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Possible mechanisms of weight loss of Siberian hamsters (*Phodopus sungorus sungorus*) exposed to short photoperiod

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Several weeks of short day photoperiod (SD) exposure promote a dramatic decrease of white adipose tissue (WAT) mass in Siberian hamsters *(Phodopus sungorus sungorus)*. This slimming effect is accompanied by changes in the adipocyte responsiveness to adrenergic stimulation that are still under debate. We investigated whether possible changes in the antilipolytic responses, and/or lipogenic activities could be involved in such lipid deposition/mobilisation imbalance. Male Siberian hamsters were exposed for 11 weeks to SD or long day photoperiod and basal or stimulated lipolytic and lipogenic activities were measured on white adipocytes. As expected, the body mass of SD-animals was decreased. Besides a slight reduction in the basal lipolysis and in the maximal response to dibutyryl-cAMP, the responses to adrenergic and non-adrenergic lipolytic agents (forskolin, adenosine deaminase) were similar in both groups. Fat mass loss was likely not resulting from changes in the lipolytic responses of adipocytes to biogenic amines (e.g. octopamine), which were unaltered, or to a direct lipolytic stimulation by melatonin or histamine, which were inactive. Antilipolytic responses to insulin or tyramine were slightly decreased in SD-adipocytes. Basal or insulin-stimulated lipid accumulation in WAT, measured by glucose incorporation into lipids, did not change after SD-exposure. However, a significant decrease in the lipoprotein lipase activity was observed in the WAT of SD-animals. Despite the observed changes, the weight loss of SD-exposed Siberian hamsters was likely not resulting only from impaired antilipolytic or *de novo* lipogenic activities in white adipocytes, but either from other dramatic changes occurring during seasonal photoperiod-sensitive body weight regulation.

Key words: Adipocyte, Lipoprotein lipase, Catecholamines, PIA, β3-adrenergic receptors, Melatonin, Photoperiod, Hamsters.

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Most animals living at temperate latitudes exhibit seasonal changes in physiology and behaviour in anticipation to climatic and environmental changes (18, 19). Prior to winter, many of them reduce their food intake and body temperature. This is the strategy utilised by the Siberian hamster *(Phodopus sungorus sungorus)* to achieve these changes (7). These seasonal changes, which affect body weight and reproductive competence, are profound and require several weeks to complete. In nature, changes in photoperiod are gradual. Nevertheless it is possible to mimic these changes in the laboratory (5). Siberian hamsters exposed in short day photoperiod (SD was 8h:16h light:dark cycle) exhibited a reduction of food intake and increase in the peripheral sympathetic nervous system, leading to an activation of the brown adipose tissue thermogenesis (16) and a decrease of the white adipose tissue mass (4). It has been recently reported that such modifications are accompanying photoperiod-induced changes in the central nervous system by a down-regulation of H3-histamine receptor expression and in an increased neuronal activity in the hypothalamic arcuate nucleus (3).

At the level of peripheral tissues, the whole fat mass is mainly involved in the regulation of the energetic metabolism by a balance between fat mobilization (lipolysis) and deposition (lipogenesis). These metabolic pathways are regulated by a number of neurotransmitters and hormones (catecholamines, insulin, and glucagon) or by a feedback control action by a local secretion of metabolites (adenosine) or transformed compounds (biogenic amines) (13). We did not observe any clear-cut increase in the lipolytic responsiveness to adrenergic stimulation of the small adipocytes of SD-housed animals when compared to LD-housed controls (LD was 16h:8h, light/dark cycle) (1), while such responsiveness was increased according to Bowers et al. (6). However, the latter was observed within a small time frame (in 5 but not 10 weeks after exposure to SD) and in small amplitude (much lower than twofold increase), while the mass of adipose depots was more than half-reduced by SD-acclimation. We therefore decided to extend the verification of the lipolytic capacities of adipocytes from either LD- or SD-housed Siberian hamsters to non-adrenergic activators of lipolysis, such as forskolin, dibutyryl cAMP (dbcAMP) or trace amines. Furthermore, we also tested several antilipolytic systems, such as that of the endogenous adenosine, which was explored by removing its inhibitory tone with the addition of adenosine deaminase (ADA) or with testing its analogue PhenylIsopropylAdenosine (PIA). We also tried to counteract the β-adrenergic lipolytic effect of isoprenaline by insulin or tyramine in the adipocytes from SD- and LD- housed hamsters. Lastly, since melatonin is increased during short "winterlike" days (5, 14, 23) and has been described to activate the sympathetic tone (12), we tested whether melatonin could also directly influence the adipocyte lipolytic and lipogenic activities. Then, in the last part of this study, we assessed the effect of 11 weeks of SD-exposure; by comparison to the same extend of LD-exposure, on the adipocyte capacity to store lipids as triglycerides. The *de novo* lipogenesis was assessed by the level of glucose incorporation into lipids of isolated adipocytes, and the flux of fatty acids from the circulation was indirectly quantified by the measurement of epididymal adipose tissue lipoprotein lipase (LPL) activity.

