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Short photoperiod reduces leptin gene expression in white and brown adipose tissue of Djungarian hamsters

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Abstract Leptin gene expression in white (WAT) and brown adipose tissue (BAT) of the Djungarian hamster (*Phodopus sungorus*) was analyzed during seasonal acclimatization. Leptin gene expression in WAT was markedly reduced during winter, independent of changes in environmental temperature. Exposure to artificial short photoperiod also decreased leptin gene expression in WAT as well as in BAT. Although specific leptin gene expression was lower in BAT, total depot expression was as high as in WAT depots, due to higher RNA content of BAT. Our results indicate that there is significant leptin synthesis in brown fat and that leptin might be involved in photoperiod mediated seasonal adaptations of mammals independent of food deprivation or overfeeding.

Key words: Leptin; *obese* gene; Seasonal acclimatization; Brown and white adipose tissue; Djungarian hamster

1. Introduction

Leptin is the product of the *obese* gene whose mutation leads to an obese phenotype in the (*ob/ob*) mouse [1]. Leptin is expressed and secreted exclusively by adipocytes and is considered to be an adipostatic signal linking energy metabolism and the regulation of food intake [1–5]. Plasma leptin levels as well as leptin mRNA levels are increased in human obesity as well as in rodent models of obesity [6]. Recently, leptin has also been described as a metabolic signal to the reproductive system involved in the neuroendocrine response to fasting [7,8].

Most studies concerning the possible function of leptin have been conducted in animal models of genetically or otherwise artificially induced obesity like the *ob/ob* and *db/db* mouse. In this study we investigated leptin gene expression in white and brown adipose tissue of the Djungarian dwarf hamster (*Phodopus sungorus*). This hamster shows a remarkable, natural 'seasonal obesity'. Its body weight of approximately 45 g in summer is reduced to 25 g in winter due to a drastic loss of body fat content. Winter acclimatization is mainly controlled by short photoperiod, i.e. an increase in night length, and includes gonadal atrophy and a display of daily torpor [9–14]. Another typical feature of the Djungarian hamster is its abundance of brown adipose tissue (BAT), a thermogenic tissue whose thermogenic capacity is increased upon cold exposure and by short photoperiod adaptation [10,14]. Our aim was to elucidate in this animal model possible seasonal variations of leptin gene expression of WAT and BAT, independent of food deprivation or overfeeding.

2. Material and methods

2.1. Animals and experimental set up

Djungarian hamsters (*Phodopus sungorus*) were bred and raised in Marburg, Germany (51°N latitude and 9°E longitude) as described [9]. All animals were housed individually after weaning and were fed a high protein hamster diet ad libitum (Altromin, Germany). Animals were killed between 8.30 and 10 a.m. by cardiac puncture after CO_2 anesthetic. Total brown fat and inguinal white fat were excised, weighed to the nearest mg and frozen in liquid nitrogen for subsequent analysis. Prior to RNA extraction and cytochrome *c* oxidase activity measurement, tissues were ground to a powder in liquid nitrogen.

For the first experiment (seasonal acclimatization), all hamsters were kept at natural photoperiod throughout the year either at 23°C which is thermoneutral (= indoors) or in an outdoor enclosure, subjected to changing ambient temperature (= outdoors). At the time of the experiments, mean minimum temperatures in June and February were 10°C and −6°C, respectively.

For the second experiment (short photoperiod adaptation), animals at least 3 months of age were used that had been kept in long photoperiod (16 h light, 8 h dark = long day, LD) at 23°C since weaning. Fourteen animals were transferred into short photoperiod (8 h light, 16 h dark = short day, SD). After 85 days 13 of these animals displayed a change in fur color from dark summer fur to light winter fur. One animal, which did not change fur color, nevertheless had atrophied testis. We thus considered all 14 animals responsive to photoperiod adaptation. SD animals were killed on day 85 or 86 of SD exposure between 8.30 and 10 a.m. (lights came on at 8 a.m.). Six control LD animals were killed at the beginning of the experiment and another six at the end of the experiment (i.e. 85 days later). As the two control groups showed no differences in any of the analyzed parameters, they were grouped for subsequent statistical analysis.

