Hypothalamic neuropeptide gene expression during recovery from food restriction superimposed on short-day photoperiod-induced weight loss in the Siberian hamster

Zoë A. Archer, Kim M. Moar, Tracy J. Logie, Laura Reilly, Valerie Stevens, Peter J. Morgan, and Julian G. Mercer

Division of Obesity and Metabolic Health, Rowett Research Institute, Aberdeen Centre for Energy Regulation and Obesity, Bucksburn, Aberdeen, Scotland

Submitted 15 May 2007; accepted in final form 25 June 2007

Archer ZA, Moar KM, Logie TJ, Reilly L, Stevens V, Morgan PJ, Mercer JG. Hypothalamic neuropeptide gene expression during recovery from food restriction superimposed on short-day photoperiod-induced weight loss in the Siberian hamster. Am J Physiol Regul Integr Comp Physiol 293: R1094–R1101, 2007. First published June 27, 2007; doi:10.1152/ajpregu.00345.2007.—Previously, 40% food restriction of male Siberian hamsters over 21 days in short-day (SD) photoperiod induced characteristic changes in expression of hypothalamic arcuate nucleus energy balance genes; mRNAs for neuropeptide Y, agouti-related peptide, and leptin receptor were upregulated, and those of proopiomelanocortin and cocaine- and amphetamine-regulated transcript were depressed. The present study examined the effect of refeeding hamsters for 6 days (~50% recovery of weight differential) or 19 days (resumption of appropriate weight trajectory). Hyperphagia continued throughout refeeding, but differences in fat pad weights and leptin levels had disappeared after 19 days. Cocaine- and amphetamine-regulated transcript gene expression was depressed by prior restriction in both refed groups. The depressive effect of prior restriction on proopiomelanocortin gene expression had disappeared after 19 days of refeeding. There was no effect of prior food restriction on neuropeptide Y or agouti-related peptide gene expression. Expression of the anorexigenic brain-derived neurotrophic factor was downregulated in the ventromedial nucleus after SD exposure for 12 wk. In the SD food restriction study, there were effects of photoperiod on brain-derived neurotrophic factor gene expression but not of prior food restriction. Hypothalamic energy balance genes in the hamster respond asynchronously to return to a seasonally appropriate body weight. The achievement of this weight rather than the weight at which caloric restriction was imposed is the critical factor. The differential responses of hypothalamic energy balance genes to food restriction and refeeding are poorly characterized in any species, a critical issue given their potential relevance to human weight loss strategies that involve caloric restriction.

seasonal; body weight; refeeding

Despite considerable progress over the past decade in identifying molecular components of the hypothalamic energy balance system and the neuroanatomic structures involved, there are a number of areas where fundamental mechanisms have received surprisingly little attention. One example is the hypothalamic response to, and recovery from, restricted food supply. The difficulty experienced by many within our own population attempting to lose weight through dieting suggests that a more detailed understanding of these events would be advantageous, as would knowledge of the means by which mammals effect "programmed" physiological changes in body weight and composition. A number of laboratories have advocated investigation of the Siberian hamster (Phodopus sungorus) as an experimental approach to the regulatory systems that encode the level of body weight that will be defended against energy imbalance and the mechanisms whereby adjustments are made to what the animal perceives to be an appropriate body weight, i.e., the level that will be defended. This mammalian model also provides a valuable perspective on the adaptations induced by food restriction and the response to refeeding.

The Siberian hamster exhibits natural, large-amplitude, programmed changes in body weight with changing season. Body weight reaches a maximum in the long days of summer and a nadir during the short days of winter. Short-day (SD) photoperiod-induced weight loss is routinely in the region of 25–30% of starting long-day (LD) photoperiod adult body weight over a 10- to 15-wk period and is accompanied by declines in adiposity and reproductive activity (10, 22). The potential power of the Siberian hamster model in the elucidation of mechanisms was highlighted by a now classical study in which food restriction was imposed on hamsters that were already losing weight as a consequence of being housed in a natural SD photoperiod (22). The superimposed food restriction accelerated the rate of weight loss, causing body weight to fall below a seasonally appropriate level. Food restriction was subsequently lifted, and the animals were again allowed to feed ad libitum. With refeeding, still in SD photoperiod, body weight increased but not to its level at the beginning of the restriction period. Rather, once body weight reached a level similar to that of control hamsters with the same photoperiodic history that had been fed ad libitum throughout, weight gain was curtailed and body weight then began to decline again in parallel to the controls. This manipulation provides some of the best evidence of direct regulation of body weight. This regulation appears to involve a seasonal time-keeping mechanism that continues to adjust the encoded appropriate body weight even during weight loss due to imposed restriction. We were able to effectively reproduce the body weight outcome of this paradigm (food restriction in SD) by following an artificial, square-wave, photoperiod switch from LD to SD photoperiod in the laboratory (14).

