Photoperiod and acute energy deficits interact on components of the thyroid hormone system in hypothalamic tanyocytes of the Siberian hamster

Annika Herwig,1 Dana Wilson,1 Tracy J. Logie,1 Anita Boelen,2 Peter J. Morgan,1 Julian G. Mercer,1 and Perry Barrett1

1Rowett Institute, University of Aberdeen, Bucksburn, Aberdeen, United Kingdom; and 2Department of Endocrinology and Metabolism, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands

Submitted 9 September 2008; accepted in final form 13 March 2009

Herwig A, Wilson D, Logie TJ, Boelen A, Morgan PJ, Mercer JG, Barrett P. Photoperiod and acute energy deficits interact on components of the thyroid hormone system in hypothalamic tanyocytes of the Siberian hamster. Am J Physiol Regul Integr Comp Physiol 296: R1307–R1315, 2009. First published March 13, 2009; doi:10.1152/ajpregu.90755.2008.—In the Siberian hamster, seasonal weight loss occurs gradually over many weeks during autumn and winter. This is driven by a regulatory mechanism that is able to integrate duration of exposure to short days (SDs) with the size of body energy reserves. After food restriction in SDs, followed by ad libitum refeeding, body weight of the hamster does not return to its former level; rather, it increases to a level defined by the length of time spent in SDs. In this report, we show that components of the thyroid hormone system that are involved in seasonal weight loss change expression in response to 48 h of starvation. Eight weeks in an SD photoperiod induced weight loss in the Siberian hamster. In the hypothalamus of these hamsters, type II deiodinase expression was decreased and type III deiodinase expression was induced, but there was no change in hypothalamic neuropeptide Y or thyrotropin-releasing hormone gene expression. For the first time, we show that the thyroid hormone transporter monocarboxylate transporter 8 is expressed in tanyocytes and is increased in response to an SD photoperiod. Food restriction (48 h of starvation) reversed the direction of gene expression change for type II and III deiodinase and monocarboxylate transporter 8 induced by SD photoperiods. Furthermore, fasting increased neuropeptide Y expression and decreased thyrotropin-releasing hormone expression. VGF, a gene upregulated in SDs in the dorsal region of the medial posterior area of the arcuate nucleus, is increased in response to starvation (7, 9, 14, 32), and as we recently demonstrated in the Siberian hamster, T3 acting in the brain regulates several aspects of seasonal physiology, including long-term seasonal energy expenditure (2). Common to both of these regulatory mechanisms is the regulation of deiodinase activities in tanyocytes lining the third ventricle of the hypothalamus. The response to starvation involves an induction of D2 deiodinase, which increases T3 availability to hypothalamic neuropeptide Y (NPY) neurons, which, in turn, leads to increased expression of NPY in the ARC and decreased expression of thyrotropin-releasing hormone (TRH) in the PVN (9, 14). In the hamster, short-day (SD) photoperiod upregulation of D3 deiodinase in tanyocytes restricts T3 availability to the hypothalamus to effect seasonal change in body weight (2, 52). In addition to body weight regulation, hypothalamic availability of T3 is also responsible for mediating the seasonal testicular response in Siberian hamsters and has been implicated in the regulation of energetically expensive reproductive activity in other seasonal animals, including birds and sheep (2, 6, 19, 37, 52, 54–56).

As well as the activity of deiodinase enzymes, availability of T3 to hypothalamic neurons will depend on other factors, including the action of thyroid hormone transporters shuttling the hormone through the blood-cerebrospinal fluid (CSF) barrier (17, 18, 49). The thyroid hormone transporter OTP1c1 was followed by reestablishment of ad libitum feeding, hamsters return to a seasonally appropriate body weight and rejoin the photoperiod-mediated downward trajectory of weight loss (1, 34, 48). This implies a regulatory system that integrates long- and short-term homeostatic mechanisms.