2.2. Body composition

For analysis of body composition the gastrointestinal tract was removed and animals dried to constant weight at 90°C. Fat free dry body mass was determined after lipid extraction with chloroform using a Soxhlet apparatus.

2.3. Cytochrome c oxidase activity (COX)

COX activity was measured polarographically in total tissue homogenates using a Clark type electrode (Hansatech system) as described [15].

2.4. Northern blot analysis

Total RNA was prepared from individual tissue depots using TRIZOL reagent (Life Technologies). 10–20 μg of total RNA was electrophoresed in 1% agarose gel containing formaldehyde and transferred to Hybond N membranes (Amersham) by capillary blotting. Equal loading of gels was checked visually by staining the membranes with bromophenol blue. Hybridization was performed using cDNA probes of the mouse leptin (*obese*) gene (donated by J.F. Friedman, Rockefeller University, New York), mouse uncoupling protein (UCP, donated by D. Ricquier, CNRS, Paris), and mouse lipoprotein lipase (LPL, donated by M. Schotz, UCLA, Los Angeles). cDNA probes

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Abbreviations: BAT, brown adipose tissue; COX, cytochrome *c* oxidase activity; iWAT, inguinal white adipose tissue; LD, long day; LPL, lipoprotein lipase; SD, short day; UCP, uncoupling protein; WAT, white adipose tissue

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were labeled by random priming with [α-^{32}P]dATP and RNA blots were hybridized at 68°C in Quick-Hyb hybridization mix (Stratagene), then washed at high stringency and exposed to Kodak X-AR film at −70°C. In some experiments slot blots were performed additionally. Autoradiographs were analyzed by scanning densitometry for quantitation of RNA signals. RNA signals were normalized using a 28S ribosomal probe.

For determination of statistical significance of differences between groups Student's unpaired *t*-test was used.

3. Results

3. . *Seasonal acclimatization*

As shown in Fig. 1, Djungarian hamsters subjected to natural changes in photoperiod displayed a reduction in body weight of 30–40% from June to December, confirming earlier findings [9]. This reduction was manifest not only in hamsters kept outdoors and thus exposed to seasonally changing ambient temperature but also in hamsters kept indoors at thermoneutrality throughout the entire year. Because both groups of hamsters were fed ad libitum this seasonal body weight reduction was clearly not the result of food restriction. The reduced body weight was mainly due to a decrease in body fat as reflected by a decrease in inguinal white fat (iWAT) weight (Table 1). Winter acclimatization was also accompanied by a marked gonadal atrophy with testis weight reduced from over 900 mg in summer to less than 50 mg in winter (Table 1).

There was a significantly higher specific leptin mRNA expression in summer than in winter (Table 1). The seasonal pattern of leptin gene expression in WAT closely paralleled seasonal changes in body weight and both followed changes in photoperiod with highest values in June and lowest in December. Due to the fact that total RNA content of iWAT was also higher in summer than in winter, this seasonal pattern was especially pronounced when total depot leptin mRNA was calculated (Fig. 1).