In our earlier laboratory study under artificial photoperiods (14), our group released SD hamsters back to ad libitum

Address for reprint requests and other correspondence: J. G. Mercer, Division of Obesity and Metabolic Health, Rowett Research Institute, Aberdeen AB21 9SB, Scotland (e-mail: J.Mercer@rowett.ac.uk).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
feeding after a period of 18 days on 40% food restriction, resulting in a body weight differential of 24% between restricted and ad libitum-fed groups. Refeeding was accompanied by a period of hyperphagia (14). Our group then examined the changes within the hypothalamus that were likely to be driving this hyperphagic episode and rebound weight gain. Hamsters were food restricted for 21 days in SD to achieve the same percent weight differential and were then killed at the point of earlier release from food restriction. Compared with ad libitum-fed SD controls, food-restricted SD hamsters had low serum leptin, increased leptin receptor gene expression in the hypothalamic arcuate nucleus (ARC), enhanced expression of orexigenic neuropeptide genes [neuropeptide Y (NPY) and agouti-related protein (AGRP)] in the ARC, and strong trends toward reductions in anorexigenic genes [proopiomelanocortin (POMC) and cocaine- and amphetamine-regulated transcript (CART)] in the same nucleus (14) (abbreviated data presented in Fig. 1). These leptin and gene expression changes summate to molecular recognition of negative energy balance (20).

Despite the induction of hyperphagia after a return to ad libitum feeding, it took ~20 days for body weight to resume a typical SD downward trajectory (14). This raises the question of how quickly do the depression of the leptin signal and the perturbations in expression of hypothalamic energy balance genes normalize as hyperphagia is established and body weight trajectories converge against a background of ongoing SD weight loss. Our hypothesis in this follow-up study was that achievement of a seasonally appropriate body weight, i.e., convergence of body weight to the SD ad libitum-fed level, would be sufficient to normalize energy balance gene expression in the hypothalamus without the requirement to return to the body weight at which restriction was introduced. To test this hypothesis and to obtain fundamental information about the functioning of the integrated regulatory system, we subjected hamsters to a similar degree of restriction to that employed previously and then refed ad libitum for 6 or 19 days, corresponding to ~50% recovery of weight differential and around the point of maximal (postrestriction) body weight and resumption of an appropriate trajectory, respectively. A panel of hypothalamic genes was then screened, including the neuropeptide, brain-derived neurotrophic factor (BDNF), which has recently been implicated in energy homeostasis in rodents (11) by evidence of inhibition of food intake and body weight on central administration and reduction in gene expression following food deprivation (24). We show here that the BDNF gene is differentially expressed in the hamster hypothalamus after photoperiod manipulation.

**MATERIALS AND METHODS**

**Animals.** All procedures were licensed under the United Kingdom Animals (Scientific Procedures) Act of 1986 and received approval from the Rowett Research Institute’s Ethical Review Committee. All hamsters were drawn from the breeding colony maintained at the Rowett Research Institute, which was held under a LD photoperiod, and sd fed ad libitum unless stated otherwise, and water was freely available. Experimental animals were individually housed and assigned to weight-matched groups to be subsequently housed in either LD or SD photoperiod (8 h of light, 16 h of dark).

In the SD food restriction study, 43 male Siberian hamsters (38.5 ± 0.6 g) were weight-matched into three groups. Nine animals remained in LD photoperiod (LD-ADLIB), and two groups of 17 animals were transferred to SD photoperiod. One of the SD groups was fed ad libitum throughout (SD-ADLIB), whereas the other was fed ad libitum for 4 wk after transfer to SD photoperiod and then had food supply restricted to 60% of the intake of the SD-ADLIB animals for 21 days (SD-REST). A measured amount of food was provided each day in the second half of the light phase. Day 0 was designated as the start of the food restriction period. On day 21, all SD-REST animals were returned to ad libitum feeding. On day 27, the LD-ADLIB animals and eight animals from each of the SD-ADLIB and SD-REST groups were killed by cervical dislocation in the middle of the light phase (designated SD-ADLIB-1 and SD-REST-1, respectively). The remaining SD-ADLIB and SD-REST animals (both n = 9 and designated SD-ADLIB-2 and SD-REST-2, respectively) were killed by cervical dislocation in the middle of the light phase on day 40. Trunk blood serum was collected, brains were removed and frozen, and testes, kidney, liver, white adipose tissue (WAT) (epididymal, retroperitoneal, and inguinal), and interscapular brown adipose tissue were dissected and weighed. One hamster from the SD-ADLIB-2 group failed to exhibit body weight change as a result of SD housing (weight loss of only 0.4 g over the 68 days in SD photoperiod, compared with an average of 7–8 g in the remainder of the population) and was excluded from all analyses.

