Seasonality of Leptin Levels in the BAT of the Common Shrew (*Sorex araneus*)

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Leptin concentrations in the interscapular brown adipose tissue (IBAT) of the common shrew (*Sorex araneus*) were measured in different seasons. The leptin concentrations in IBAT were much higher than in the liver, where leptin is supposed to be of blood origin. In the heart muscle no detectable amount of leptin was found. There were clear seasonal variations in the leptin concentrations in IBAT. Leptin levels in IBAT were the lowest in November at the beginning of the winter. The concentrations increased, however, strongly after the onset of the permanent snow cover, and the highest concentrations were measured in December-January, when the weight of the animals was very low. In April-May, at the time when shrews attain sexual maturity, leptin concentrations in IBAT were lower than in the mid-winter, but significantly higher than in November. In overwintered adults the leptin concentrations were at the same level as in nonwintered subadults. Leptin originating from BAT may inform the central nervous system about the amount of nonshivering thermogenesis as well as the amount of feeding necessary for survival in the winter months.

Introduction

Leptin is an adipocyte-derived hormone discovered in 1994 by the positional cloning of the murine *obese* (*ob*) gene, which is highly conserved among vertebrates (Zhang et al., 1994). Leptin is secreted primarily by the white adipose tissue (WAT) (Cinti et al., 1996), although the ventricular mucosa also seems to be a source of leptin (Bado et al., 1998). The production of leptin by brown adipose tissue (BAT) of adult mammals remains controversial (Cinti et al., 1996, Tsuruo et al., 1996), even though it has been demonstrated that the leptin gene is expressed in rat BAT at birth (Dessolin et al., 1997).

Leptin seems to be the signal that informs the central nervous system (CNS) of mammals about the energy reserves of the body thus controlling the feeding behaviour of animals (Collins et al., 1996). In CNS leptin e.g. decreases the production of hypothalamic neuropeptide Y, which causes an increase in food intake and a decrease in thermogenesis (Glaum et al., 1996). Leptin is taken into the CNS by a saturable transport system (Karonen et al., 1998, Koistinen et al., 1998). It is also the trigger for the onset of puberty (Cheung et al., 1997), as the normal onset of puberty in mammals requires a sufficient amount of energy reserves as fat (Frisch and McArthur, 1974) to proceed.

In BAT leptin increases thermogenesis via the stimulation of the production of uncoupling protein 1 (UCP1) (Scarpace et al., 1997) and uncoupling protein 3 (UCP3) (Muzzin et al., 1999). Leptin also has direct effects on BAT, where the intravenous administration of exogenous leptin increases the utilization of glucose *in vivo* (Siegrist-Kaiser et al., 1997). The induction of UCP1 gene expression in BAT by leptin seems to be dependent on sympathetic innervation, especially the β3-adrenergic receptor (Scarpace and Matheny, 1998). Melnyk and Himms-Hagen (1998) have previously demonstrated that partial ablation of brown adipocytes leads to obesity and unexpected hyperphagia in transgenic mice due to a deficit of thermogenesis. They have also suggested that BAT might generate a signal acting independently of leptin in inverse relationship to ambient temperature. In infant rats, however, it has been demonstrated that exogenous leptin is able to disinhibit BAT thermogenesis during cold exposure, which has suggested a role for leptin in the modulation...
of thermogenesis and energy utilization in the postnatal period of rats (Blumberg et al., 1999).

Leptin research has concentrated largely on the causes and possible amelioration of human obesity by leptin. In nature the key adaptation is not to avoid obesity but to survive the periods of food and energy shortage. It has been suggested that the function of leptin in natural situations might be its ability to regulate the neuroendocrine response to fasting including a prolonged dioestrus and delayed oestrus of female mammals, lowered levels of serum testosterone, luteinizing hormone and thyroxin and increased levels of serum corticosteroids and ACTH (Ahima et al., 1996). As leptin levels fall with decreasing adipose tissue mass, the increased production of neuropeptide Y in hypothalamus triggers this neuroendocrine response crucial to the survival of animals in the time of inadequate nutrition, e.g. in winter.

The common shrew (Sorex araneus L.) is a small insectivore living in Northern Europe and Asia. It has a short life span lasting about one and a half years at most. It has been shown that during the life of the common shrew its body lenght and certain other dimensions display a significant range of seasonal variation (Dehnel, 1949; Pucek, 1955; Hyvärinen, 1969). The mean body weight of young subadult shrews decreases in the autumn after the molt and is much lower in the winter than in the summer. The shrews shorten their spine in the winter by decreasing the volume of the nuclei pulposi in the intervertebral discs of the spinal column (Hyvärinen, 1969). Sexual maturity is attained in the spring after overwintering, and the adult common shrew is about 60% heavier than the wintering subadult and about 30–50% heavier than a young subadult in the previous summer (Dehnel, 1949; Pucek, 1955; Hyvärinen, 1969).