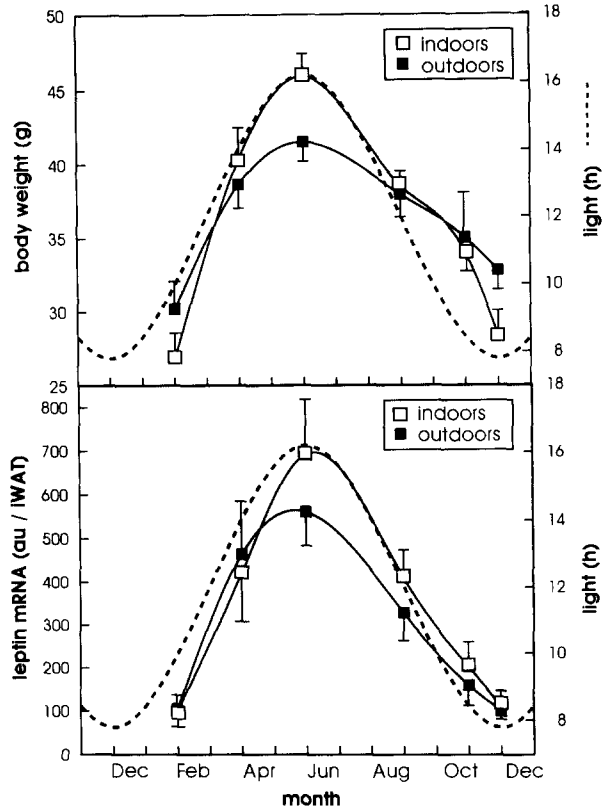

Fig. 1. Seasonal changes in body weight (upper panel) and total leptin mRNA levels in inguinal white fat (lower panel) of Djungarian hamsters kept at natural photoperiod at either 23°C (indoors: open squares) or in an outdoor enclosure (outdoors: black squares), subjected to changing ambient temperature. The hatched line indicates the natural day length (hours of light) throughout the year. Hamsters were killed at different times of the year, total RNA was prepared from individual inguinal WAT tissue depots and Northern blot hybridization was performed using a leptin cDNA probe as described in Section 2. Data are means ± S.E.M., $n = 6$–13 per group (au: arbitrary units).

3 2. *Short photoperiod adaptation*

This experiment was performed in order to clarify whether changes in leptin gene expression observed during seasonal acclimatization were triggered by photoperiod or were due to an endogenous rhythm. As shown in Table 2, after 85 days of SD adaptation, hamsters had decreased their body weight by 17% and undergone a gonadal atrophy comparable to the winter animals in the first experiment. The reduction of body weight in SD was entirely due to a reduction in body fat content as total dissectable white fat mass was reduced by 50% and fat free dry body mass was not different between LD controls and SD animals (Table 2).

Table 2 shows the specific expression of the leptin, lipoprotein lipase (LPL), and uncoupling protein (UCP) genes in WAT and BAT upon SD adaptation. The only significant effect was a reduction of leptin mRNA in both WAT and BAT. However, the leptin mRNA levels per unit total RNA were 4–5 times lower in BAT than in WAT.

Table 1
Seasonal changes in testis weight and white fat weight, RNA content and gene expression of Djungarian hamsters

Parameter		February	April	June	September	November	December
Testis weight	indoors	10 ± 1	570 +30	960 ± 60	560 ± 100	50 ± 10	40 ± 10
(mg)	outdoors	180 ± 1	730 ± 70	900 ± 50	610 ± 230	50 ± 10	70 ± 20
iWAT weight	indoors	0.69 ± 0.11	1.59 ± 0.16	2.04 ± 0.26	1.97 ± 0.36	0.99 ± 0.16	0.63 ± 0.11
(g)	outdoors	0.41 ± 0.08	1.24 ± 0.12	1.76 ± 0.09	1.34 ± 0.16	0.72 ± 0.09	0.53 ± 0.08
iWAT RNA	indoors	76 ± 8	141 ± 17	230 ± 18	193 ± 28	107 ± 13	82 ± 15
(μg)	outdoors	99 ± 14	155 ± 21	177 ± 12	167 ± 18	111 ± 8	94 ± 9
Leptin mRNA	indoors	750 ± 140	1530 ± 320	1640 ± 230	1260 ± 170	1010 ± 280	680 ± 140
(au/μg RNA)	outdoors	570 ± 230	1540 ± 310	1810 ± 270	1010 ± 140	740 ± 190	670 ± 150

Djungarian hamsters were kept at natural photoperiod in Marburg, Germany (51°N latitude and 9°E longitude) at constant 23°C which is thermoneutral (indoors) or in an outdoor enclosure (outdoors), subjected to changing ambient temperature. All animals were killed between 8.30 and 9.30 a.m. at the beginning of the light cycle. Total RNA was prepared from individual tissue depots and Northern blot hybridization performed using a cDNA probe to mouse leptin (*obese*) gene as described in Section 2. Data are means ± S.E.M., $n = 6$–16 per group. au, arbitrary units.