Thyroid hormones [triiodothyronine (T3) and thyroxine (T4)] have long been recognized to be involved in regulating energy expenditure in peripheral tissues (31) and, more recently, have been demonstrated to trigger homeostatic mechanisms directly in the brain (for review, see Ref. 24). T3 is considered to be the bioactive form of the hormone and is produced as a result of 5'-deiodination activity of type I (D1) or II (D2) deiodinase on the precursor T4. Conversion of T4 to bioactive T3 can be prevented by 3'-deiodination to reverse T3 (rT3) by type III (D3) deiodinase, which also catalyzes active T3 to an inactive 3,3'-diodothyronine (T2) molecule. T3 can act on hypothalamic neurons of the arcuate nucleus (ARC) and the paraventricular nucleus (PVN) as a short-term signal of energy deficit imposed by starvation (7, 9, 14, 32), and as we recently demonstrated in the Siberian hamster, T3 acting in the brain regulates several aspects of seasonal physiology, including long-term seasonal energy expenditure (2). Common to both of these regulatory mechanisms is the regulation of deiodinase activities in tanyocytes lining the third ventricle of the hypothalamus. The response to starvation involves an induction of D2 deiodinase, which increases T3 availability to hypothalamic neuropeptide Y (NPY) neurons, which, in turn, leads to increased expression of NPY in the ARC and decreased expression of thyrotropin-releasing hormone (TRH) in the PVN (9, 14). In the hamster, short-day (SD) photoperiod upregulation of D3 deiodinase in tanyocytes restricts T3 availability to the hypothalamus to effect seasonal change in body weight (2, 52). In addition to body weight regulation, hypothalamic availability of T3 is also responsible for mediating the seasonal testicular response in Siberian hamsters and has been implicated in the regulation of energetically expensive reproductive activity in other seasonal animals, including birds and sheep (2, 6, 19, 37, 52, 54–56).

The mechanism of regulation of thyroid hormone availability in seasonal animals has been examined in three species (Japanese quail, Saanen goat, and Soay sheep) in addition to the Siberian hamster, and in each of these, regulation of deiodinases is likely to be a key event in facilitating photoperiod-induced switches in seasonal physiology (19, 55, 56).

As well as the activity of deiodinase enzymes, availability of T3 to hypothalamic neurons will depend on other factors, including the action of thyroid hormone transporters shuttling the hormone through the blood-cerebrospinal fluid (CSF) barrier (17, 18, 49). The thyroid hormone transporter OTP1c1 was...
previously considered to be a potential regulatory component
of the thyroid hormone system in the Japanese quail. Although
expression was observed in tanyctyes lining the third ventricle,
no photoperiodic regulation was found (38).

The monocarboxylate transporter 8 (MCT8) has been iden-
tified as a high-affinity and selective thyroid hormone trans-
porter (17) that is highly expressed in tanyctyes of the third
ventricle (26). At this location, MCT8 is in a strategic position
to facilitate transport of thyroid hormones to the hypothalamus
and has not previously been studied in relation to location and
regulation in seasonal species.

We hypothesized that if hypothalamic thyroid hormone
availability is important to acute energy deficits and the sea-
sonal body weight trajectory of the Siberian hamster, appro-
priate regulation may be imposed on the components of the
thyroid hormone system in tanyctyes of the third ventricle by
starvation.

Within the hypothalamus of the photoperiod-responsive Si-
berian hamster, we have identified changes in gene expression
when hamsters are switched from long-day (LD) to SD pho-
toperiod exposure (1, 3, 4, 35, 36, 45). Our studies have
revealed that a majority of photoperiod-induced gene expres-
sion changes occur in the dorsal region of the medial posterior
area of the hypothalamic ARC (dmpARC). VGF (nonacron-
ymic) is one gene that is highly induced in an SD photoperiod
in the dmpARC, and a peptide fragment of this protein
(TLQP21) has been shown to decrease body weight and food
intake in LD hamsters (27), suggesting that VGF may be a
component of the mechanism of long-term SD-induced body
weight loss. This gene was chosen for analysis to determine
whether starvation may impact the dmpARC and act as a
mediator of starvation on the thyroid hormone system in the
Siberian hamster.

In this study, we show that components of the hypothalamic
thyroid hormone system that are involved in the initial pathway
in the photoperiodic regulation of seasonal physiology react to
starvation-induced changes in energy balance in a photoperiod-
dependent manner.

