obesus*. *P* = 0.004 compared with DRHE and DPLE (Mann–Whitney test); **P* < 0.001 compared with DRHE and DPLE (ANOVA with post hoc LSD test); #P* = 0.012 compared with DRHE (ANOVA with post hoc LSD test).
degree of insulin resistance and levels of beacon in the hypothalamus. Further studies are required to investigate the relationship between these factors, including whether beacon contributes directly to insulin resistance, or secondary to increased accumulation of body fat.

The 16-week-old DPHE animals were heavier (243 ± 11 g) than both DPLE (200 ± 11 g; P = 0.008) and DRHE animals (212 ± 7 g; P = 0.026). The DPHE animals were also hyperglycaemic (12.4 ± 3.4 mmol/l) relative to both the DPLE (3.8 ± 0.1 mmol/l; P = 0.004) and DRHE groups (4.5 ± 0.3 mmol/l; P = 0.010). There was no difference in serum insulin concentration or hypothalamic beacon gene expression between the three groups, although beacon expression tended to be higher in DP animals (1.41 ± 0.12 vs 1.03 ± 0.21 arbitrary units; P = 0.099). Thus it appears that by 16 weeks of age, when the growth rate of the animals has slowed dramatically, the differences in hypothalamic beacon gene expression are less pronounced. It is possible that a major function of beacon could be to contribute to determination of the steady-state body weight at which the adult animal will be in energy balance, also known as the body weight 'set-point'. If further studies prove that this is the case, beacon would be an attractive target for early intervention to prevent the accumulation of body fat and the development of obesity that, once established, is very difficult to effectively treat.

General linear modelling was used to investigate factors that contribute to the variation in the level of hypothalamic beacon gene expression. Beacon gene expression did not change with age either within each of the three groups of Psammomys obesus, or when all animals were combined (P = 0.870). Overall, beacon gene expression was higher in DP animals compared with DR animals regardless of diet (DP 1.39 ± 0.05 vs DR 1.07 ± 0.08 arbitrary units; P = 0.001), consistent with our hypothesis that a metabolic defect in this genetic line causes both insulin resistance and elevated hypothalamic expression of beacon. When age, line (DR or DP) and diet (LE or HE) were included in a linear model, only line (F = 16.5, P < 0.001) and diet (F = 5.0, P = 0.029) were independently associated with beacon gene expression (adjusted $r^2 = 0.183$), and no significant interactions were detected between the three variables. Inclusion of body weight, serum glucose and insulin as covariates into the model did not alter these results.

In summary, $P. obesus$ from the DP line appear to have a metabolic defect that, in the presence of an energy-dense diet, results in insulin resistance and elevated hypothalamic beacon gene expression by 4 weeks of age. This overexpres-
sion of beacon is likely to result in excessive accumulation of body fat leading to obesity, which in turn exacerbates the insulin resistance and precipitates the development of type 2 diabetes. Of particular note is the fact that feeding the animals a low energy density diet prevented the development of obesity and diabetes. Alternatively, overexpression of beacon in the DF animals may in fact be the trigger for the subsequent metabolic events that lead to the development of obesity and type 2 diabetes in P. obesus. We suggest that beacon may play a key role in the development of obesity and type 2 diabetes.

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References