We confirm that, after 11 weeks of exposure to a SD-photoperiod, the hor-

monal regulation of lipolysis of adult Siberian hamsters was somewhat modified but not deeply altered when compared to LD- exposed animals. Moreover, the capacity of fat cells to incorporate glucose into lipids was not modified, while the lipoprotein lipase activity was dramatically reduced.

Materials and Methods

Chemicals.– (-)Isoprenaline-hydrochloride, dibutyryl cyclic AMP (dbcAMP), (-)adrenaline-bitartrate, bovine serum albumin, bovine insulin, and collagenase type II (C-6885) were purchased from Sigma Chemical Company (St Louis, MO, U.S.A.). Adenosine deaminase, PIA, glycerol kinase and glycerol phosphate dehydrogenase were obtained from Boehringer Mannheim.

Animals and protocols.– Adult male Siberian hamsters (*Phodopus sungorus sungorus*) were raised on a long-day cycle in our breeding colony. For the comparison between LD and SD, age-matched animals were housed individually at 20-24°C and exposed during 11 weeks before sacrifice to the studied photoperiod. The animals were weighed weekly. At the end of the photoperiod exposure, hamsters were killed by decapitation between 9 and 10 a.m. In each set of experiment, the animals submitted to SD-exposure presented a testicular involution. White adipose tissues (WAT), epididymal (EWAT), perirenal (PWAT), inguinal (IWAT) and retroperitoneal (RWAT), were removed, dissected and pooled for adipocyte isolation and analysis of lipolytic and lipogenic responses, or frozen for furthers experiments.

Isolation of white adipocytes and lipolysis measurement.– Adipocytes were isolated according to the method of Rodbell (21) with only minor modifications (2). A pool of all the fat deposits was incubated with collagenase (1mg/ml), in Krebs-Ringer bicarbonate buffer containing BSA 3.5 %. Adipocytes were immediately used for determination of lipolytic activity in Krebs-Ringer solution buffered by 15 mM sodium bicarbonate, 10 mM HEPES, pH 7.5, and containing 5 mM glucose 3.5 % albumin (KRBHA) by measurement of glycerol released during 90 min as previously described (20). The mean blank value was substracted to the assays performed with cells to obtain the net release of glycerol originating from fat cells. The lipid content was determined gravimetrically after extraction, as described (10).

Incorporation of glucose into cellular lipids.– Lipogenesis was assessed by the incorporation of [^{3}H]-glucose into lipids separated by extraction in an organic mixture for scintillation counting according to MOODY and coworkers (17). Expression of results was performed as previously described (11).

Determination of lipoprotein lipase activity.– Samples of approximately 250 mg of EWAT were thawed and the heparin-releasable lipoprotein lipase (LPL) activity was measured according to the method of SAVARD *et al.* (22).

Statistical analysis.– Values are means ± s.e.m. Unless otherwise stated, unpaired Student's *t* tests were used for comparisons, differences being considered significant when p was smaller than 0.05.

Results

Effect of photoperiod on body weight gain.– Siberian hamsters were kept in SD photoperiod for 11 weeks and their body

mass was compared to that of age-matched animals exposed to LD photoperiod. Prolonged SD-exposure stopped the natural growth observed in animals exposed to LD (body weight gain: 3.0 ± 1.3 g) and even induced a decrease of around 15 % in body mass: body weight loss at 11 weeks was 5.0 ± 0.9 g in SD group (Fig. 1).

