Effects of Manipulating Hypothalamic Triiodothyronine Concentrations on Seasonal Body Weight and Torpor Cycles in Siberian Hamsters

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Siberian hamsters display photoperiodically regulated annual cycles in body weight, appetite, and reproduction. Previous studies have revealed a profound up-regulation of type 3 deiodinase (DIO3) mRNA in the ventral ependyma of the hypothalamus associated with hypophagia and weight loss in short-day photoperiods. DIO3 reduces the local availability of T_3, so the aim of this study was to test the hypothesis that decreased hypothalamic T_3 availability underlies the short-day-induced catabolic state. The experimental approach was to determine whether a local increase in T_3 in the hypothalamus of hamsters exposed to short days could reverse the behavioral and physiological changes induced by this photoperiod. In study 1, microimplants releasing T_3 were placed bilaterally into the hypothalamus. This treatment rapidly induced a long-day phenotype including increased appetite and body weight within 3 wk of treatment and increased fat mass and testis size by the end of the 10-wk study period. In study 2, hypothalamic T_3 implants were placed into hamsters carrying abdominal radiotelemetry implants. Again body weight increased significantly, and the occurrence of winter torpor bouts was dramatically decreased to less than one bout per week, whereas sham-implanted hamsters entered torpor up to six times a week. Our findings demonstrate that increased central T_3 induces a long-day metabolic phenotype, but in neither study was the molt cycle affected, so we infer that we had not disrupted the initial detection of photoperiod. We conclude that hypothalamic thyroid hormone availability plays a key role in seasonal regulation of appetite, body weight, and torpor. **(*Endocrinology* 153: 101–112, 2012)**

Many animals respond to the annual change in day length by modifying their body weight and ingestive behavior in anticipation of the altered energetic demands of their seasonal environment. Siberian hamsters have been widely used as a model organism to understand the underlying processes because they display profound seasonal cycles in energy metabolism, food intake, body weight, and reproductive ability, which can all be induced by changing only their ambient photoperiod in a laboratory setting (1). Hamsters exposed to short winter photoperiods [short days (SD)] enter a catabolic state in which they become hypophagic and catabolize abdominal fat reserves such that they lose up to 40% of total body weight. Additionally, hamsters exposed to SD molt to a white winter coat, display bouts of torpor, and are reproductively inactive (2). Hamsters maintained in long summer photoperiods [long days (LD)] are relatively obese, hyperphagic, and reproductively active; thus, these natural photoperiod-generated lean and fat states provide a valuable rodent model for the study of obesity (1).

A number of candidate genes have been identified recently in the hypothalamus that show photoperiod-regu-

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Abbreviations: CLAMS, Comprehensive laboratory animal monitoring system; DIO3, type 3 deiodinase; DIO2, type 2 deiodinase; LD, long days; SD, short days; T_b, core body temperature; VO_2, oxygen consumption.

lated patterns of expression and are associated with the transition from the catabolic SD state to the anabolic LD state. Consistent across a variety of seasonal species including sheep (3), F344 rats (4), quail (5), and Syrian hamsters (6) are changes in expression of the genes encoding type 2 deiodinase (DIO2) and/or type 3 deiodinase (DIO3). These deiodinase genes are very selectively expressed in tanycytes in the mediobasal hypothalamus, and their role in regulating the conversion of T_4 to biologically active T_3 or inactive rT_3 is well understood (7, 8). These observations of photoperiod-induced changes in hypothalamic deiodinase gene expression are consistent with studies carried out for over 70 yr demonstrating that seasonal cycles are disrupted by removal of the thyroid gland and can be restored by appropriate hormonal replacement (see Ref. 9 for review). Although the precise regulation of DIO2 and DIO3 differs between species, the common feature is that LD induce a state whereby local T_3 production is facilitated, whereas SD promote production of rT_3 and degradation of T_3 to 3,3′-diiodothyronine in the hypothalamus (see Ref. 1 for review). In the Siberian hamster, this is achieved principally through up-regulation of DIO3 by SD, with the peak of DIO3 expression coinciding with the nadir of body weight (10).