MATERIALS AND METHODS

Animals and housing. Male 3- to 6-mo-old Siberian hamsters
(Phodopus sungorus) were bred and raised under a 16:8-h light-dark
photoperiod at the Rowett Research Institute. All research using
animals was licensed under the Animals (Scientific Procedures) Act of
1986 and received ethical approval from local ethical review commit-
tees.

![Fig. 1. Quantification of type II (D2) and III (D3) deiodinase (Dio2 and Dio3) mRNA expression in the 3rd ventricular tanyctye layer of Siberian hamster in
long-day (LD) or short-day (SD) photoperiod with or without 48 h of starvation. A and C: representative autoradiographs of coronal brain sections from each
of the 4 treatment conditions hybridized with a probe for Dio2 and Dio3. B and D: quantification of autoradiograph signals for Dio2 and Dio3 mRNA expression.
Expression was normalized to the highest value obtained for each probe. Solid bars, ad libitum-fed hamsters; gray bars, fold-restricted (48-h-starved) animals.
*P < 0.05; ***P < 0.001 by 2-way ANOVA and Tukey’s post hoc test for Dio2 and by t-test for Dio3.](http://ajpregu.physiology.org/)
Siberian hamsters were individually housed in an LD (16:8-h light-dark cycle) or an SD (8:16-h light-dark cycle) photoperiod for 8 wk at 20°C. Hamsters were fed ad libitum (n = 5 in LD, n = 6 in SD) or were food deprived (n = 6 in LD, n = 6 in SD) for 48 h before they killed in the middle of their respective light phases. None of the hamsters in the SD or the LD group showed signs of torpor at the time they were killed. Body weight and postmortem testes weights were recorded. Only one hamster in the SD group did not respond to SD photoperiod exposure and was excluded from the study. Trunk blood was collected for the preparation of serum, which was stored at −80°C until analysis.

Riboprobes. Plasmids and generation of riboprobes for Dio2, Dio3, NPY, TRH, and VGF have been described previously (2, 4, 12, 33). A 516-bp DNA fragment encompassing a region of the MCT8 (monocarboxylic acid transporter SLC16A2) mRNA sequence was generated by PCR amplification from Siberian hamster hypothalamic cDNA with primers based on rat and mouse MCT8 sequence data (GenBank accession nos. NM_147216 and NM_009197, respectively): 5′-CGGTCCATCTTCCGATCCTAA (forward) and 5′-AGGGCCGCTGAGTGGTACGARAGC (reverse). The resultant fragment was cloned into pGEM-TEasy (Promega).

A 521-bp fragment encompassing a fragment of vimentin was generated by PCR amplification from Siberian hamster hypothalamic cDNA with primers based on mouse and rat vimentin sequences (GenBank accession nos. NM_011701 and NM_031140): 5′-AGAACACCCGCACAAACGAGAAGG (forward) and 5′-AGCCAGGCGACGCRTGAGGTC (reverse). The resultant fragment was also cloned into pGEM-TEasy.

Sense or antisense transcripts were generated on linearized templates in the presence of 35S-labeled UTP for radioactive in situ hybridization or digoxigenin-labeled UTP for dual in situ hybridization.

In situ hybridization. In situ hybridization was carried out on serial coronal sections (14 μm) of PVN (−1.34 to −0.46 mm, with bregma as reference) or ARC (−2.54 to −1.34 mm, with bregma as reference), as described previously (12). Frozen brain sections mounted on glass slides were fixed in 4% paraformaldehyde in 0.1 M PBS and acetylated in 0.25% acetic anhydride in 0.1 M triethanolamine (pH 8). Radioactive probes (106 counts/min) were applied to the slides in 70 μl of hybridization buffer containing 0.3 M NaCl, 10 mM Tris-HCl (pH 8), 1 mM EDTA, 0.05% transfer RNA, 10 mM dithiotreitol, 0.02% Ficoll, 0.02% polyvinylpyrrolidone, 0.02% BSA, and 10% dextran sulfate. Hybridization was carried out overnight at 55°C. After hybridization, slides were washed in 4× standard saline citrate at 60°C. Slides were dried and apposed to Biomax MR film together with a 14C microscale for 5–7 days.