Influence of short photoperiod-induced emaciation on non-adrenergic lipolytic activitation in isolated adipocytes. – Unexpectedly, we observed a small but significant ($p < 0.05$) reduction in the basal lipolysis when expressed as µmoles of glycerol released per 100 mg lipids (Fig. 2A). The response to forskolin (which directly activates adenylyl cyclase) remained unaltered while the response to dbcAMP (which promotes direct PKA activation) could be slightly impaired. The antilipolytic effect of PIA, a non-hydrolysable analogue of adenosine, lowered the glycerol release to the same levels in both groups (Fig. 2A). Lastly, the lipolytic activation due to simple removal of endogenous inhibitory adenosine, by a maximal (Fig. 2A) or by increasing (Fig. 2B) doses of ADA, promoted the same lipolysis in LD- and SD-treated animals.

Influence of biogenic amines and antilipolytic agents on lipolytic activity.–

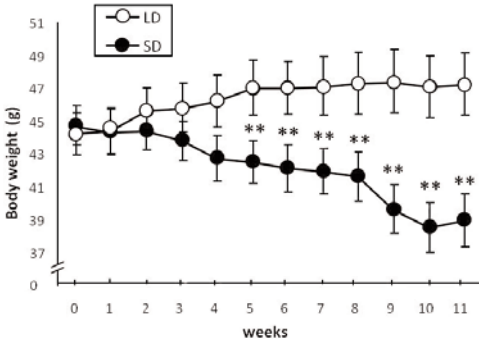

Fig. 1. *Effect of 11 weeks exposure to short day (SD) or long day (LD) photoperiod on body weight evolution of Siberian hamsters.*
Results are means ± s.e.m. of 6 to 8 animals.
**: p<0.01 *vs.* corresponding LD control.

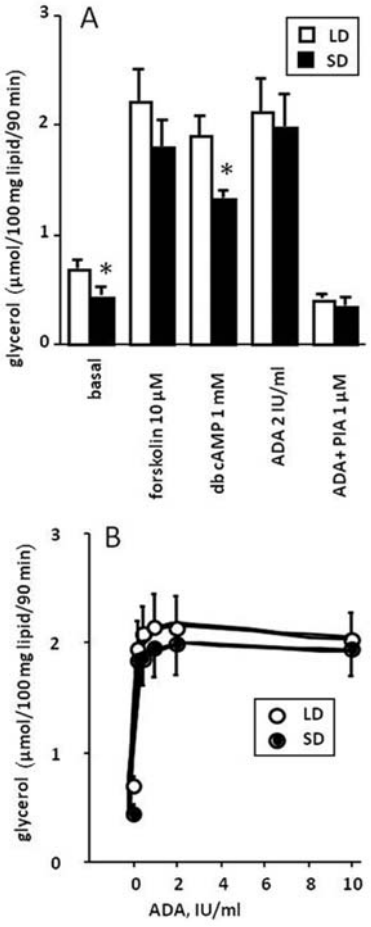

Fig. 2. *Influence of short photoperiod-induced emaciation on lipolytic activity of isolated adipocytes.*
A. Lipolysis activation by non-adrenergic lipolytic agents: forskolin, dibutyryl cyclic AMP (dbcAMP), adenosine deaminase (ADA) alone or with phenylisopropyladenosine (ADA+PIA). B. Dose-response curves for ADA-stimulated lipolysis.
Values are means ± s.e.m. of 5 experiments. *p<0.05 *vs.* LD.

Alongside the non-adrenergic agents tested above, rare biogenic amines have been reported to influence adipocyte biology. Among the biogenic amines tested, several increased lipolysis, but no difference was detected between LD- and SD-groups (Table I). Thus, octopamine completely reproduced the lipolytic action of the β-adrenergic agonist isoprenaline, taken as reference. On the opposite, 2-phenylethylamine, tyramine, and histamine were inactive (Table I).