The seasonal changes in deiodinase gene expression in tanycytes appear to be driven by melatonin acting on the neighboring pars tuberalis, with local paracrine production of TSH-β being the intermediate signal (3). This understanding, combined with the historical knowledge of the importance of the intact thyroid gland for seasonal cycles, has led to the hypothesis that changes in central T_3 availability are the key mechanism underlying seasonal cycles of energy balance and the reproductive axis. Thus, high central T_3 availability is thought to promote a summer anabolic state, whereas decreased T_3 availability is thought to induce the winter catabolic state. Although it has not proved technically feasible to measure local concentrations of T_3 in the hypothalamus, there is some limited evidence to support this hypothesis based on the findings that central administration of T_3 to quails in SD stimulates gonadal growth (5), and direct infusion of T_3 into the hypothalamus induces hyperphagia in rats (11). Our initial studies in Siberian hamsters also provide some support, because local implants releasing T_3 placed in the hypothalamus were initially able to block SD-induced weight loss (10). We now report a much more rigorous test of this hypothesis in which hamsters exposed to SD, which would be expected to have high DIO3 expression and thus low hypothalamic T_3 availability, received local implants releasing T_3. Across two separate studies, we found that local delivery of T_3 increased food intake, promoted body weight gain, prevented daily torpor bouts, and induced testicular recrudescence. These findings support the hypothesis that the winter catabolic phenotype induced by SD is a consequence of reduced hypothalamic T_3 availability.

Materials and Methods

Animals and housing

All experiments were carried out on male Siberian hamsters obtained from a colony bred in-house (12). Animals were housed individually at constant temperature (21 ± 1 C) and 40–50% humidity in study 1 and at 18 ± 1 C in study 2 after the experimental surgery such that hamsters could be monitored for torpor bouts. Animals were allowed *ad libitum* access to food (9% fat, 19% protein extruded laboratory chow: Teklad 2019, Harlan, Shardlow, UK) and water throughout the studies. Animals in SD were exposed to a lighting regime of 8 h light, 16 h dark (lights on at 0300 h in study 1 and 2200 h in study 2) and animals under LD conditions were housed under 16 h light, 8 h dark (lights on at 1900 h in study 1 and 1400 h in study 2). Throughout the duration of the experiment, body weight was monitored at least once weekly. Pelage was scored on a scale of 1–4, where 4 represented a full LD brown coat and 1 signified a full SD white coat [scale modified from Duncan *et al.* (13)]. All animal procedures were approved by the University of Nottingham Local Ethical Review Committee and were carried out in accordance with the United Kingdom Animals (Scientific Procedures) Act of 1986 (Project license PPL 40/3065).

T_3 administration

T_3 implants were made by thoroughly mixing crystalline T_3 (Sigma-Aldrich, Poole, UK) with medical-grade silicone/SILASTIC (Dow Corning, Midland, MI) adhesive (Med-1137; Polymer Systems Technology Ltd., High Wycombe, UK) in a 1:9 ratio as described previously (10). The mixture was packed into polyethylene tubing (0.5 mm inner diameter, Sims Portex Ltd., Hythe, UK) and allowed to cure at room temperature for 24 h. The solid SILASTIC-brand polymer was extracted when dry from the tubing and cut into 0.5-mm lengths. The total T_3 content of the implants was approximately 10 μg per implant, and *in vitro* incubation studies indicated that the initial release rate of the implants (*i.e.* over the first 4 wk) was approximately 100 pg/d (Jethwa, P., and M. Murphy, unpublished data). Sham implants were made in the same way using 100% SILASTIC adhesive.