Image analysis. Slides containing serial brain sections for the complete experiment were exposed to a single sheet of autoradiographic film. Autoradiographic films were scanned at 600 dpi on a Umax scanner linked to a personal computer running Image-Pro PLUS version 4.1.0.0 analysis software (Media Cybernetics, Wokingham, UK). For D2 and D3 deiodonase, MCT8, and NPY riboprobes, three to four sections spanning a selected region of the hypothalamus (approximately −2.54 to −1.82 mm, with bregma as reference) were chosen for image analysis. TRH was quantified from four sections (approximately −1.34 to −0.46 mm, with bregma as reference) on two consecutive sets of slides.

Integrated optical density for each selected region was obtained by reference to a standard curve generated from the 14C microscale. The integrated optical densities for each section of each animal were added, and an average (with SE of the mean) was obtained for each animal and, subsequently, every specific treatment. The highest values of one treatment in an experiment were set to 100% expression value, and other treatment values were calculated accordingly.

Thyroid hormone levels. Total (bound + free) serum T3 and T4 were measured by RIAs (53). To prevent interassay variation (6.2% for T3 and 7.3% for T4), all samples of one experiment were measured within the same assay (intra-assay variability of 3.6% for T3 and 6.6% for T4). For the measurement of T3, all available serum samples were assayed. However, because of a shortage of serum for some hamsters, the sample sizes for T3 were as follows: n = 5 for LD ad libitum and SD ad libitum, n = 6 for LD starved, and n = 3 for SD starved.

Statistical analysis. Values are means ± SE. All data were analyzed using SigmaStat 3.1.1. (Systat). Statistical tests applied in this study were t-tests or two-way ANOVA with photoperiod and feeding conditions as factors. Differences revealed by the two-way ANOVA were tested with Tukey’s post hoc test for multiple comparisons as appropriate.

**RESULTS**

**Body weights.** After 8 wk in LD (16:8-h light-dark cycle) or SD (8:16-h light-dark cycle), the body weight of ad libitum-fed hamsters averaged 38.5 ± 1.7 and 27.9 ± 1.4 g, respectively. Complete food deprivation for 48 h (starvation) reduced the body weight of LD animals by 13.5% to 31.7 ± 2.0 g, while the body weight of SD hamsters decreased by 14.8% to 25.7 ± 0.8 g (P < 0.001 for effect of photoperiod and P < 0.004 for effect of starvation, with no interaction). Within SD, however,
starvation did not result in a significant difference in body weight because of the range of body weights in each group as they approach their nadir. Nevertheless, analysis of weight loss over the 48-h period when the food was removed shows the weight loss to be significantly different (0.95 ± 0.21 and 2.0 ± 0.28 g for ad libitum-fed and starved animals, respectively, *P = 0.014 by t-test).

**Deiodinase expression in Siberian hamster hypothalamus.**

D2 and D3 deiodinase mRNAs are expressed in tanycytes of the ependymal layer lining the third ventricle. D2 deiodinase

Fig. 3. Dual in situ hybridization of MCT8 with vimentin and D3 deiodinase. A: antisense digoxigenin-11-UTP-labeled probe for MCT8 was cohybridized with a 35S-labeled probe for the intermediate filament protein vimentin. Arrows show colocalization MCT8 and D3. Majority of MCT8-expressing tanycytes also express D3. B: an antisense digoxigenin-11-UTP-labeled probe for MCT8 was cohybridized with a 35S-labeled probe for D3 deiodinase. Arrow shows one of many tanycytes hybridizing with vimentin (a marker of tanycytes) and confirms expression of MCT8 in this group of cells. All vimentin-expressing tanycytes also express MCT8. Scale bar, 10 μm.

