Impact of obesity and leptin treatment on adipocyte gene expression in *Psammomys obesus*

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Abstract

We examined the effects of leptin treatment on the expression of key genes in adipocyte metabolism in *Psammomys obesus* (*P. obesus*), a polygenic rodent model of obesity. Lean and obese *P. obesus* were given three daily intraperitoneal injections of either saline or leptin (total of 45 mg/kg per day) for 7 days. In lean animals, leptin treatment led to reductions in food intake, body weight and fat mass. Pair-fed animals matched for the reduction in food intake of the lean leptin-treated animals demonstrated similar reductions in body weight and fat mass. In obese *P. obesus*, leptin treatment failed to have any effect on body weight or body fat mass, indicating leptin resistance. Lipoprotein lipase, hormone-sensitive lipase and peroxisome proliferator activated receptor gamma 2 mRNA levels were significantly reduced in lean leptin-treated animals, whereas pair-fed animals were similar to lean controls. Uncoupling protein 2 and glycerol phosphate acyltransferase were also reduced in the lean leptin-treated animals, but not significantly so. Obese animals did not show any gene expression changes after leptin treatment. In conclusion, high circulating concentrations of leptin in lean *P. obesus* resulted in decreased gene expression of a number of key lipid enzymes, independent of changes in food intake, body weight and fat mass. These effects of leptin were not found in obese *P. obesus*.

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Introduction

Adipose tissue is a dynamic organ integrating multiple signals provided by hormones, proteins and endocrine factors involved in energy homeostasis (Spiegelman & Flier 1996). Leptin, an adipocyte hormone, has been shown to be an important regulator of energy balance (Zhang *et al.* 1994, Pelleymounter *et al.* 1995, Tartaglia *et al.* 1995). Leptin is mainly secreted from adipocytes (Masuzaki *et al.* 1995) in proportion to body adiposity in both humans and animals (Maffei *et al.* 1995, Considine *et al.* 1996), although the control of leptin gene expression, secretion and signaling is complex (Friedman 1998). Circulating leptin concentrations are communicated primarily to the hypothalamus, via the long isoform of the leptin receptor (Tartaglia *et al.* 1995), triggering a number of adaptive responses, including decreased food intake and increased energy expenditure. In addition, there is evidence of peripheral actions of leptin, including increased lipolysis in adipocytes (Fruhbeck *et al.* 1997, Siegrist-Kaiser *et al.* 1997) and increased lipid oxidation in pancreatic islets and skeletal muscle (Lee *et al.* 1997, Muoio *et al.* 1997).

Despite the evidence suggesting that leptin may impact on adipocyte metabolism in a paracrine fashion, the mechanisms involved in leptin regulation of fatty acid synthesis/mobilization and gene expression within adipose tissue are poorly understood. There are a number of key transcriptional regulators of fatty acid metabolism in adipocytes. Adipocyte differentiation and subsequent phenotypic gene expression are regulated, in part, by the induction of the peroxisome proliferator activated receptor gamma 2 (PPARγ2) (Tontonoz *et al.* 1994, Spiegelman & Flier 1996). After PPARγ2 activation, there is a rapid induction of lipoprotein lipase (LPL) in maturing adipocytes, which mediates fatty acid influx. Similarly, glycerol phosphate acyltransferase (GPAT) which catalyzes the first step in fatty acid esterification to triglycerides (Zhou *et al.* 1997), and hormone sensitive lipase (HSL) (Schoonjans *et al.* 1996), which catalyses the hydrolysis and release of fatty acids from the cell, are both upregulated with adipocyte differentiation and maturation. Recent studies have provided evidence of regulated uncoupled protein expression in adipose tissue. The distribution of uncoupling protein 1 (UCP-1) appears to be restricted to thermogenic brown adipose tissue, whereas uncoupling protein 2 (UCP-2) expression is widespread in white adipose tissue (Zhou *et al.* 1997). *UCP-2* gene expression in white adipose tissue increases with adipocyte

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differentiation (Aubert *et al.* 1997). Modulations in the gene expression of each of these genes may be important events mediating the actions of leptin on adipocyte lipid metabolism.

In an effort to gain insight into the role of leptin in adipocyte fat metabolism we examined the effect of increased circulating leptin concentrations on adipocyte gene expression *in vivo*. In this study, we utilized a polygenic gerbil model of obesity and type II diabetes, *Psammomys obesus* (*P. obesus*). In their native, semi-arid environment, these animals remain lean and free of diabetes. Transition to normal laboratory rodent diets results in population variability, with a proportion of these animals remaining lean and free of diabetes and others developing a range of metabolic abnormalities that include obesity, hyperinsulinemia, glucose intolerance, hyperleptinemia, and frank diabetes (Barnett *et al.* 1994*a*,*b*, Collier *et al.* 1997, Walder *et al.* 1997). The spectrum of metabolic responses to, and the variability in the susceptibility to development of, obesity in *P. obesus* enables the examination of exogenous leptin treatment on adipocyte gene expression in a model that is more analogous to human obesity than are single gene obesity models (Shafrir & Gutman 1993). In the present study, the actions of exogenous supraphysiological leptin treatment on adipocyte gene expression were analysed in lean and obese *P. obesus*.