The hamsters were anesthetized with a mixture of ketamine (1 mg/kg Vetalar 100 mg/kg ip; Fort Dodge Animal Health Ltd., Southampton, UK) and medetomidine (Dormitor 1 mg/kg ip; Pfizer Ltd., Kent, UK) in a ratio of 1:4. Analgesia was maintained via sc injection of carprofen (5 mg/kg Rimadyl; Pfizer) and administered before surgery. Animals were placed in a stereotaxic frame (David-Kopf Instruments, Buffalo, NY) with the incisor bar positioned level with the interaural line. Implants were placed bilaterally into the hypothalamus at 0.65 cm below the surface of dura after deflection of the superior midsagittal sinus, ±0.05 cm from the third ventricle. The skin was closed over the wound using Michel clips. The anesthetic was reversed by treatment of atipamezole (Antisedan 1 mg/kg sc; Pfizer), and fluid replacement (0.9% saline) was given. After surgery, animals

were given daily injections of analgesia and extra fluids for 7 d or until any resulting weight loss had stopped and body weight was stable.

Study 1: effects of hypothalamic T_3 implants on body weight, feeding, and metabolic parameters

Male Siberian hamsters (n = 35) were exposed to SD photoperiod for 10 wk. and body weight, food intake in the home cage, and pelage were monitored weekly. After 10 wk in SD, T_3 or sham implants were placed bilaterally in the hypothalamus as explained above. After surgery, the hamsters were subdivided such that one group receiving sham implants (n = 8) and one group receiving T_3 (n = 10) remained in SD, and a second group receiving sham implants (n = 8) or T_3 implants (n = 9) were immediately transferred to LD. For the purposes of analysis of body composition, three separate batches of hamsters were also set up with sham implants and exposed to SD (n = 8) or transferred to LD for 6 wk (n = 5) or received T_3 implants and remained in SD (n = 7). Dietary supplementation was given immediately after surgery comprising wet lab chow and assorted grains and sunflower seeds; thus, measures of food intake were not taken for the first 2 wk after surgery. At 3, 5, and 9 wk after surgery, randomly selected hamsters from each of the main groups (n = 8) were assessed for 48 h using a modified open circuit calorimeter [comprehensive laboratory animal monitoring system (CLAMS); Linton Instrumentation, Linton, UK, and Columbus Instruments, Columbus, OH] as described by Warner *et al.* (14). Ground chow (also Teklad 2019; Harlan, UK) was provided in the hoppers, and water was available *ad libitum*. All data were collected and initially analyzed using the OxyMax Windows software version 4.2 (Columbus Instruments). Metabolic parameters including oxygen consumption (VO_2), carbon dioxide production, and respiratory exchange ratio (CO_2 production divided by VO_2) were measured at 9-min intervals. Various parameters of eating behavior, including frequency and duration of feeding, as well as locomotor activity were measured. The removal of 0.02 g or more of food from the hopper was defined as a meal. Infrared beams allowed the detection of movement within the cages, and the disruption of two or more consecutive beams was classed as locomotor activity.

Animals were euthanized after the final CLAMS session by ip injection of sodium pentobarbitone (Euthatal; Rhone Merieux, Harlow, UK). Their brains were frozen on dry ice, and stored at −80 C until analysis of implant placement and *in situ* hybridization studies. The wet weights of testis, epididymal fat pads, brown adipose tissue, and liver were recorded. For the cohorts that were euthanized at 6 wk after implantation/change of photoperiod, carcasses were dried and finely blended, then fat was extracted from the dried carcass samples of known weight by petroleum ether using rapid Soxhlet extraction (Gerhardt Soxtherm fat analyzer), and total fat content was calculated.

Study 2: effects of hypothalamic T_3 implants on core body temperature and torpor

Core body temperature and activity were measured using radiotelemetry devices (TA10TA-F20) and receivers (RPC-1) and ART version 2.1 software (Data Sciences International, St. Paul, MN). Animals (n = 12) were castrated and telemetry devices were surgically inserted into the peritoneal cavity under general anesthetic as described above. Postsurgery care included daily injections of analgesia with additional fluids for 7 d as well as varied additional food and removal of wound clips after 1 wk.