Fig. 4. Quantification of VGF, neuropeptide Y (NPY), and thyrotropin-releasing hormone (TRH) mRNA expression in the hypothalamus of the Siberian hamster. A: representative autoradiographs of coronal brain sections from each of the treatment conditions hybridized with a probe for VGF. B: quantification of VGF mRNA expression in the dorsal region of the medial posterior area of the arcuate nucleus (dmpARC) of hamsters in the SD photoperiod (no expression in LD) with and without 48 h of starvation. C: autoradiographs of coronal brain sections from each of the treatment conditions hybridized with a probe for NPY. D: quantification of NPY expression in the arcuate nucleus of hamsters held in LD and SD photoperiods with and without 48 h of starvation. E: autoradiographs of coronal brain sections from each of the treatment conditions hybridized with a probe for TRH. F: quantification of TRH mRNA expression in the paraventricular nucleus. Expression was normalized to the highest value for each probe. Solid bars, ad libitum-fed hamsters; gray bars, starved hamsters. *P < 0.05; ***P < 0.001 by 2-way ANOVA and Tukey’s post hoc test.
expression was significantly affected by photoperiod \( (P = 0.010 \text{ by 2-way ANOVA}) \): it was reduced by \( \sim 60\% \) in SD under ad libitum conditions relative to LD ad libitum conditions \( (P = 0.001 \text{ by Tukey’s test; Fig. 1, A and B}) \). In LD, D2 deiodinase expression was reduced by 30\% after starvation \( (P = 0.037 \text{ by Tukey’s test}) \), whereas the SD starved group showed an increase of \( \sim 70\% \) in D2 deiodinase mRNA levels relative to the SD ad libitum-fed group \( (P = 0.028 \text{ by Tukey’s test}) \). D3 deiodinase expression was markedly affected by photoperiod: it was absent in LD but highly expressed in SD. Starvation reduced D3 deiodinase mRNA expression in SD by \( \sim 40\% \) \( (P = 0.001 \text{ by } t\text{-test; Fig. 1, C and D}) \).

**MCT8 expression in Siberian hamster hypothalamus.** MCT8 is highly expressed in cells of the ependymal layer lining the third ventricle, as well as cells of the median eminence (Fig. 2A). Dual in situ hybridization studies showed that MCT8 and vimentin, a marker of tanycytes constituting the ependymal wall, completely colocalize (Fig. 3A). Dual in situ hybridization also shows that D3 deiodinase colocalized with MCT8 mRNA in the majority of tanycyte cells of this layer (Fig. 3B). Photoperiod significantly affected expression of MCT8 (Fig. 2B; \( P = 0.001 \text{ by 2-way ANOVA}) \). Relative to the LD ad libitum-fed group, MCT8 expression was upregulated by \( \sim 130\% \) \( (P = 0.001 \text{ by Tukey’s test}) \) in SD ad libitum-fed hamsters. Food availability significantly influenced MCT8 expression \( (P = 0.001 \text{ by 2-way ANOVA}) \). Starvation had no effect in LD (Fig. 2B) but significantly decreased MCT8 mRNA levels in SD by \( \sim 20\% \) \( (P = 0.001 \text{ by Tukey’s test}) \), but this reduced expression level was still greatly elevated relative to that in LD ad libitum-fed hamsters \( (P = 0.001 \text{ by Tukey’s test}) \).

**VGF expression in Siberian hamster dmpARC.** After 8 wk in SD, VGF mRNA was strongly upregulated in the dmpARC \( (P = 0.001 \text{ by Tukey’s test}) \). Food restriction for 48 h had no effect on expression levels of VGF mRNA (Fig. 4, A and B) in SD.

**NPY expression in Siberian hamster ARC.** There was no effect of 8 wk in SD on NPY mRNA expression in the arc. However, consistent with the known response of hypothalamic NPY to food deprivation, NPY mRNA levels in LD and SD were upregulated by \( \sim 50\% \) by 48 h of starvation \( (P = 0.001 \text{ by Tukey’s test; Fig. 4, C and D}) \).

**TRH expression in Siberian hamster PVN.** There was no effect of 8 wk in SD on TRH expression in the PVN. However, starvation downregulated TRH expression in LD and SD \( (P = 0.050 \text{ by Tukey’s test; Fig. 4, E and F}) \).