For examination of the effect of photoperiod on expression of the BDNF gene, adult male Siberian hamsters were allocated to one of four weight-matched groups that were then exposed to either LD or SD for either 4 wk (n = 10) or 12 wk (n = 13). All animals were killed by cervical dislocation in the middle of the light phase, and brains and blood serum were collected, frozen on dry ice, and stored at −70°C.

**Radioimmunoassay.** Serum concentrations of leptin were measured with the Linco multispecies kit (catalog no. XL-85K; Biogenesis, Poole, UK), as described previously (14). The sensitivity of the assay was stated as 1.0 ng/ml, the interassay coefficient of variation was 7.8%, and the intra-assay coefficient of variation was 6.3%. The specificity of the assay was 100, 67, 61, 73, and 3% for human, pig, rat, mouse, and dog leptin, respectively, according to the manufacturer’s data.

**Hypothalamic gene expression.** Hypothalamic gene expression was quantified by in situ hybridization, using a protocol described in detail elsewhere (13, 21). Riboprobes complementary to partial fragments of NPY, AGRP, POMC, CART, BDNF, and leptin receptor long form (OBRb) were generated from cloned cDNAs as previously described (1, 2, 9, 15). Hypothalamic sections (20 μm) were collected from the very caudal extent of the ARC through to the rostral extent of the hypothalamic arcuate nucleus (ARC) from male Siberian hamsters (n = 8) fed ad libitum during short-day photoperiod (SD-ADLIB group) for 49 days or held in SD photoperiod with restricted food (60% of ad libitum) from day 28 onward (SD-REST group). Values (means ± SE) are expressed as percentages of values in SD-ADLIB hamsters. NPY, neuropeptide Y; AGRP, agouti-related peptide; POMC, proopiomelanocortin; CART, cocaine- and amphetamine-regulated transcript; OB-Rb, leptin receptor long form. *P < 0.05. Adapted from Fig. 8 in Mercer et al. (14).
paraventricular nucleus (PVN) onto two sets of eight slides. Adjacent sections were mounted on consecutively numbered slides so that on each slide adjacent sections were separated by 160 μm. A single slide thus contained representative sections throughout the hypothalamic structure of interest. Accordingly, the first set of slides spanned the full extent of the ARC, approximating −2.7 to −1.46 mm, relative to Bregma, according to the atlas of the mouse brain (16). The second set of slides continued through to −0.58 mm relative to Bregma and included both rostral and caudal extents of the PVN. Sections were fixed, acetylated, and hybridized overnight at 58°C using 35S-labeled antisense riboprobes (1–1.5 × 106 dpm/ml). Slides were treated with RNase A to remove unhybridized probe and then desalted with a final high stringency wash in 0.1/11003 saline-sodium citrate at 60°C for 30 min. The slides were air dried and exposed to Biomax MR film (Sigma). Autoradiographic images were quantified with the Image-Pro Plus system (Media Cybernetics). This system determined the intensity and area of the hybridization signal on the basis of set parameters; the integrated intensity was then computed with standard curves generated from 35S autoradiographic microscales (Amersham).

Image analysis was performed on four or five sections spanning the ARC for NPY, AGRP, CART, POMC, and OBRb gene expression. BDNF gene expression was also analyzed in three or four sections from the ventromedial hypothalamic nucleus (VMH), along with OBRb gene expression. BDNF gene expression was also analyzed in the PVN and amygdala.

Statistical analysis. Data were analyzed by one-way (food restriction/refeeding study) or two-way (4 or 12 wk photoperiod study) ANOVA followed by post hoc t-tests. Where data failed normality tests, they were analyzed by Dunn’s one-way ANOVA on ranks. We used SigmaStat statistical software (Jandel, Erkrath, Germany) for all statistical analyses. Results are presented as means ± SE, and differences are considered significant at P < 0.05.

RESULTS

Restriction and refeeding in SD photoperiod: in vivo and postmortem measures. During the 21-day food restriction period, the body weight of SD-REST hamsters fell by ∼32% compared with the SD-induced weight loss in the SD-ADLIB groups of 11% (Fig. 2A), establishing a body weight differential of 23%. Removal of the imposed food restriction resulted in body weight increasing by 11.6% (SD-REST-1) by day 27 (6 days of ad libitum feeding) and 14.5% (SD-REST-2) by day 40 (19 days of ad libitum feeding). On day 27, body weights of the SD-ADLIB-1 and SD-REST-1 animals were similar, but both groups weighed less than LD-ADLIB hamsters (SD-ADLIB-1: 31.3 ± 0.87 g; SD-REST-1: 27.8 ± 1.03 g; LD-ADLIB: 38.5 ± 1.65 g; P < 0.001).