Castration and telemetry surgery were carried out while hamsters were maintained on LD (16 h light, 8 h dark, lights on 1400 h). After the animals had recovered, they underwent telemetry recordings for 1 wk before being moved to SD; their home cages were placed on receivers and data collected for 10 sec every 2 min. One week of telemetry readings were also taken after 3 wk exposure to SD to establish a baseline core body temperature (T_b) for each individual. At wk 10 of SD, T_3 (n = 6) or sham (n = 6) implants were inserted into the hypothalamus as described above. Additional telemetry recordings were made continuously from wk 12–24. Body weight, food intake, and pelage were measured weekly in the home cage throughout the study. At the end of the study, brains were collected, frozen, and then sectioned at a thickness of 12 μm and the location of the implants confirmed. All hamsters had implants that were considered to be located within an appropriate region of the hypothalamus.

In situ hybridization

In situ hybridization for prepro-TRH mRNA was carried out on the brains from study 1 as described previously (15, 16). Briefly, 12-μm coronal sections were taken through the hypothalamus. Plasmids containing prepro-TRH gene sequences were linearized by restriction enzyme digestion. Antisense or sense RNA sequences were transcribed with SP6 or T7 polymerase as appropriate in the presence of [^{35}S]UTP. Hybridization was performed at 58 C overnight. After hybridization with the antisense (one slide containing four to five sections from the appropriate region per animal) or sense probe (one slide from an animal in each treatment group), slides were treated with ribonuclease A and washed to a stringency of 0.1× saline-sodium citrate (1× saline-sodium citrate = 0.15 M NaCl, 15 mM sodium citrate) at 60 C. Slides were apposed to a single sheet of autoradiographic film. Films were scanned with a CCD camera (KY-F55B, JVC, Yokohama, Japan), captured on a AcQuis framegrabber card (Synoptics Ltd., Cambridge, UK) and analyzed using Scion Image/NIH Image version 1.62 software provided by Scion Corp. (Frederick, MD). Because the hybridizations with the sense sequences did not reveal any signal, two sections through the paraventricular nucleus hybridized with the antisense probe were analyzed for each animal. The density of particles above background plus 3 SD was measured. The mean value of the density of hybridization signal in the sections from hamsters with sham implants kept in SD was then defined as 100%, and the value in sections derived from the other groups was expressed as a percentage of this value.

Statistical analysis

Data were analyzed using repeated-measures ANOVA and Bonferroni *post hoc* tests (Prism version 4.0; GraphPad, San Diego, CA). Food intake from CLAMS, tissue weights, and TRH OD were analyzed using a one-way ANOVA and Tukey's *post hoc* test (Prism version 4.0; GraphPad). In all cases, $P < 0.05$ was considered statistically significant. In study 2, a torpor bout was defined as a drop in body temperature for a minimum of 2 h to at least 3 SD below the mean for each individual as assessed over their third week of exposure to SD, which is well before any torpor bouts would be expected.

Results

Study 1: effects of hypothalamic T_3 implants on body weight

As expected, before treatment, all hamsters exposed to SD showed a substantial spontaneous loss of body weight despite being maintained on *ad libitum* feed at constant temperature (Fig. 1A); thus, after 10 wk exposure to SD, body weight had fallen by an average of 9 g, a loss of over 20% of initial body weight. Treatment with hypothalamic T_3 implants in the 11th week on SD had a striking effect on body weight (Fig. 1A; group × time interaction $F = 4.36$; $P < 0.001$). Hamsters treated with T_3 but remaining in SD increased their weight almost immediately, and body weight was significantly elevated compared with hamsters receiving sham implants within 1 wk of implan-