**Circulating thyroid hormone levels.** In ad libitum-fed hamsters, photoperiod affected total serum T3 and T4 levels, which were increased after 8 wk in SD \( (P = 0.004 \text{ by Tukey’s test for } T_3 \text{ and } P = 0.002 \text{ by Tukey’s test for } T_4; \text{ Fig. 5}) \). Starvation decreased T3 serum levels in LD and SD \( (P = 0.001 \text{ by Tukey’s test}) \). T4 remained unaffected in LD starved animals, whereas it was depleted by starvation in SD hamsters \( (P = 0.001 \text{ by Tukey’s test}) \). Body weights were plotted against T4 and T3 levels for individual hamsters. Regression analysis revealed a negative correlation in all comparisons (Fig. 6) but was only significant for body weight against T4 in the ad libitum-fed animals \( (r^2 = -0.69, P = 0.02 \text{ for body weight vs. } T_4 \text{ in ad libitum-fed animals}) \); \( r^2 = -0.07, P = 0.836 \) for body weight vs. T4 in starved animals; \( r^2 = -0.469, P = 0.173 \) for body weight vs. T3 in ad libitum-fed animals; \( r^2 = -0.575, P = 0.139 \) for body weight vs. T3 in starved animals).

**DISCUSSION**

We previously demonstrated that a decline in thyroid hormone availability to the hypothalamus is a key determinant in the progression of reproductive quiescence and body weight loss induced by SD photoperiod in the Siberian hamster (2). Studies in other rodents have shown that hypothalamic thyroid hormone availability is a component of the energy balance regulation mechanism responding to acute energy deficits (9, 10). Common to the mechanisms involved in these physiolog-
ical responses is the regulation of deiodinase enzymes in tanycytes lining the ependymal wall adjacent to the hypothalamus. In the Siberian hamster, regulation of seasonal physiological responses, including body weight, is dependent on photoperiod-induced change in expression of D2 and/or D3 deiodinase in tanycytes (2, 52). In rats and mice, D2 deiodinase expression in tanycytes is increased upon fasting (9, 10). We hypothesized that these two mechanisms might converge when food restriction is imposed on SD body weight loss in the Siberian hamster. We therefore analyzed the expression of components of the thyroid hormone system in LD and SD Siberian hamsters in response to 48 h of starvation.

The duration of experimental photoperiod chosen for this study was 8 wk, inasmuch as we previously showed that the thyroid hormone-catabolizing enzyme D3 deiodinase reaches a high mRNA expression level at about this time before a subsequent decline (2). Moreover, at 8 wk in SD, Siberian hamsters do not yet spontaneously enter torpor (13-46). Although starvation can induce torpor in either photoperiod (48), none of the hamsters used in this study displayed torpor at the time they were killed, perhaps because the animals were weighed ~3 h before they were killed. This would have aroused hamsters from a torpor bout. Therefore, we cannot completely rule out the possibility that there may have been an influence of torpor on gene expression changes reported here, even though we think it is unlikely.

At 8 wk in the LD or SD experimental photoperiod, 48 h of starvation elicited a clear response to starvation on hypothalamic gene expression and circulating thyroid hormone concentrations. As expected, NPY expression in the ARC increased, whereas TRH expression in the PVN decreased, compared with ad libitum-fed hamsters. Starvation decreased serum levels of T3 in LD and SD, but a reduction in serum T4 concentration was found only in SD hamsters. These results are consistent with the effect of fasting in other rodent models (9, 44).

In SD ad libitum-fed hamsters, expression of D2 deiodinase in tanycytes decreased compared with LD ad libitum-fed hamsters. Previously we found no change in D2 deiodinase expres-
D2 deiodinase expression in rats, where this has been shown to be a component of the increase in D2 deiodinase mRNA in food-deprived mice and expression in SD starved hamsters is consistent with the increase in D2 deiodinase mRNA expressed in the third ventricular tanycytes of the Syrian hamster exposed to SD for 10 wk, although in this latter case the decrease is far greater (42).