Another cause of facilitated lipid mobilization could be the removal of antilipolytic regulators. Since the adenosine-dependent inhibition of lipolysis appeared to be as functional in SD- than in LD-treated hamsters (see Fig. 2A), it was tested whether responsiveness to the antilipolytic hormone of reference, insulin, was modified. Surprisingly, insulin was poorly efficient since it reduced the ADA-induced activation by only 26.6 ± 3.0 % in LD-treated hamsters (n = 5). This weak antilipolytic insulin effect was even reduced to 12.3 ± 1.9 % inhibition in SD-treated hamsters ($p<0.01$). The combination of 1 mM tyramine plus 0.1 mM vanadate, known to mimic insulin effects in adipocytes (15) was also altered in a similar way by short photoperiod: it fell from 31.0 ± 1.5 % inhibition in LD-treated to 15.4 ± 1.9 % inhibition in SD-hamsters (n = 5; $p<0.01$).

Table I. *Influence of biogenic amines (at 10 µM concentration) on lipolytic activity of Siberian hamster adipocytes.*

	Glycerol release	
	LD	SD
isoprenaline	100.0±2.3	100.0±3.4
adrenaline	84.5±3.4	95.2±3.3
octopamine	90.1±3.7	91.9±3.2
phenylethanolamine	55.5±9.4	60.9±7.9
tyramine	12.2±2.0	13.0±4.2
2-phenylethylamine	9.5±2.8	4.4±1.2
histamine	2.5±2.2	1.6±5.4

White adipocytes isolated from a pool of adipose tissues were treated as described in Materials and Methods. Maximal response to 10 µM isoprenaline taken as 100% reference were 2.9 ± 0.3 and 2.9 ± 0.1 µmoles glycerol released/90 min/100 mg lipid in LD- and SD-animals, respectively. Values are means ± s.e.m. of 4 to 5 experiments. No significant difference was found between LD and SD.

Effect of melatonin on lipogenic activity in isolated adipocytes.– In Siberian hamster adipocytes, insulin was more efficient in activating glucose incorporation into lipids than in inhibiting triglyceride breakdown (Fig. 3A). Since melatonin can reproduce the effects of SD on body mass regulation, it was verified whether this hormone could directly interact with the insulin lipogenic action in control Siberian hamsters grown under LD conditions. However, melatonin was inactive on short-term, direct regulation of lipid synthesis (Fig. 3A) as it was lacking of effect on lipolysis (not shown). Again, melatonin did not modify directly the adipocyte maximal response to 1 µM insulin (not shown) but slightly altered insulin sensitivity. The intermediate response to the insulin dose of 1 nM fell from 64.3 ± 2.1 % of maximal response with insulin alone to 50.9 ± 0.2 % in the presence of 1 µM melatonin (n = 4, $p<0.01$) (Fig. 3B).

Effect of SD-exposure on de novo *lipogenesis and on lipoprotein lipase activity*. - As shown in Fig. 4A, 10 nM insulin reached the submaximal activation in both groups: 84.1 ± 9.2 and 83.6 ± 3.6 %, respectively (n = 5; NS). The basal lipogenesis was also similar between LD- and SD-treated groups (29 ± 3 and 40 ± 7 nmoles of glucose incorporated/100 mg lipid/90 min; n = 5; NS). The insulin