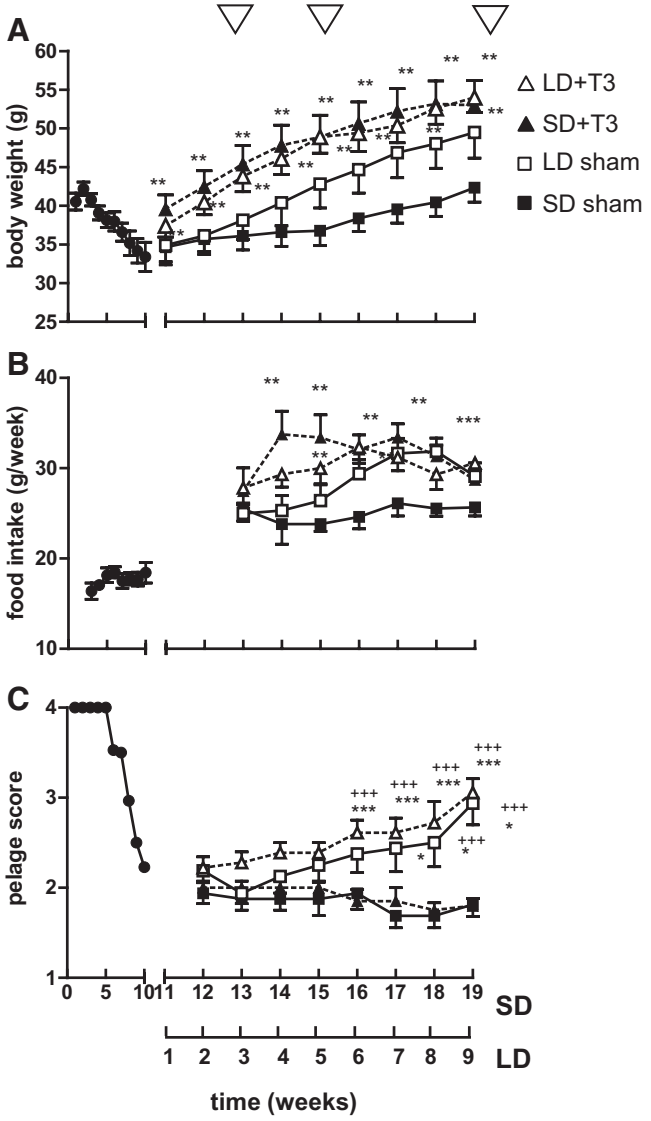

FIG. 1. Study 1: body weight (A), food intake in the home cage (B), and pelage score (C) in male hamsters placed in SD at wk 0 and then receiving sham (■ and □) or T_3 (▲ and △) implants in the hypothalamus at wk 10–11. Hamsters either remained in SD (■, sham n = 8; ▲, T_3 n = 9) or were moved to LD (□ sham, n = 8; △, T_3 n = 10). *Inverted arrows* indicate occasions when hamsters were removed from their home cages and placed in the CLAMS for 48 h. Note that the hypothalamic T_3 implants induce a more rapid gain in body weight and food intake than exposure to LD alone but that pelage scores are unaffected by T_3 treatment and are solely determined by photoperiod. Values are group mean ± SEM. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$ *vs.* SD-sham group; +, $P < 0.05$; ++, $P < 0.01$; +++, $P < 0.001$ *vs.* SD-T_3 group.