Testes and kidney weights were reduced in all SD groups compared with LD-ADLIB controls (P < 0.05) but did not differ between SD groups (Table 1). Epididymal WAT, retropitoneal WAT, inguinal WAT, and interscapular brown adipose tissue weights and the weights of pooled WAT depots were reduced in all SD groups compared with LD-ADLIB controls (P < 0.05) and were further reduced in the SD-REST-1 group compared with the SD-ADLIB-1 group (P < 0.05) but not in the SD-REST-2 group compared with the SD-ADLIB-2 group (Table 1). There was a significant effect of treatment on liver weight, with weights being higher in the LD-ADLIB group than in the SD-ADLIB-1 or SD-REST-2 groups (P < 0.05).

Food intake by hamsters in the SD-REST groups averaged 63.8% of that consumed by animals in the SD-ADLIB groups during the 21-day restriction period, whereas intake by SD-ADLIB hamsters averaged 88.2% of that of LD-ADLIB controls over the same period (Fig. 2B). When released from the imposed food restriction, SD-REST hamsters expressed voluntary hyperphagia; during the first 6 days after the restoration of ad libitum food (days 22–27), hamsters in the SD-REST groups consumed 28.3% more food than animals in the SD-ADLIB groups. During the subsequent two 6-day periods, SD-REST-2 hamsters consumed 25.6% and 24.4% more food than SD-ADLIB-2 hamsters (days 28–33 and 34–39, respectively).

There was a strong effect of treatment group on serum leptin (Dunn’s test; P < 0.001), with lower concentrations in all SD groups compared with LD-ADLIB controls (P < 0.05; Fig. 2C). Leptin concentrations were depressed by prior food restriction in the SD-REST-1 group compared with the SD-ADLIB-1 group weight loss in the SD-ADLIB groups (data not shown). After 4 wk, the LD-ADLIB group and 2 of the short photoperiod groups (SD-ADLIB) continued to be fed ad libitum, whereas the 2 SD-REST groups had food supply restricted to 60% of the intake of the SD-ADLIB animals for 21 days. Day 0 was designated as the start of the food restriction period. On day 21, all SD-REST animals were returned to ad libitum feeding. On day 27, the LD-ADLIB, SD-ADLIB-1, and SD-REST-1 groups were killed. The SD-ADLIB-2 and SD-REST-2 groups were killed on day 40. Values are means ± SE; n = 8 or 9 animals; for clarity, error bars have been omitted from B. In C, means without a common letter differ, P < 0.05.
ADLIB-1 group but were not significantly lower in the SD-REST-2 group compared with the SD-ADLIB-2 group. Leptin levels were positively correlated with both final body weight ($y = 1.179x - 28.026$, $R^2 = 0.70$) and weight of pooled dissected WAT ($y = 0.0084x - 3.3859$, $R^2 = 0.81$).

Restriction and refeeding in SD photoperiod: hypothalamic gene expression. Hypothalamic sections were subjected to in situ hybridization using $^{35}$S-labeled riboprobes to assess expression levels of a panel of energy balance-related genes (Fig. 3). There was a strong effect of treatment on CART gene expression ($P < 0.001$), with significant differences between SD-ADLIB-1 and both LD-ADLIB and SD-REST-1 and between SD-ADLIB-2 and SD-REST-2 ($P < 0.05$). CART gene expression remained elevated in the SD-ADLIB-2 group compared with LD-ADLIB controls. There was an effect of treatment on POMC gene expression (Dunn’s test; $P < 0.05$), with higher expression levels in the LD-ADLIB group and SD-ADLIB-1 group than in the SD-REST-1 group ($P < 0.05$) and a trend toward lower expression in the SD-ADLIB-2 group compared with the LD-ADLIB group ($P = 0.09$). There was no overall effect of treatment on either NPY or AGRP gene expression in the ARC, although both genes mRNA levels in the SD-ADLIB-1 group, but not in SD-REST-1, were lower than in LD-ADLIB controls ($P < 0.05$). There were no differences between SD-REST-1 and SD-REST-2 groups at either time point. There was no overall treatment effect on the expression of the long form of the leptin receptor (OBRe) in the ARC, but the differences between the LD-ADLIB and SD-ADLIB-2 groups ($P = 0.11$) and SD-ADLIB-2 and SD-REST-2 groups ($P = 0.10$) came close to achieving statistical significance, with a reduction in gene expression due to SD exposure and an increase due to prior restriction, respectively. In the VMH, there was an effect of treatment on OBRb gene expression ($P < 0.01$), with lower mRNA levels in SD-ADLIB-1 and SD-REST-1 than in LD-ADLIB hamsters, and an increase in the SD-REST-2 group compared with the SD-REST-1 group ($P < 0.05$).