Table 2
Effects of short day adaptation on organ weights, RNA content and gene expression of inguinal WAT and BAT

Parameter		Long day	Short day	P-value
Body weight (g)		39.9 ± 1.18	33.0 ± 1.35	< 0.001
Testis weight (mg)		846 ± 80	50 ± 6.8	< 0.0001
Fat free, dry body mass (g)		7.24 ± 0.29	6.86 ± 0.24	n.s.
Inguinal WAT weight (g)		1.524 ± 0.111	0.814 ± 0.145	< 0.005
Total WAT weight (g)		5.42 ± 0.36	2.68 ± 0.44	< 0.0001
BAT weight (g)		1.311 ± 0.102	0.996 ± 0.075	< 0.02
Total RNA	iWAT	194 ± 19.5	129 ± 13.2	< 0.01
(μg/tissue)	BAT	582 ± 47.6	503 ± 26.6	n.s.
LPL mRNA	iWAT	7643 ± 931	6513 ± 850	n.s.
(au/μg RNA)	BAT	3633 ± 209	3728 ± 223	n.s.
UCP mRNA	iWAT	–	–	–
(au/μg RNA)	BAT	5983 ± 441	6343 ± 317	n.s.
Leptin mRNA	iWAT	5181 ± 450	3326 ± 675	< 0.05
(au/μg RNA)	BAT	1331 ± 147	619 ± 80	< 0.001

All animals were raised and kept in long photoperiod (16 h light/8 h dark) at 23°C (thermoneutrality). For short day adaptation animals were transferred into a clima chamber with 8 h light/16 h dark (23°C) for 85 days. All animals were killed between 8.30 and 9.30 a.m. at the beginning of the light cycle. Analysis of body composition and Northern blots were performed as described in Section 2. Data are means ± S.E.M., $n = 11$–14 animals per group. P-value corresponds to the long day group compared to the short day group by t-test. au, arbitrary units.

Fig. 2 shows gene expressions per total tissue depot, as well as markers for brown adipose tissue thermogenic capacity, i.e. COX activity and UCP gene expression. The reduction of leptin gene expression is even more pronounced because total RNA of iWAT and BAT also decreased upon SD adaptation. Brown fat COX activity, however, was largely increased by SD adaptation, whereas UCP and LPL mRNA levels did not change. It should be noted that leptin mRNA levels of total BAT (Fig. 2B) are quite comparable to those of total iWAT, due to the much higher RNA content of BAT (Table 2).

Fig. 3 shows that leptin gene expression in BAT as well as in WAT correlates closely with the tissue mass (Fig. 3A) as well as body weight (Fig. 3B).

4. Discussion

The Djungarian hamster displays drastic seasonal fluctuations in many physiological parameters. Our data (Table 1, Fig. 1) corroborate previous findings that seasonal changes in body weight and gonadal, i.e. reproductive, status are almost exclusively triggered by changes in photoperiod [9–15]. Changes of iWAT mass could also be attributed for almost 90% to photoperiod with ambient temperature having only a minor modulating function (Table 1).

Leptin gene expression in iWAT (Fig. 1) showed almost the same seasonal pattern as body weight. Interestingly, leptin mRNA levels followed even more closely the decline of day length in fall than body weight which was still quite high in November, when leptin expression was already very low. Ambient temperature had no significant effect on seasonal changes in leptin expression, corroborating findings in rats where chronic cold exposure also did not change leptin expression [14], although an acute cold stress seems to suppress leptin gene expression in white and brown fat [16,17]. Hamsters living outside throughout the year have metabolic requirements that are about doubled in summer and tripled in winter compared to hamsters living at thermoneutrality [9]. This means that leptin gene expression in WAT does not indicate the metabolic status of an animal.