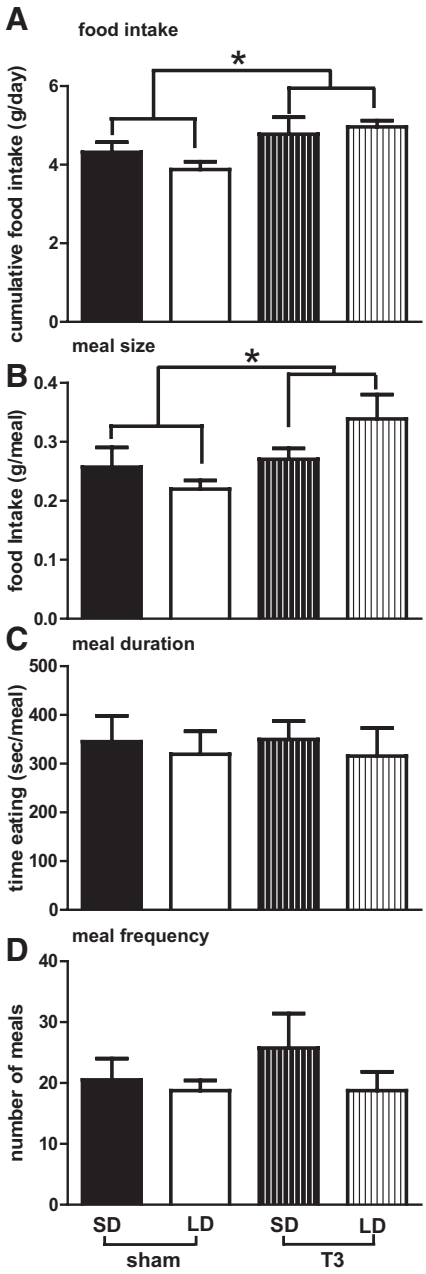

FIG. 2. Feeding behavior in adult male hamsters studied at wk 13 SD (same as wk 3 LD) expressed as total food intake (A), meal size (B), meal duration (C), and meal frequency (D), values are group mean ± SEM (n = 8) for the final 24 h of the CLAMS sessions. *, $P < 0.05$. Note that overall food intake (A) is increased in the T_3-treated animals under both photoperiods, reflecting an increase in meal size (B), whereas no significances were found in meal duration (C) or meal frequency (D).

tation (Fig. 1A). This was a more rapid increase in body weight than that seen in hamsters with sham implants that were transferred to LD as a positive control; body weight in the LD-sham group was not significantly increased relative to the SD-sham group until 3 wk after the change in photoperiod (Fig. 1A). The hamsters transferred to LD that were additionally treated with T_3 also increased their body weight more rapidly than the sham group that had been transferred to LD (Fig. 1A). By the end of the study, however, body weight was similar in both the T_3-implanted groups and not significantly different from the sham controls moved to LD (Fig. 1A). Although, as expected, the hamsters in SD with sham implants showed a gradual increase in body weight toward the end of the study, hamsters in the two T_3-implanted groups and the LD-sham group were significantly heavier than those in the SD-sham group at the end of the study (wk 19 SD/wk 9 LD; Fig. 1A).

Study 1: effects of hypothalamic T_3 implants on food intake

Hamsters treated with T_3 significantly increased their food intake in the home cage relative to those bearing sham implants (treatment group × time interaction $P < 0.001$; Fig. 1B). By wk 14 in SD, the T_3-treated group had increased its average food intake by approximately 10 g/wk relative to the SD-sham group ($P < 0.01$), and this significant increase in food intake was maintained until wk 18 (Fig. 1B). During the course of the experiment, T_3-implanted hamsters in LD also increased their food intake relative to hamsters in the SD-sham group; however, they did this more gradually (Fig. 1B). In the ninth week after implant (wk 19 SD/wk 9 LD), the two T_3-implanted groups and the LD-sham group all ate more than the hamsters in the SD-sham group, with a difference in intake of approximately 5 g/wk (Fig. 1B). A microanalysis of food intake was carried out during the three CLAMS analyses. In wk 3 after treatment, the T_3 implants had induced a significant ($P < 0.05$) increase in overall food intake compared with the intake of groups with sham implants (Fig. 2A). This reflected a significant ($P < 0.05$) increase in mean meal size (Fig. 2B), but average meal duration (Fig. 2C) and frequency (Fig. 2D) were not affected. By wk 5 after implantation, there was a marginal effect on overall food intake and significant effects of T_3 treatment on