Distribution of BDNF mRNA in the hamster hypothalamus. The regional distribution of BDNF mRNA in the hamster hypothalamus and adjacent brain areas revealed particularly intense expression in the VMH, PVN, and amygdala. These areas were selected for analysis of regulation (Fig. 4A).

Regulation of BDNF gene expression by photoperiod and food restriction. SD photoperiod exposure reduced terminal body weights at both 4 and 12 wk compared with that shown in respective LD controls (4 wk LD: 39.35 ± 0.99 g, 4 wk SD: 36.60 ± 0.72 g, $P < 0.05$; 12 wk LD: 38.82 ± 1.26 g, 12 wk SD: 30.69 ± 0.75 g, $P < 0.001$). There were significant (depressive) effects of both SD photoperiod ($F_{1,42} = 25.33$, $P < 0.0001$) and weight of pooled dissected WAT ($y = 0.0084x - 3.3859$, $R^2 = 0.81$).

Table 1. Tissue weights from male Siberian hamsters held in LD or SD photoperiod and fed ad libitum throughout or subjected to a period of food restriction

<table>
<thead>
<tr>
<th>Testes</th>
<th>Liver</th>
<th>Kidney</th>
<th>EWAT</th>
<th>RWAT</th>
<th>IWAT</th>
<th>Pooled WAT</th>
<th>IBAT</th>
</tr>
</thead>
<tbody>
<tr>
<td>LD-ADLIB</td>
<td>663±63 *</td>
<td>1,409±63 *</td>
<td>481±16 a</td>
<td>1,241±52 b</td>
<td>182±20 a</td>
<td>1,190±110 a</td>
<td>2,674±173 a</td>
</tr>
<tr>
<td>SD-ADLIB-1</td>
<td>67±13 b</td>
<td>1,242±41 b</td>
<td>354±11 b</td>
<td>682±55 a</td>
<td>94±9 b</td>
<td>759±54 a</td>
<td>1,536±94 b</td>
</tr>
<tr>
<td>SD-REST-1</td>
<td>56±2 b</td>
<td>1,252±46 b</td>
<td>347±12 b</td>
<td>392±61 b</td>
<td>41±7 b</td>
<td>382±52 b</td>
<td>816±111 b</td>
</tr>
<tr>
<td>SD-ADLIB-2</td>
<td>51±5 b</td>
<td>1,214±69 b</td>
<td>351±17 b</td>
<td>545±64 b</td>
<td>78±20 c</td>
<td>630±136 b</td>
<td>1,263±215 b</td>
</tr>
<tr>
<td>SD-REST-2</td>
<td>78±26 b</td>
<td>1,171±36 b</td>
<td>332±13 b</td>
<td>453±48 b</td>
<td>69±10 b</td>
<td>561±77 b</td>
<td>1,075±134 b</td>
</tr>
</tbody>
</table>

Values (in mg) are means ± SE; $n = 8$ or 9 animals. EWAT, RWAT, and IWAT, epididymal, retroperitoneal, and inguinal white adipose tissue (WAT), respectively; IBAT, interscapular brown adipose tissue. The long-day (LD) photoperiod group (LD-ADLIB) and the 4 short-day photoperiod groups were all fed ad libitum (ADLIB) for the first 4 wk following photoperiod manipulation (data not shown). After 4 wk, the LD-ADLIB group and 2 of the short photoperiod groups (SD-ADLIB) continued to feed ad libitum, whereas the 2 SD-REST groups had food supply restricted to 60% of the intake of the SD-ADLIB animals for 21 days (REST). Day 0 was designated as the start of the food restriction period. On day 21, all SD-REST animals were returned to ad libitum feeding. On day 27, the LD-ADLIB, SD-ADLIB-1, and SD-REST-1 groups were killed. The SD-ADLIB-2 and SD-REST-2 groups were killed on day 40. Means without a common letter differ, $P < 0.05$.