BAT as a thermogenic, i.e. energy dissipating, organ has the opposite physiological function from the energy storing white fat. Its thermogenic function is due to a high respiratory capacity and the presence of the mitochondrial UCP which uncouples respiration from ATP synthesis [18]. Short photoperiod adaptation induced a considerable increase in BAT thermogenic capacity which was manifest in an increase of total tissue COX activity, a marker for mitochondrial respiratory capacity (Fig. 2B). UCP gene expression was not increased by short photoperiod, which is in accordance with previous findings that UCP content of BAT is primarily stimulated by cold exposure and to a much lesser degree by short photoperiod [18]. It was also shown in vitro that UCP gene expression is mainly activated by the sympathetic nervous system acting through β_3-adrenergic receptors [19].

We found significant leptin gene expression in BAT which was also suppressed by short photoperiod adaptation (Table 2, Figs. 2B and 3). Although specific leptin mRNA levels were much lower in BAT than in WAT, total depot leptin mRNA levels were quite comparable in both tissues, due to higher RNA levels in BAT (Fig. 3). It is thus highly improbable that leptin expression in BAT resulted from contaminating white adipocytes, especially since in vitro studies showed that over 90% of hamster BAT adipocytes express UCP and are thus truly brown adipocytes [20]. A significant leptin gene expression has previously also been shown in BAT of rats [16].

WAT and BAT have opposing physiological functions for energy storage and energy dissipation, and as shown previously, LPL is differently regulated during seasonal acclimatization [21]. Accordingly, photoperiod regulation of LPL gene expression was different in both tissues (Fig. 2A,B). Leptin mRNA levels, however, were regulated the same way in both tissues. Additionally, in both fat types total depot leptin mRNA content correlated much better with depot mass than the specific leptin RNA content (Fig. 3A). This indicates that leptin gene expression in fat is indeed an internal signal of body fat content and thus body weight. Indeed, leptin mRNA levels in iWAT and BAT also correlated very strongly with body weight (Fig. 3B).

Acute cold exposure acting through the sympathetic ner-

vous system has been found to suppress leptin gene expression in white fat of mice [17]. Our findings imply that suppression of leptin gene expression by short photoperiod is regulated via a different pathway and not mediated by the sympathetic nervous system, since cold exposed hamsters from outdoor conditions had the same leptin mRNA levels as hamsters living at thermoneutrality. Interestingly, the reduction of leptin gene expression in natural and artificial short photoperiods was concomitant with gonadal atrophy, fitting nicely with recent observations that leptin stimulates the reproductive endocrine system [7,8]. Further studies should elucidate if leptin does indeed have an effect on reproductive status of the Djungarian hamster.

Leptin mRNA expression in white as well as in brown fat correlated closely with tissue mass and body weight in our animal model of natural, non-pathological fluctuations in body fat content. Despite low leptin gene expression during the winter, i.e. after short photoperiod adaptation, Djungarian hamsters did not become hyperphagic. This indicates that in normal physiological situations leptin does not primarily act as a satiety factor but may serve as an internal signal of body weight, independent of the actual set point for body mass (Fig. 3B). This hypothesis is supported by the fact that very high doses of recombinant leptin are required to induce a body weight reduction [2,3], although recombinant human leptin seems to be virtually identical with the wild type protein [22].