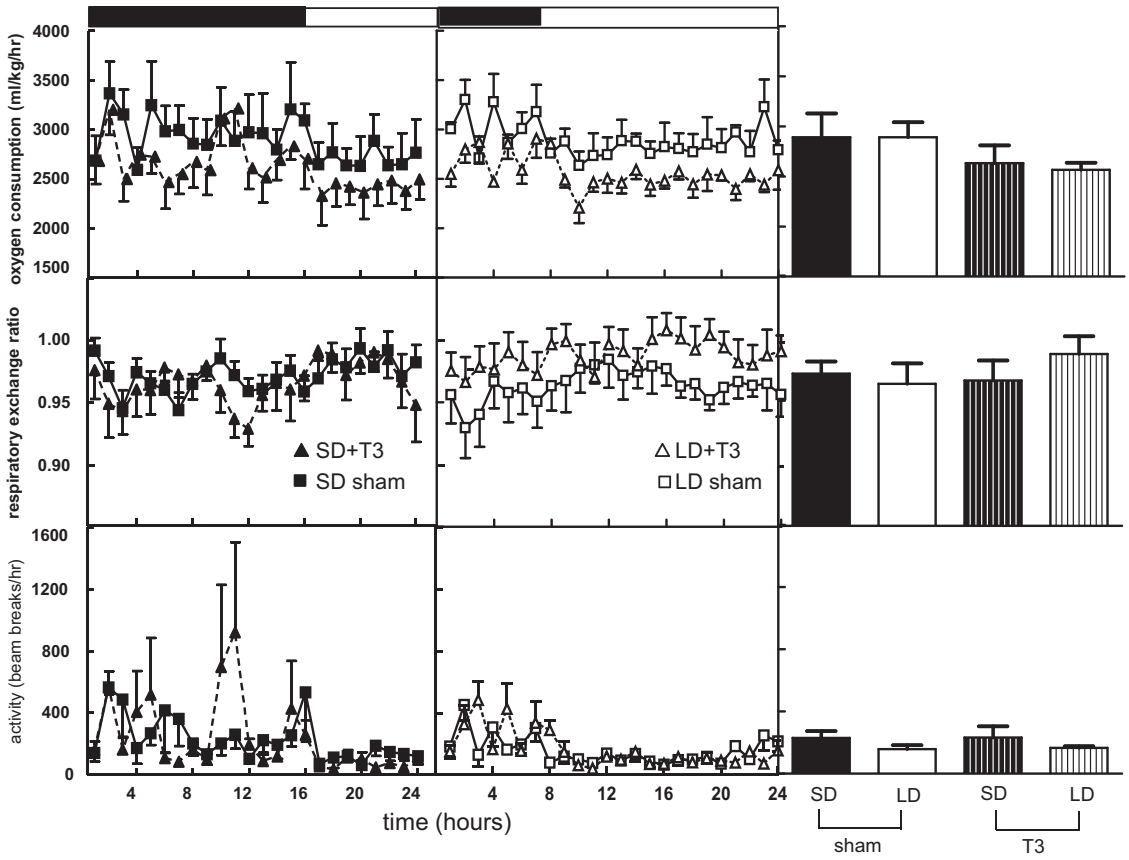

FIG. 3. Oxygen consumption (*top*), respiratory exchange ratio (*middle*), and locomotor activity (*bottom*) in adult male hamsters at wk 13 SD/wk 3 LD with sham (■ and □) or T_3 (▲ and △) hypothalamic implants. Lighting regimes are represented by *horizontal bars*: *solid bar*, dark phase; *open bar*, light phase. Values are group mean ± SEM; n = 8, as calculated for 1-h epochs over the final 24 h of the CLAMS session.

meal size and duration but not meal frequency (Supplemental Fig. 1, published on The Endocrine Society's Journals Online web site at http://endo.endojournals.org), but by wk 9 after implantation, there was a significant effect of T_3 on meal size only in the hamsters transferred to LD (Supplemental Fig. 1).

Study 1: effects of hypothalamic T_3 implants on metabolic parameters

Figure 3 shows data collected for metabolic parameters during the third week after T_3 implantation. Two-way ANOVA revealed that VO_2 was slightly but significantly decreased in the hamsters bearing T_3 implants (main effect of T_3 $F = 11.8$; $P = 0.014$). There were no significant effects of T_3 or photoperiod on the respiratory exchange ratio, values for all hamsters were in the range 0.9–1 (Fig. 3). There was a trend toward increased activity in the hamsters maintained in SD, but this did not reach significance, and there was no significant effect of T_3 treatment on activity (Fig. 3). No significant effects of T_3 treatment or photoperiod were observed on VO_2 at wk 5 and 9 after surgery (data not shown), and likewise there were no significant differences between the groups in respiratory exchange ratio or locomotor activity at these time points (data not shown).