DISCUSSION

Our earlier study (14) of food restriction superimposed on SD photoperiod-induced weight loss in the Siberian hamster established that the additional weight loss generated a profile of regulated ARC gene expression (Fig. 1) characteristic of a state of negative energy balance (3, 4, 20). In the present study, using a food restriction challenge of very similar magnitude, and consequence for body weight trajectory, we investigated the effect of refedding to ad libitum intake on food intake, tissue weights, leptin concentration, and expression levels of a panel of hypothalamic energy balance genes. Curiously, there is a paucity of information on the effects of refedding after extended food restriction in any mammalian...
species; however, on the basis of our understanding of hypothalamic energy balance systems and hormonal feedback onto these systems, it would be anticipated that perturbations to hypothalamic gene expression would be progressively normalized as body weight, fat stores, and leptin levels recover; that is, orexigenic gene expression would be downregulated from its level of overexpression and anorexigenic gene expression would be increased back to a normal level. It is not clear whether these changes would be synchronous. In the case of food restriction of SD-housed Siberian hamsters, there is an additional level of sophistication because recovering body weight is not restored to its previous level but to an adjusted seasonally appropriate level. The duration of refeeding was timed so that hamsters that had previously been subjected to food restriction would be killed at two time points: corresponding to when the body weight differentials between ad libitum-fed and formerly restricted SD groups were approximately halved and corresponding to when body weight stabilized at a level that was appropriate to photoperiodic history, i.e., a level equivalent to that of SD hamsters fed ad libitum throughout. Our earlier studies had indicated that this was likely to take 15–20 days under the precise experimental paradigm being examined (14). Consequently, refed hamsters were killed after 6 days of refeeding (~50% recovery of weight differential; SD-REST-1 group) and around about the point of maximal (postrestriction) body

Fig. 3. Neuropeptide and receptor gene expression in the hypothalamic ARC, ventromedial nucleus (VMH), or paraventricular nucleus (PVN) of male Siberian hamsters from the experimental groups detailed in Fig. 2. Values are means ± SE, expressed as percentages of values in LD-ADLIB hamsters; n = 8 or 9 animals. Means without a common letter differ, P < 0.05.
Means without a common letter differ, shown in all of the other groups (gene expression after 12 wk of LD exposure was elevated compared with that A12 wk (B). SD photoperiod exposure of either duration suppressed serum leptin Bphotoperiods for either 4 or 12 wk and compared with respective LD controls. Brain sections were drawn from a study in which hamsters were housed in SD photoperiods for 12 wk of SD photoperiod exposure (LD vs. SD panels). Brain structures [VMH, PVN, and amygdala (AMYG)] and downregulation of VMH expression by 12 wk of SD photoperiod exposure (BDNF) gene expression in the Siberian hamster hypothalamus and adjacent structures [VMH, PVN, and amygdala (AMYG)] and downregulation of VMH expression by 12 wk of LD photoperiod exposure (LD vs. SD panels). Brain sections were drawn from a study in which hamsters were housed in SD photoperiods for either 4 or 12 wk and compared with respective LD controls. B: SD photoperiod exposure of either duration suppressed serum leptin concentrations. BDNF gene expression in the VMH was downregulated after 12 wk (A and C) but not after 4 wk (C) of SD exposure; however, in the PVN, gene expression after 12 wk of LD exposure was elevated compared with that shown in all of the other groups (D). Means without a common letter differ, P < 0.05.

weight and resumption of an appropriate trajectory (19 days of refeeding; SD-REST-2 group). At the refeeding time points examined, relative hyperphagia was still apparent in the SD-REST groups and had declined only slightly by the end of the study in the SD-REST-2 group. Long-term food restriction during the SD photoperiod may induce hyperphagia as hamsters approach their body weight nadir, whereas short-term complete food deprivation in either photoperiod does not. This behavioral measure, in agreement with our earlier observations where food intake also remained elevated over this time scale (14), did not appear to depend on serum leptin concentrations. Leptin levels reflected body weight and adiposity, being decreased during SD and by prior food restriction in the SD-REST-1 group; however, there was no effect of prior restriction in the SD-REST-2 group after refeeding for 19 days. However, the trend toward lower adipose tissue weights in this latter group compared with the SD-ADLIB-2 group suggest that subtle differences in body composition could still exist.

The anorexigenic genes encoding CART and POMC both exhibited the depressive effects of prior food restriction after 6 days of refeeding (SD-ADLIB-1 vs. SD-REST-1); i.e., they were not completely normalized. In the case of CART, but not POMC, this effect of prior restriction was still apparent after 19 days of refeeding (SD-ADLIB-2 vs. SD-REST-2). Comparison of rapid upregulation of CART gene expression in the ARC in SDs has been repeatedly observed in Siberian hamsters from our breeding colony (12) but has not been confirmed by other laboratories doing similar studies (18, 19). The depressive effect on CART gene expression of prior food restriction was further substantiated by the absence of a significant difference between both SD-REST groups and the LD-ADLIB controls. In contrast to the situation referred to above for CART, there is agreement across all laboratories and studies that POMC gene expression in the ARC is downregulated by SD exposure (1, 14, 15, 17, 19) but only after lengthy exposure periods, a finding that provides the context for the trend observed in the SD-ADLIB-2 group for this gene compared with the LD-ADLIB controls. Despite the extensive coexpression of CART and POMC in ARC neurons (7), their respective expression profiles differ considerably, as a consequence of both photoperiod effect and chronological response to refeeding after restriction.