Materials and Methods

Animals

Mixed-sex 16-week-old *P. obesus* were housed in individual cages in a temperature-controlled facility (22 ± 1 °C) with a 12-h light : 12-h darkness cycle and given free access to a standard laboratory chow (Baristoc, Pakenham, Australia). The energy composition of the chow comprised 12% energy from fat, 63% energy from carbohydrate and 25% energy from protein. All experimental procedures were performed according to institutional (Deakin University Animal Ethics Committee) and the National Health and Medical Research Council of Australia guidelines.

Experimental procedure

Animals were categorized into two groups – a lean (body weight <190 g) and an obese (body weight >190 g) group – as previously described (Walder *et al.* 1999). Previous studies have shown that obese animals have increased body fat content, hyperinsulinemia and hyperleptinemia compared with their lean counterparts (Walder *et al.* 1999). All animals were monitored for at least a 7-day run-in period, to establish baseline data for food intake (measured by rate of disappearance of food), body weight, and blood glucose, plasma insulin and leptin concentrations. At the end of the run-in period, lean and obese animals were further subdivided into two treatment groups (saline treated control or leptin treated) and matched for body weight and sex, and blood glucose, plasma insulin and leptin concentrations. Animals were given intraperitoneal injections, three times per day (at 0800, 1600 and 2400 h), of either saline or a 'supraphysiological' dose of recombinant murine leptin (total of 45 mg/kg per day) for 7 days. Leptin was generously supplied by Amgen Inc. (Thousand Oaks, CA, USA). Body weight and food intake were measured daily. A further group of lean animals were pair-fed the same amount of chow as was consumed by the lean leptin-treated animals.

At the completion of the study, animals were killed by an overdose of pentobarbitone (120 mg/kg). A number of tissues were removed and weighed; these included a selection of fat depots (interscapular, perirenal, epididymal, mesenteric and intermuscular fat taken from the hind leg), to provide us with a estimate of body fat content. Interscapular fat mass, a major variable adipose depot in *P. obesus* was utilized in the present study.

RNA extraction, cDNA synthesis and comparative RT-PCR quantitation

Total RNA was extracted from 40 mg interscapular fat by the RNeasy Mini Spin Column methodology (Qiagen, Hilden, Germany). Aliquots of total RNA (2 μg) were used for first-strand cDNA generation, using the oligo d(T) primer in first-strand cDNA synthesis kit (Promega, Madison, WI, USA). We amplified 1 μl reverse transcription reaction mix using primers designed specifically for LPL, HSL, GPAT, UCP-1 and UCP-2, and PPARγ2. The primer sequences used were:

LPL: forward 5′-CCA GCT GGA CCT AAC TTT GAG-3′; reverse 5′-CCT CCA TTG GGG TAA ATG TC-3′

HSL: forward 5′-GGC TTT GTG GCA CAG ACC TC-3′; reverse 5′-CCA GGA AGG AGT TGA GCC AT-3′

GPAT: forward 5′-AAG GCA CCA TTT CTC TGC CC-3′; reverse 5′-AGC AAT CTC GCT GCT CCT CA-3′

UCP-1: forward 5′-TCC AAG GTG AAG GCC AGG CT-3′; reverse 5′-CTG TGG TGG CTA TAA CTC TG-3′

UCP-2: forward 5′-AAC AGT TCT ACA CCA AGG GC-3′; reverse 5′-AGC ATG GTA AGG GCA CAG TG-3′

PPARγ2: forward 5′-CGC ACT GGA ACT AGA TGA CA-3′; reverse 5′-GGT GAA GGC TCA TGT CCG TC-3′

Primer pairs were designed by aligning sequences for rat, mouse and human (where possible) and looking for

conserved regions for each gene of interest. PCR products for each primer combination were sequenced and confirmed using sequences obtained from Genebank. All PCR reactions were optimized and duplicate aliquots amplified in the linear phase. β-actin mRNA was used as an internal standard for normalizing data. PCR products were electrophoresed on a 1·5% agarose gel, visualized using ethidium bromide and quantitated by computer-integrated densitometry using Kodak's Digital Science electrophoresis documentation and analysis system (Eastman Kodak Company, Rochester, NY, USA).