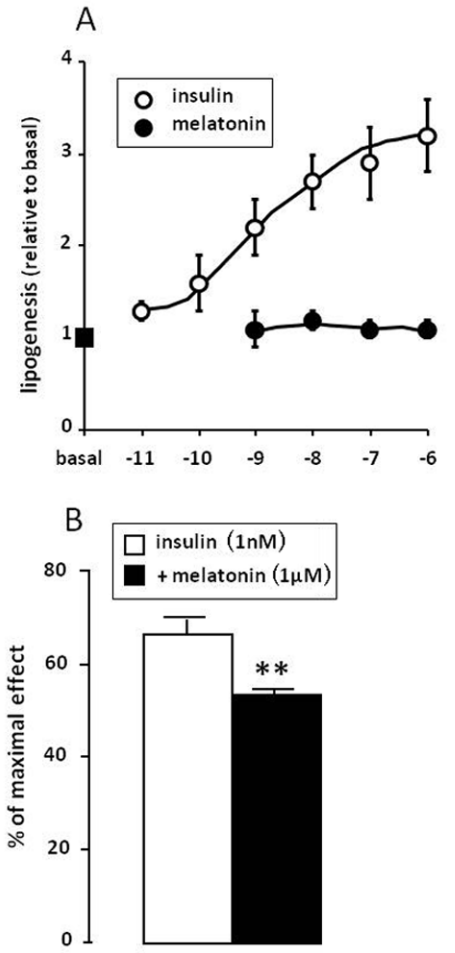

Fig. 3. *Lipogenic action of insulin and melatonin on white adipocytes of Siberian hamsters.*
A. Dose-responses curves for the hormones tested separately. Results are expressed as increase over basal lipogenesis, with basal set at 1 (square). B. Impairment of the submaximal effect of insulin by melatonin. Values are mean ± s.e.m. of 4 experiments performed on white adipocytes from hamsters breeded in long day and warm environment.

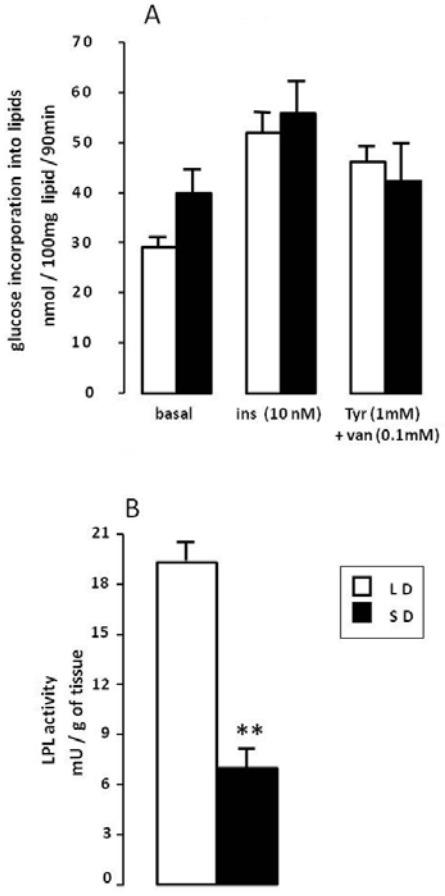

Fig. 4. *Lipogenesis in white adipose tissue.*
A. Comparison of the effects of 10 nM insulin and 1 mM tyramine plus 0.1 mM vanadate on *de novo* lipogenesis in Siberian hamster adipocytes after long exposure to short (SD) or long (LD) photoperiod. Glucose incorporation into lipids was measured on 90 min without (basal) or with indicated agents. B. Comparison of basal lipoprotein lipase (LPL) activities in epididymal adipose depots of SD- compared to LD-exposed animals. Means ± SEM of 5 experiments **, $p<0.001$ *vs.* LD.

activation of glucose incorporation into lipids was partly reproduced with tyramine (1 mM) plus vanadate (0.1 mM) and exhibited a tendency to be hampered in the short photoperiod group (Fig. 4B). The determination of the lipoprotein lipase activities in epididymal adipose depots of SD- and LD-exposed animals clearly showed a marked decrease in the activity of the SD-adipocytes: from 19.2 ± 2.4 to 7.6 ± 1.8 mU/g of tissue) (Fig. 4B).

Discussion

We confirmed, in this study, that Siberian hamsters exposed for more than ten weeks in a short winter-like photoperiod present a negative body weight gain compared to animals housed to a long day photoperiod (1). Moreover, the physiological adaptation to SD photoperiod was also attested by testes involution (not shown).

A dramatic increase in the lipolytic activity of isolated adipocytes was expected to occur in the SD-group since Siberian hamsters loose more than fifteen percent of their fat mass. Such increased lipid mobilization would have largely contributed to fat store reduction, leading to an increased basal and/or stimulated lipolysis in isolated fat cells when compared to LD-group. However, neither the basal lipolysis nor the capacity to respond to lipolytic stimulators was increased in fat cells from SD-group, as we already reported (1) and as observed by others in several but not all fat depots (6). The response to forskolin, which directly activates adenyl cyclase, remained unaltered while the response to the cAMP analogue was slightly impaired. Moreover, the effect of ADA, which promotes the endogenous antilipolytic feedback maintained by released adenosine was also unaltered. Lastly, the antilipolytic effect of PIA, a non-hydrolysable adenosine analogue similarly lowered glycerol release in both groups. All observations confirmed that the fat mass decrease observed after 11 weeks exposure to SD did not deeply alter the *in vitro* lipolytic regulation.