In contrast to the anorexigenic neuropeptide genes, the substantial upregulation of both NPY and AGRP gene expression in the ARC seen in our previous study (14) on equivalent SD food restriction (Fig. 1) was already dissipated in the present study after 6 days of refeeding, during which time hyperphagia had reduced the body weight differential between the respective groups by ~50%. However, the observation that prior food restriction apparently counteracted the depressive effect of SD photoperiod at the first time point provides circumstantial evidence of an overall upward pressure on expression of these genes by the earlier period of food restriction. The effects of SD photoperiods on NPY and AGRP gene expression reported previously in Siberian hamsters are restricted in magnitude. For NPY, the only statistically significant effects reported are at 8 wk, where a reduction in gene expression was observed in SD photoperiod (17). In addition to the present data, we have recently obtained similar findings (depressed NPY mRNA in the ARC) in a large group of hamsters after 12 wk in SD (C. Ellis and J. G. Mercer, unpublished observations). For AGRP, the only significant effect of photoperiod reported is upregulation after 18 wk in SD (15), whereas in the present study SD photoperiod downregulated AGRP gene expression at 55 days, an observation again substantiated in a separate study (Ellis and Mercer, unpublished observations). The similarity in expression profile of NPY and AGRP, which are coexpressed in ARC neurons in the hamster (15), is striking.
The analysis of expression of the four “classical” ARC energy balance genes (NPY, AGRP, CART, and POMC) reveals that normalization of expression on refeeding after food restriction is not synchronous but does appear to segregate broadly on the basis of overall effect of the gene product on energy balance. Orexigenic gene expression (NPY and AGRP) was substantially normalized within 6 days, whereas expression of anorexigenic genes (CART and POMC) continued to reflect prior restriction, although with gene-specific chronology. Because leptin levels are also recovering back to appropriate levels during refeeding, these observations may provide further support for the postulate that orexigenic pathways are more sensitive to changes in leptin feedback than are anorexigenic pathways (20) and that the latter may prevail in normal weight maintenance.

Previously, we saw a near doubling of OBRb gene expression in the ARC after food restriction during SD photoperiod (Fig. 1), at which time serum leptin concentrations were assayed at 2.15 ng/ml compared with at 7.33 ng/ml in the ad libitum-fed SD group (14). This upregulation of the leptin receptor gene had disappeared within the first 6 days of refeeding in the present study, after which the respective leptin concentrations were 3.95 and 7.96 ng/ml. This suggests that the direction of change in circulating leptin concentration rather than the concentration per se may be important for the regulation of leptin receptor gene expression. We have reported previously a downregulation of OBRb gene expression in the ARC of hamsters after SD exposure for 12 (14) or 18 wk (15). Downregulation in the ARC was not observed in the present study, in which SD exposure was restricted to 55 or 68 days. The effects of SD photoperiod and food restriction on OBRb gene expression were more apparent in the VMH, although from previous studies an increase in leptin receptor gene expression would again be expected in the SD-REST-1 group against a background of low leptin levels (14). Thus, although OBRb gene expression in the ARC and VMH responds similarly to food restriction (14), there may be differences between the two nuclei in response to refeeding. Indeed, recent studies suggest that leptin receptors in the VMH may play an important role in the regulation of body weight (6), although the mechanisms involved remain to be established.

There is growing evidence of a physiological role for BDNF in energy homeostasis in rodents (11), with central administration of BDNF decreasing food intake and body weight and food deprivation decreasing BDNF gene expression in the VMH (24). BDNF mRNA had a distribution in the hypothalamus of the hamster similar to other rodent species, with expression in important hypothalamic structures involved in energy balance (such as the PVN and VMH) and in part of the reward circuitry (the amygdala). There was robust downregulation of gene expression in the VMH after SD exposure for 12 but not for 4 wk in the photoperiod study. However, the timing of this regulation, after the establishment of photoperiod-driven body weight differentials, and the directional change, downregulation in SDs, make it unlikely that this presumed anorexigenic peptide has direct involvement in SD-induced weight loss. Inclusion of BDNF in the panel of genes for the food restriction study allowed assessment of the interaction between photoperiodic and imposed changes in body weight. SD exposure in the food restriction study was limited to 55 or 68 days, and the trend was for SD exposure to reduce BDNF gene expression in the VMH, although the effect was less pronounced than that observed at 12 wk in the photoperiod study. There was no apparent effect of prior food restriction. In the PVN, the pattern of suppression of gene expression was similar, albeit of greater magnitude, and again the preceding period of food restriction was without clear effect. We are not aware of any published data describing the effects of food restriction on hypothalamic BDNF gene expression.