The common shrew has developed adaptations for surviving winters with extremely low ambient temperatures. Shrews (Sorecidae) are very small mammals and their specific insulation is poor (Hissa and Tarkkonen, 1969). Shrews also have a very high basal metabolic rate (Nagel, 1994). We have chosen the common shrew as a model of seasonal changes in the leptin levels of small actively wintering mammals, because practically all the adipose tissue of the common shrew is BAT (Hyvärinen, 1994). This has enabled us to study the possible expression of leptin protein by the BAT of adult mammals as well as to determine the leptin levels of the animals according to the seasonal changes in nutrition and environmental temperature.

Materials and Methods

For this study 39 common shrews were collected between August 1998 and July 1999 using traps that killed the animals immediately. The traps were checked daily to avoid any postmortal denaturation of peptides. The specimens were then frozen in −20 °C. It has been demonstrated that leptin remains stable for two months in +4 °C and at least five cycles of freezing and thawing can be tolerated without errors in assay results (Ma et al., 1996).

The winter in Eastern Finland, where the specimens were collected, was slightly colder than on average. The lakes froze over and permanent snow fell in late November offering some shelter from the falling temperatures. The coldest months were January and February and there was a week in January and another week in February when the temperatures remained at −30 °C for several days (Fig. 1). The thaw began in March, and the snow melted in April.

Due to the small size of the common shrew it was impossible to obtain enough plasma for the measurements of leptin levels in the circulation of the animals. Therefore, the animals were weighed and the interscapular brown adipose tissue (IBAT) was dissected and homogenized in 1 ml of assay buffer breaking the cell membranes and gaining access to the intracellular proteins. The assay buffer we used was a commercial 0.05 phosphosulphate pH 7.4 buffer from the multi-species leptin radioimmunoassay kit® containing 0.025 mM EDTA, 0.1% sodium azide, 0.05% Triton X-100 and 1% radioimmunoassay grade bovine albumin (Anon., 1998). The homogenized samples were centrifuged at 1000xg and the water-soluble fraction was extracted and then used for analysis. We also homogenized three heart and three liver samples in the same way to obtain control tissues for the analysis.

The leptin levels were measured using the radioimmunoassay method developed by Ma et al. (1996). We used the multi-species leptin radioimmunoassay kit® developed by Linco research (Anon., 1998). For the actual measurements the
gamma counter of the Central Hospital of Joensuu was used.

The results from the gamma counter were expressed as ng/l in water-soluble fraction of the extract. From these results, the leptin concentrations as ng/g BAT and as ng in BAT/g body weight were subsequently calculated. The results are expressed as the mean ± SE.

The statistical analysis was performed using one-way analysis of variance (ANOVA) followed by a post hoc Duncan’s test (p<0.05). Some groups were also compared to each other using a two-tailed t-test to test possible statistically significant differences in groups showing borderline differences in the Duncan’s test (p<0.05). Correlations, standard deviations and average values were calculated in the usual manner.

Results

The weight of the animals fell significantly from August to November and stayed at a low level until spring. The overwintered adults had a mean body weight significantly greater than the other specimens (Table I).

The absolute weight of the IBAT was the lowest in the specimens caught in November and the highest among the overwintered adults caught in the summer, but the differences were not statistically significant. The relative weight of the interscapular BAT (IBAT/body weight) showed no significant seasonal fluctuations (Table I).

The leptin concentrations in all IBAT samples were above detection limit. The multi-species radioimmunoassay kit we used is not specific to the leptin of the common shrew. Therefore, our results might show leptin concentrations considerably lower than the actual concentrations present in the BAT cells. The leptin concentrations in IBAT were between 0.70 ng/g in November and 55.70 ng/g in January. These values are comparable to the leptin levels found in human and rodent (mouse and rat) plasma (see Maffei et al., 1995).

In the three heart muscle samples no detectable amounts of leptin were found. Two of the three livers analyzed yielded small but detectable amounts of leptin (1.64, 0.00 and 2.31 ng/g liver tissue). The amounts were about one fifth of the levels measured in the IBAT of the same individuals (7.57, 13.33 and 11.08 ng/g IBAT, respectively). The detectable leptin in the liver probably represents the large amount of blood in liver tissue.