With starvation, the SD reduction of D2 deiodinase expression in tanycytes was partially reversed. Therefore, in SD hamsters, fasting increases the deiodinase enzyme for the synthesis of T3 and decreases the deiodinase enzyme for catabolism of T3, suggesting a local increase of T3 availability in the hypothalamus. The increase in D2 deiodinase mRNA expression in SD starved hamsters is consistent with the increase in D2 deiodinase mRNA in food-deprived mice and rats, where this has been shown to be a component of the mechanism to increase NPY gene expression in response to starvation (9, 10).

Takanashi, in SD hamsters the appropriate responses of NPY, TRH, and serum T3 concentrations to 48 h of starvation, together with reversal in the photoperiod-programmed responses of D2 and D3 deiodinase, are evidence of recognition of energy deprivation by the hamster. These data suggest that SD hamsters have taken appropriate measures at the level of the hypothalamus and periphery that have the potential to alter the drive to appetite and weight loss (9), even though the Siberian hamster in SD reduces food intake and body weight (16, 30).

Expression of D2 deiodinase in tanycytes decreased in 48-h-starved LD hamsters compared with ad libitum-fed LD hamsters. The increase in NPY and decrease in TRH in these hamsters are the expected hypothalamic responses of these genes to starvation. However, T3 is unlikely to be involved in this response, inasmuch as the observed decrease in D2 deiodinase expression is likely to reduce hypothalamic T3 levels in starved LD hamsters. In the fat LD hamsters, other hormonal systems may play a role in the overall response to starvation. In this regard, reduced levels of insulin or leptin, which affect central NPY expression (8, 34, 40, 50), are candidate hormones for the observed response.

In this study, we demonstrate for the first time that the thyroid hormone transporter MCT8 is expressed and colocalized with vimentin and D3 deiodinase in cells of the ependymal layer in the lower third of the third ventricle, where tanycytes constitute the majority of cells (43) and show downregulation of vimentin in SD (50).

In the SD photoperiod, MCT8 mRNA expression was substantially upregulated, suggesting augmented T3 and T4 transport into hypothalamic tanycytes. This appears counterintuitive, inasmuch as low levels of T3 are required to trigger the SD response in body weight and reproduction (2). One hypothesis to explain these results is that a rise in MCT8 would facilitate an increase in the transport of T4, which could be converted to rT3 by D3 deiodinase. T4 and rT3 are inhibitory to the activity of D2 deiodinase (28) and, together with SD induction of D3 deiodinase, may serve as part of a mechanism to enhance the decline in hypothalamic T3 concentrations required to induce SD physiology (2, 19, 56). Starvation in LD had no effect on MCT8 mRNA expression, but 48 h of starvation in SD reduced MCT8 mRNA expression. With the above hypothesis in mind, an elevation of D2 deiodinase with concomitant decreases in D3 deiodinase and MCT8 in SD may be an attempt to increase supply of hypothalamic T3. Clearly, with the added complexity of this counterintuitive change in MCT8 expression and the temporal changes in D2 and D3 deiodinase gene expression, additional studies are required to determine the actual hypothalamic concentrations of T3 and metabolites. A model for the interactions of the components of the thyroid hormone system in tanycytes under different conditions of photoperiod and food availability is presented in Fig. 7.

Our previous studies have identified a significant number of genes, the expression of which is regulated by photoperiod. These include several well-known energy balance-related genes, including the competent signaling form of the leptin receptor (ObRb) suppressor of cytokine signaling protein SOCS3, which negatively regulates leptin signaling, and proopiomelanocortin (giving rise to the anorectic peptide α-melanocyte-stimulating hormone following processing), in the ARC (35, 50). However, the evidence does not support a role for the many known hypothalamic factors in photoperiodic regulation of body weight (4, 11, 36). A possible area for a role in

Fig. 7. Components of the thyroid hormone system and their possible responses to photoperiod and starvation. For clarity, uptake of T4 and possible fates of this molecule in tanycytes of ad libitum-fed hamsters in LD and SD photoperiods and starved hamsters in SD photoperiod are shown. In each of the SD conditions (ad libitum-fed and food-restricted), because of the activity of D3 deiodinase to convert T3 to T2 and T4 to rT3, increased MCT8 transport could result in an increase in T2 and rT3 to act as inhibitors (–ve) of D2 deiodinase enzyme action. rT3, reverse T3.
photoperiod-induced physiological responses is the dmpARC, where we found the largest number genes to be up- or down-regulated in SD (4, 39, 45). This includes VGF, which is substantially upregulated in SD. Furthermore, a peptide fragment of this protein (TLQP21) can reduce body weight in the Siberian hamster (4, 27).