As alluded to above, studies of the effects of long-term food restriction on hypothalamic systems are far less numerous than those of shorter-term complete food deprivation, which is a near routine test to demonstrate involvement of genes of interest in energy balance regulation. In fact, comparison of the two manipulations reveals differential effects on hypothalamic energy balance gene expression (3, 4). Furthermore, information on the time course of restoration of gene expression after imposed caloric deficit is also surprisingly sparse, for any species and/or manipulation, given its potential importance to human weight loss strategies and observed differences in the pattern of recovery of body composition on refeeding after food deprivation or restriction (8). This issue has been addressed in the case of complete food deprivation for 24 h in mice by assay of gene expression after different periods of refeeding (23); refeeding for 6 h (but not 2 h) was sufficient to reduce, but not normalize, NPY mRNA levels, which were elevated after 24 h of food deprivation, but had no effect on elevated AGRP gene expression and conversely restored suppressed POMC levels to the level of ad libitum-fed controls. Furthermore, NPY gene expression remained elevated after 26 h of refeeding, whereas AGRP and POMC were normalized (23). Although presumed postabsorptive events evidently begin to restore normal levels of gene expression quite rapidly as animals recover from this short-duration fast, it is generally assumed that, during recovery from any negative energy balance, relative hyperphagia, depression of the leptin signal, and perturbations to downstream hypothalamic systems will proceed until body weight or composition is normalized relative to that shown for controls (5). In the case of food restriction in the SD-exposed hamster, our hypothesis was that the key factor would be the reestablishment of an appropriate body weight trajectory rather than the return to the weight at which restriction was imposed, which would be the cue for reinstatement of normal activity in the homeostatic signaling systems. This emphasis on the size of the deviation from appropriate body weight or energy stores would suppose that the hypothalamic homeostatic systems are more heavily influenced by a hypothetical system that allows current body weight/composition to be compared with a continually adjusted, seasonally appropriate target than they are by any memory of the severity of the imposed energy restriction or body weight/composition at the point when restriction was applied. This is in line with the original observation that LD hamsters return to their prerestriction body weight when released back to ad libitum feeding, whereas SD hamsters do not (22).

However, the hypothesis that achievement of a seasonally appropriate body weight would be sufficient to normalize energy balance gene expression in the hypothalamus was not entirely supported. The effect of prior food restriction in SD-exposed animals appeared to be more robust for the anorexigenic peptides than for their orexigenic counterparts, with the latter (NPY and AGRP) being restored to SD-ADLIB gene expression by 10.220.33.1 on June 29, 2017 http://ajpregu.physiology.org/ Downloaded from
levels after only 6 days and before the recovery of an appropriate body weight, body composition, or leptin level. Thus, as appears to be the case for 24-h food deprivation (23), different energy balance genes appear to be restored to normal levels of activity over different time scales and the feedback signals involved in this restoration are also likely to be different. Certainly, serum leptin concentrations cannot be the sole regulatory input. Normalization of CART gene expression, for example, which continues to be perturbed by prior food restriction even after 19 days of refeeding, may require a more accurate restoration of an appropriate body composition or elimination of the relative hyperphagia that was still evident at that time.

It is clear from a review of the literature that we still know comparatively little about 1) the response of hypothalamic energy balance systems to food restriction, an analogous situation to the caloric restriction diets undertaken by many human subjects trying to lose weight, and 2) the consequences of perturbations to these signals and the speed of their reversal once caloric restriction is relaxed. There is a dearth of information here for any species, representing a significant gap in our knowledge base. The differential response of different components of the homeostatic system to these manipulations argues for a more systematic description of sensitivity and recovery at a molecular level. Such evidence may help shape future calorie-controlled interventions targeting weight loss or weight maintenance.

ACKNOWLEDGMENTS
We thank Dr. G. Horgan, Biomathematics and Statistics Scotland, for advice on statistical analysis.

GRANTS
This work was supported by the Scottish Executive Environment and Rural Affairs Department and the European Union as part of Framework VI: LSHM-CT-2003-503041, “Diabesity” Integrated Project.

REFERENCES