In our experimental conditions, lipolysis was studied in a "steady state" relative to body weight evolution, since weight loss reached a plateau between the tenth and the twentieth weeks (18). However, it has been argued that the reduction of adipose tissue in Siberian hamsters held under short photoperiod is mediated by enhanced lipolysis through sympathetic activation of adipose tissue, and by increased sensitivity to noradrenergic stimulation (23) before 5 weeks of exposure, when both body and lipid mass decrease rapidly and independently of food intake (25). BOWERS and coworkers found significant changes in the lipolytic responses of SD-adipocytes to β-adrenergic compounds mainly during this transition phase (6). However, the claimed increase in the potency of β-adrenergic stimulation by noradrenaline is less marked than the clear-cut SD-induced changes in adipocyte size. Similarly, the response to the selective β_3- agonist BRL 37344 did not exhibited impresive shift to the left in adipocytes from SD-animals in both studies (1, 6). In the same way, the dose-response curves for the lipolytic activation by ADA reported in the present study were superposed in SD- and LD-groups.

The antilipolytic effect of tyramine, previously described in other species (8) led us to test other biogenic or rare amines. Several of them were found to increase lipolysis, e.g. octopamine (9), but no difference was detected between LD- and SD-groups. None of these amines showed antilipolytic activity like tyramine did, or any difference in effectiveness relative to photoperiod exposure. Lastly, melatonin, one of the most important amine regarding regulation of circadian rhythms, was not directly active on adipocyte lipolysis despite the fact that: 1) there are functional receptors for melatonin in the neuronal sympathetic outflow from brain to adipose tissue; 2) their stimulation increases lipid mobilization (23).

Thus, in the absence of substantial changes in the lipolytic and antilipolytic responses, a decrease in lipogenesis was suspected to support the fat mass loss. Since melatonin reproduces the effect of SD on body mass (12), it was verified whether this hormone could impair the insulin lipogenic action in control Siberian hamsters grown under LD conditions. Like for lipolysis, melatonin did not modify directly the lipogenic activity, but, at micromolar dose, it slightly altered the insulin sensitivity. We hypothesized that such impairment of insulin lipogenic effect could be attributed to antioxidant properties of melatonin (24), as we were able to mimic the effect of insulin on lipogenesis of Siberian hamster by the use of mixture of hydrogen peroxide or tyramine and vanadate. However, the lack of difference between the stimulating effects of insulin on *de novo* lipogenesis in white adipocytes of LD- and SD-hamsters indicated that the increased melatonin of SD-animals was not sufficient to impair insulin action.

Lipid storage in WAT also results from fatty acids captured and re-esterified in adipocytes after the breakdown of circulating lipids by lipoprotein lipase. So, the lack of fat deposition observed after SD exposure could be explained by the decrease in adipose tissue LPL activity found in the EWAT. However, one needs to carefully extrapolate to the whole SD-acclimated animal whether the fall in LPL is a cause or a consequence of the slimming effect of SD-exposure because our measurements were performed only on one adipose depot (without any digestion process), in which the size (and probably the number) of adipocytes was decreased after SD-exposure, and because it is well established that LPL activity is strongly correlated with adipose cell size (22).

To summarize, *ex-vivo* responses of adipocytes to adrenergic and non-adrenergic activators, or to lipolysis inhibitors, or either to *de novo* lipogenesis activation did not clearly differ between LD- and SD-housed Siberian hamsters, in spite of the reduction of WAT found in the latter. We suspect that whatever the anatomical location of adipocytes, the changes in their environment are much more driving their lipid depletion in SD acclimation rather than dramatic changes in their own hormonal responsiveness, at least regarding to the above mentioned functions. Accordingly, recent results (3) clearly demonstrate that the neuroendocrine system is mainly involved in the photoperiod-induced reduction of adipose stores. The sympathetic nervous system activity is directly influenced by photoperiod-induced changes in the expression of H3-histamine receptors located on neurons of the dorsomedial posterior arcuate nucleus. These results are of the utmost importance to better understand the neuroendocrine stimuli regulating the fat depletion/repletion seasonal cycle which accompanies numerous other physiological adaptations of body weight.

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