Statistical analysis

All results are expressed as means ± S.E.M. Statistical significance was evaluated using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test, and a two-way unpaired *t*-test was used where appropriate. In all instances, probability values of $P>0{\cdot}05$ were considered significant.

Results

Anthropometric and blood variables

We have described previously (Walder *et al.* 1999) the results of the current study showing that leptin treatment significantly increased (24 ± 6 ng/ml to 2048 ± 552 ng/ml and 57 ± 14 ng/ml to 1921 ± 334 ng/ml) circulating plasma leptin to similar concentrations in lean and obese animals, respectively. Increased circulating leptin concentrations were associated with significant reductions in food intake (12·5 ± 0·7 g/day to 9·7 ± 0·9 g/day), body weight (164 ± 6 g to 160 ± 7 g) and fat mass (2·7 ± 0·5% to 1·3 ± 0·4%) in lean animals only. Likewise, pair-fed animals matched for the reduction in food intake of the lean leptin-treated animals also showed similar reductions in body weight (166 ± 3 g to 160 ± 4 g) and fat mass (2·7 ± 0·5% to 1·5 ± 0·2%). In contrast, leptin treated obese animals failed to show any effect on food intake, body weight or fat mass in comparison with the obese controls.

Gene expression

To determine the effect of leptin treatment on adipocyte gene expression, we investigated a number of key enzymes involved in lipid metabolism. Leptin treatment in the lean animals resulted in significant reductions for *LPL*, *HSL* and *PPARγ2*, whereas *UCP-2* and *GPAT* were reduced, but not significantly (Fig. 1). Lean pair-fed animals showed no gene expression changes in comparison with their lean counterparts. No effect of leptin treatment was found in the obese animals for any of the genes investigated, although the tendency was for decreased gene expression.

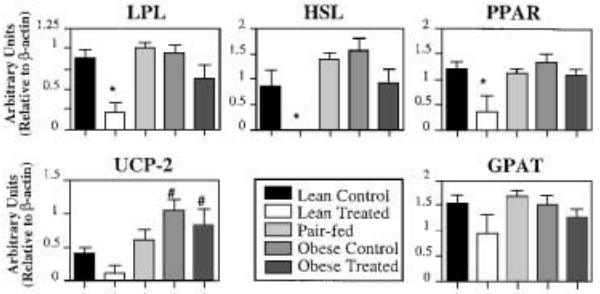

Figure 1 Expression of mRNA for LPL, HSL, PPAR, UCP-2 and GPAT from lean and obese *P. obesus* and the effect of leptin treatment or pair-feeding ($n=8$ in all groups). All values are the mean ± S.E.M. *$P>0{\cdot}05$ compared with lean animals; #$P>0{\cdot}05$ compared with control and lean leptin-treated animals.

For all genes analyzed, only *UCP-2* was expressed in significantly greater amounts in obese animals compared with lean *P. obesus*. *UCP-1* was not detected in any samples, indicating no brown adipose tissue (BAT) contamination or transformation of white adipose tissue into BAT.

Discussion

The results of this study demonstrate that treatment with supraphysiological concentrations of leptin in lean *P. obesus* decreased the expression of key genes involved in adipocyte fat metabolism independent of changes in food intake, body weight and percent fat mass. Identical leptin treatment in obese *P. obesus* for 7 days failed to alter adipocyte gene expression, body weight or body fat mass.

P. obesus is a polygenic animal model of obesity and diabetes. This species displays a wide spectrum of body weight and metabolic responses after dietary manipulations, comparable to observations in human populations (Shafrir & Gutman 1993). We have previously shown that, as a species, *P. obesus* is comparatively more leptin insensitive than other rodent models (Walder *et al.* 1997). A high dose of leptin (animals received a total of 45 mg/kg per day) was required before any impact on food intake, body weight and fat mass was observed (Walder *et al.* 1999). In addition, these effects were found only in lean animals, indicating that obese *P. obesus* have pronounced leptin resistance.

The current study examined the impact of very high circulating concentrations of leptin on adipocyte gene expression in intrascapular white adipose tissue. In lean *P. obesus*, high circulating leptin concentrations reduced the expression of a number of genes important in adipocyte metabolism, including *LPL*, *HSL* and *PPARγ2*, whereas *UCP-2* and *GPAT* tended to be decreased, although these changes were not significant. Interestingly, in pair-fed animals that were matched for the reduction in food intake