Analysis of VGF expression in this experiment showed that VGF is upregulated in the dmpARC by 8 wk in SD photoperiod expression. This was sufficient to assess the effect of 48 h of starvation. However, 48 h of starvation did not affect VGF mRNA expression. This would imply that if the VGF in the dmpARC is involved in seasonal changes in physiology, including body weight, then acute energy deficits do not impinge on a system to govern long-term changes in physiology. However, this does not rule out the susceptibility or involvement of other genes expressed in the dmpARC to the effect of acute energy deficits caused by starvation.

Serum concentrations of T3 were higher in SD ad libitum-fed than LD ad libitum-fed hamsters. Although photoperiodic differences were not observed in the study of O’Ilie and Bartness (41), our measurements are consistent with a previous study on the seasonal pattern of serum T3 and T4 concentrations in the Siberian hamster (47). Regression analysis for gene expression data on the components of the thyroid hormone system with body weight did not reveal significant correlations (data not shown). However, there was a significant inverse correlation of body weight with total (bound + free) serum T4 levels in ad libitum-fed hamsters, with a similar trend for T3. The basal metabolic rate of hamsters in comparison of LD and SD conditions with the same ambient temperature has not been studied; therefore, our results cannot be put into the context of this important physiological response. However, increases in serum T4 or T3 do not necessarily translate to increases in metabolic rate, inasmuch as intracellular concentration of T3 is the important determinant and will depend on the cellular uptake and the conversion of T4 to T3. However, one hypothesis for an increase in T3 would be to facilitate the potential for an increase in thermogenesis (for review see Ref. 5) driven by a melatonin-induced increase in brown adipose tissue mass (20, 21) as an adaptation to the seasonal cold climate of their natural habitat.

Perspectives and Significance

Reduced T3 availability to the hypothalamus is a critical component of SD-induced loss of body mass, including adipose tissue. Our data provide evidence that the reduction in T3 availability to the hypothalamus involves inverse temporal changes in D2 and D3 deiodinase expression in tanyocytes lining the ependymal wall of the third ventricle. The data reported here also illustrate how the Siberian hamster can adapt this mechanism when presented with an acute energy deficit.

The presence and photoperiodic regulation of the T3 transporter MCT8 in tanyocytes should be considered another component of the thyroid hormone system that is involved in the response to SD photoperiod and provides additional evidence of the importance of the thyroid hormone system to the photoperiod-induced changes in the physiology of the hamster. The regulation of MCT8 was counterintuitive, and so the significance of this remains to be established, but with the presence of D3 deiodinase in the same tanyocytes, there is a possibility that metabolites of T3 and T4 may play a role in the physiological responses to SD photoperiods in the Siberian hamster. It will be important for future studies to try and address where and to what extent are the local changes in T3, T4, and potential metabolites.

Taken together these data suggest that regulation of hypothalamic T3 in an SD photoperiod is an important event in obtaining a sustainable weight reduction during autumn and winter. The adaptations made to the components of the thyroid hormone system in SD photoperiod may be important to a seasonal mammal that requires T3 to maintain adequate lipid stores during a period of limited food supply.

GRANTS

This work was supported by the Scottish Government Rural and Environment Research and Analysis Directorate and the European Union as part of Framework VII: LSHM-CT-2003-503041, “Diabesity integrated project.”

REFERENCES

by Siberian hamsters.

Vitam Horm Thyroid hormone trans-

Ancestral TSH mechanism signals summer in
adipose tissue

Phodopus sungorus.

Daily torpor