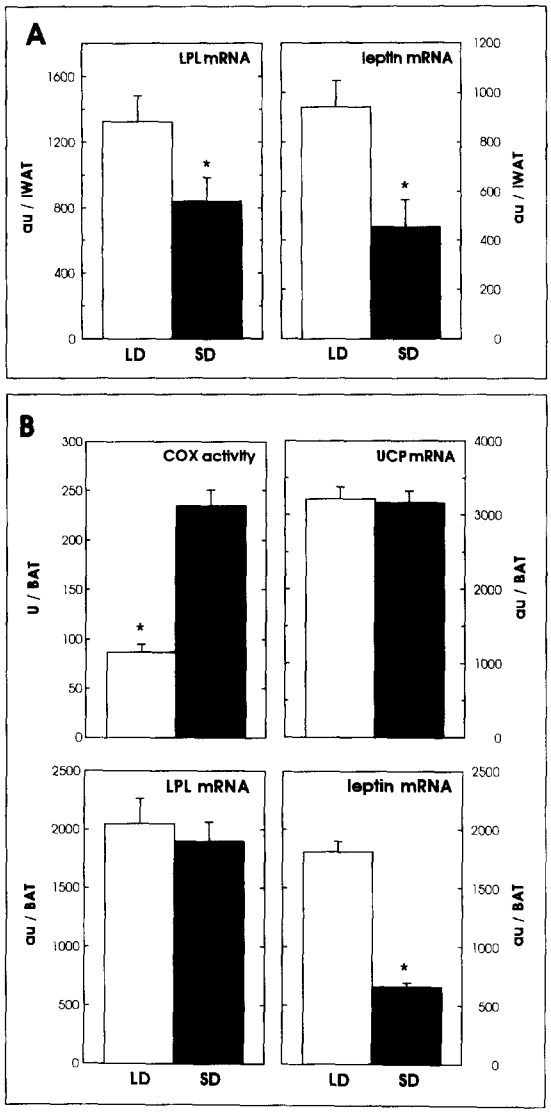

Fig. 2. Effects of artificial short day on white and brown fat of Djungarian hamsters. For treatments of animals see Table 2. LD: long day (open columns); SD: short day (black columns). Asterisks indicate significant differences between LD and SD animals ($P<0.05$). A: Gene expression in iWAT. The left panel shows the LPL mRNA and the right panel the leptin mRNA levels of the total iWAT depot. B: Thermogenic activity and gene expression in BAT. The upper left hand panel shows the COX activity of total BAT as an indicator of thermogenic capacity. The upper right hand panel shows UCP mRNA expression of total BAT and the lower half LPL mRNA (left) and leptin mRNA (right) levels.

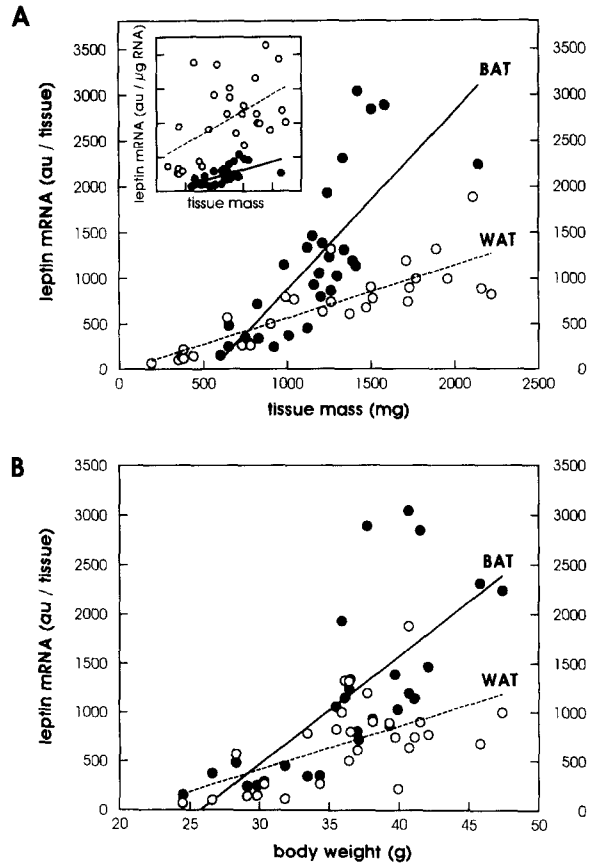

Fig. 3. Correlation of leptin mRNA expression in white and brown adipose tissue with depot mass and body weight. Open circles and hatched line: iWAT; black circles and solid line: BAT. A: Total leptin mRNA levels in iWAT and BAT correlate with tissue mass ($r=0.83$, $P<0.0001$ for WAT, and $r=0.77$, $P<0.0001$ for BAT). The inset shows specific leptin mRNA expression, i.e. per unit total RNA in relation to tissue mass. For both iWAT and BAT there is also a significant, though lower correlation ($r=0.55$, $P=0.028$ for WAT and $r=0.59$, $P=0.0007$ for BAT). B: Total leptin mRNA levels in iWAT and BAT also correlate with body mass ($r=0.57$, $P<0.002$ for WAT, and $r=0.72$, $P<0.0001$ for BAT).

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