of the lean leptin-treated animals, no significant changes in adipocyte gene expression were demonstrated. These results indicated that reductions in *LPL*, *HSL* and *PPARγ2* gene expression in lean animals were due to a specific effect of leptin, independent of changes in food intake, body weight and fat mass. It is unclear from the present study whether the actions of supraphysiological leptin on adipocyte gene expression were mediated via peripheral or central mechanisms. In support of a direct peripheral action of leptin, adipocytes have been shown to express low levels of both the long and short isoforms of the leptin receptor (Lee *et al.* 1996, Fei *et al.* 1997). At these low levels of expression, sufficient signaling capacity is present to initiate cellular responses via the Janus kinases (JAK) and signal transducers and activators of transcription (STAT) pathways (Tartaglia 1997). Direct actions of leptin in isolated adipocytes have been demonstrated to include the activation of the JAK/STAT pathway, increased lipolysis, decreased *LPL* gene expression, decreased acetyl-CoA carboxylase and lipid synthesis, and impaired insulin action (Bai *et al.* 1996, Fruhbeck *et al.* 1997, Muller *et al.* 1997, Siegrist-Kaiser *et al.* 1997). However, studies using rat adipocytes and 3T3-L1 adipocytes have not demonstrated any direct action of leptin treatment on LPL enzyme activity and glucose transport, respectively (Ranganathan *et al.* 1998, Zierath *et al.* 1998).

In the present study, no significant changes in mRNA levels of either UCP-2 and GPAT were demonstrated in lean leptin-treated animals (Fig. 1). In contrast, Zhou *et al.* (1997) isolated islets and adipocytes obtained from lean Zucker diabetic fatty rats made hyperleptinemic by recombinant adenovirus leptin infusion and cultured them with 20 ng/ml leptin for 48 h, which resulted in large increases in mRNA levels of UCP-2 and reductions in GPAT, indicating fuel partitioning towards oxidation instead of storage. It is not apparent from the data presented in the current study why leptin treatment in *P. obesus* failed to alter *UCP-2* and *GPAT* gene expression. Scarpace *et al.* (1998) recently reported that, in rats, leptin treatment for 1 week induced UCP-2 mRNA in epididymal adipose tissue, but not in perirenal or BAT. Therefore, leptin treatment may exert differing regional effects on *UCP-2* gene expression. Acute treatment, however, may have a transient action to stimulate uncoupled respiration, with preferential trafficking of fatty acids towards oxidation, whereas longer-term treatment, with decreased body weight and fat mass, elicits a compensatory normalization of levels of expression of *UCP-2* and *GPAT*.

PPARγ2 mRNA levels are significantly reduced after leptin treatment. PPARs have been implicated in mediating the expression of a number of key genes involved in lipid metabolism, including *LPL*, *UCP-2*, fatty acid binding protein and acetyl-CoA synthase (Schoonjans *et al.* 1996). Likewise, PPARs have themselves been shown to be subject to regulation by a number of factors including fatty acids (Green 1995), prostaglandin derivatives (Forman *et al.* 1995, Kliewer *et al.* 1995), thiazolidinediones (Lehmann *et al.* 1995, De Vos *et al.* 1996), insulin (Zhang *et al.* 1994) and tumor necrosis factor alpha (TNFα) (Beier *et al.* 1997, Zhang *et al.* 1996). Our data raise the possibility that the actions of leptin may be mediated via interactions with PPARγ2.

In contrast to the efficacy of leptin treatment in modulating adipocyte gene expression in lean *P. obesus*, the high circulating leptin concentrations had no significant effect in the adipocytes of obese *P. obesus*. These results suggest that, obese animals are resistant, not only to the hypothalamic actions of leptin to regulate food intake, but also to the effects of leptin on gene expression in adipocytes. Centrally administered leptin has previously been reported to promote adipocyte apoptosis and regulate PPARγ, TNFα, UCP-2 and the CCAAT/enhancer binding protein family (Qian *et al.* 1998*a*, *b*). Support for the necessity of a functionally centrally mediated axis was found in a recent study by Koyama *et al.* (1998). This group demonstrated that, in ventromedial hypothalamus-lesioned rats, adenovirally induced hyperleptinemia had no effect on food intake, body weight or adipocyte fat mass. The requirement for an intact hypothalamic axis is supported by unpublished data in which comparable mRNA levels of both leptin receptor isoforms are found in adipocytes isolated from either lean or obese *P. obesus* (A de Silva, K Walder & G R Collier, personal communication). Further studies are required to determine whether the lack of efficacy of leptin treatment in obese *P. obesus* on adipocyte gene expression is due to defective leptin action in either the central nervous system or peripheral tissues.

In summary, high circulating leptin concentrations in lean *P. obesus* resulted in decreased gene expression of a number of key enzymes central to adipocyte lipid metabolism, independent of changes in food intake, body weight and fat mass. These observed reductions in gene expression suggest that chronic leptin treatment down-regulates many aspects of adipocyte function. These effects of leptin treatment were not found in leptin-resistant obese animals.

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