There was a significant fall in the mean leptin concentration of the BAT from June-August (summer subadults) to November. After November
there was a rise in the leptin concentrations and the levels reached about 23 ng/g in December-January. The mean of leptin levels in April-May was about 16 ng/g. After that the leptin levels decreased a little, but not significantly. The leptin concentrations of wintered adults in April-May were, however, nearly significantly higher than in nonwintered subadults in June-August (p=0.09, t-test) (Fig. 2). The most clearly distinguishable change was the significant fall of leptin levels in November followed by the rise in the mid-winter (Fig. 2). The leptin concentrations in relation to the body weight of the specimens showed also clearly the statistically significant rise in the mid-winter (Fig. 2). Unlike previously observed in rodents and humans (Maffei et al., 1995; Considine et al., 1996; Stamogiannou et al., 1997), the leptin concentrations in the BAT of the shrew showed no significant correlation with the weight of the specimens (r = 0.124).

**Discussion**

The winter is a challenge for small mammals in two ways. The availability of nutrition diminishes and the low temperatures pose a threat to survival. The relative importance of non-shivering thermogenesis (NST) of the BAT is greater in small mammals that are cold acclimated (Horwitz, 1989). NST is very important to the common shrew. Compared to other mammals they consume food at a high rate because of their higher basic metabolism and poor specific insulation (Hyvärinen, 1994). Heat production by the BAT is enhanced by various agents, such as insulin, which is also an activator of the leptin-producing *ob*-gene. It is also induced by leptin itself (Scarpae et al., 1997).

The role of leptin in the seasonal adaptation of mammals remains unclear, but it seems that the leptin levels are also influenced by changes in the photoperiod (Mercer et al., 2000). In the garden dormouse (*Eliomys quercinus*) it has been shown that exogenous melatonin increases leptin gene expression (Ambid et al., 1996). The leptin levels in the shrew, however, do not simply seem to follow the photoperiod. If photoperiodism had been the main determinant we would have expected to see a rise in the leptin levels as early as in November, when the leptin levels in fact decreased and rose significantly only thereafter in December-January.

There is a direct correlation between the body-mass index (BMI) and leptin concentration in the plasma of humans and rodents (Maffei et al., 1995; Considine et al., 1996; Stamogiannou et al., 1997). The weight of the shrews was, however, very low in December-January, when the leptin levels were the highest. The leptin concentration in IBAT was very low in November, although the shrews faced a cold environment at that time, too. The influence of the sympathetic nervous system (SNS) might also be of importance here. In rats it has been observed that the SNS suppresses the leptin gene expression in BAT (Li et al., 1997). Due to the cold ambient temperature in November there could be increased SNS outflow to BAT, which would lead to increased lipid depletion or utilization in BAT. This could explain the slight although statistically insignificant decrease in IBAT weight in November (Table I). After November, the snow cover protects the animals from the cold. This would lead to a decrease in the SNS outflow and thus disinhibition of the leptin gene expression resulting in the observed rise of leptin levels during the months of snow cover (Fig. 2).

On the other hand, it has been suggested that there is a physiological signal informing mice and other small mammals about how much to eat at different temperatures. This signal should originate from the tissue sensitive to changes in temperature, BAT, and be generated in inverse relationship to ambient temperature (Melyn and Himms-Hagen, 1998). Because the leptin levels in IBAT are the highest in the mid-winter, we sugg...
gest, that leptin functions as a signal produced by the BAT in the shrew to inform the central nervous system about the amount of nonshivering thermogenesis needed for survival. As leptin has been shown to be able to enhance thermogenesis in rat infants (see Blumberg et al., 1999), it could have the same function in wintering subadult shrews that unlike rats remain dependent on nonshivering thermogenesis for their whole lives.

Leptin is also a potent inhibitor of the production of neuropeptide Y, which inhibits nonshivering thermogenesis in BAT (Billington et al., 1994). There is a probable relationship between leptin, NPY and the energy expenditure in BAT (Boyer et al., 1996). The high leptin levels in the midwinter keep the production of NPY inhibited, which could be one of the possible pathways of leptin action on BAT of the shrew.

Thus the leptin levels in the BAT of the common shrew are not simply determined by the amount of fat present in their bodies. Instead of this the decisive factor for the amount of leptin produced in the winter could be a decrease in the body temperature, e.g. in the hypothalamus, caused by the fall of the ambient temperature. The effects could be mediated via the central nervous system by feeding behaviour or paracrinically in the BAT itself (see Siegrist-Kaiser et al., 1997). The SNS could also play an important role here by down-regulating the amount of leptin produced during the crucial period of cold without snow cover in November. In the common shrew it seems that leptin plays an adaptive function not only related to the control of body-weight but also to the survival of insectivores in the boreal climate by regulation of thermogenesis.

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