Study 1: effects of hypothalamic T_3 implants on pelage

In all the hamsters exposed to SD before experimental manipulation, the molt to winter pelage had started by wk 10 (Fig. 1C). Subsequent T_3 treatment did not affect the pelage score of the hamsters in either photoperiod (Fig. 1C), because the normal photoperiodic response was observed in both sham- and T_3-implanted groups. In the hamsters that continued to be kept in SD, pelage continued to progress toward a score of 1 representing a full white winter coat (Fig. 1C), whereas scores in sham- and T_3-implanted hamsters transferred to LD returned toward 4, reflecting a molt to a full summer coat. A significant divergence was evident by wk 16, that is 5 wk after the change in photoperiod, with the pelage scores of the LD-sham and LD-T_3 groups being significantly different from the SD-sham group ($P < 0.05$ and $P < 0.001$, respectively).

Study 1: effects of hypothalamic T_3 implants on organ weights

Hamsters were euthanized in wk 20 SD/wk 10 LD. The testes weights of the LD-sham, SD-T_3, and LD-T_3 groups were all significantly heavier than in SD-sham animals (Fig. 4A). Similarly, the epididymal fat pads of hamsters receiving T_3 implants or exposed to LD were significantly heavier compared with hamsters in the SD-sham group (Fig. 4B). No significant differences were found between groups with regards to the wet weights of brown adipose tissue (Fig. 4C) or liver (Fig. 4D). Whole carcass composition to ascertain the total body fat was also assessed for the cohort of hamsters euthanized 6 wk after implantation. Body weight and epididymal fat pad mass were both found to correlate closely with total body fat (Supplemen-

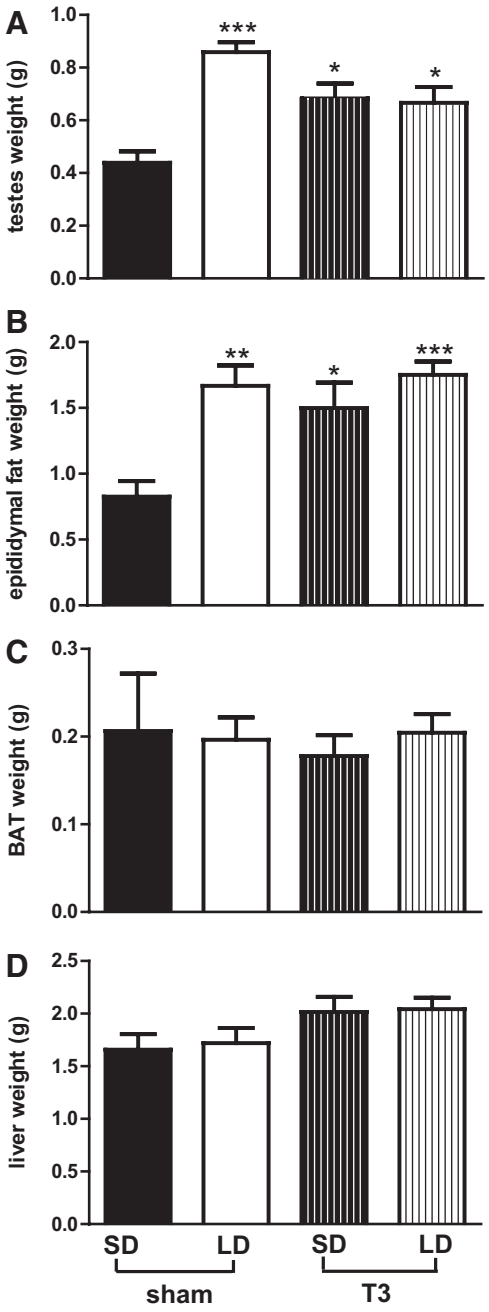

FIG. 4. Wet tissue weights at the end of study 1 (SD wk 20, LD wk 10) for hamsters with sham implants in SD (n = 8) and LD (n = 8) or with T_3 implants in SD (n = 9) and LD (n = 10). A, Weights for paired testes; B, epididymal fat pads; C, intrascapular brown adipose tissue; D, liver. Values are group mean ± SEM. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$ *vs.* SD-sham group.

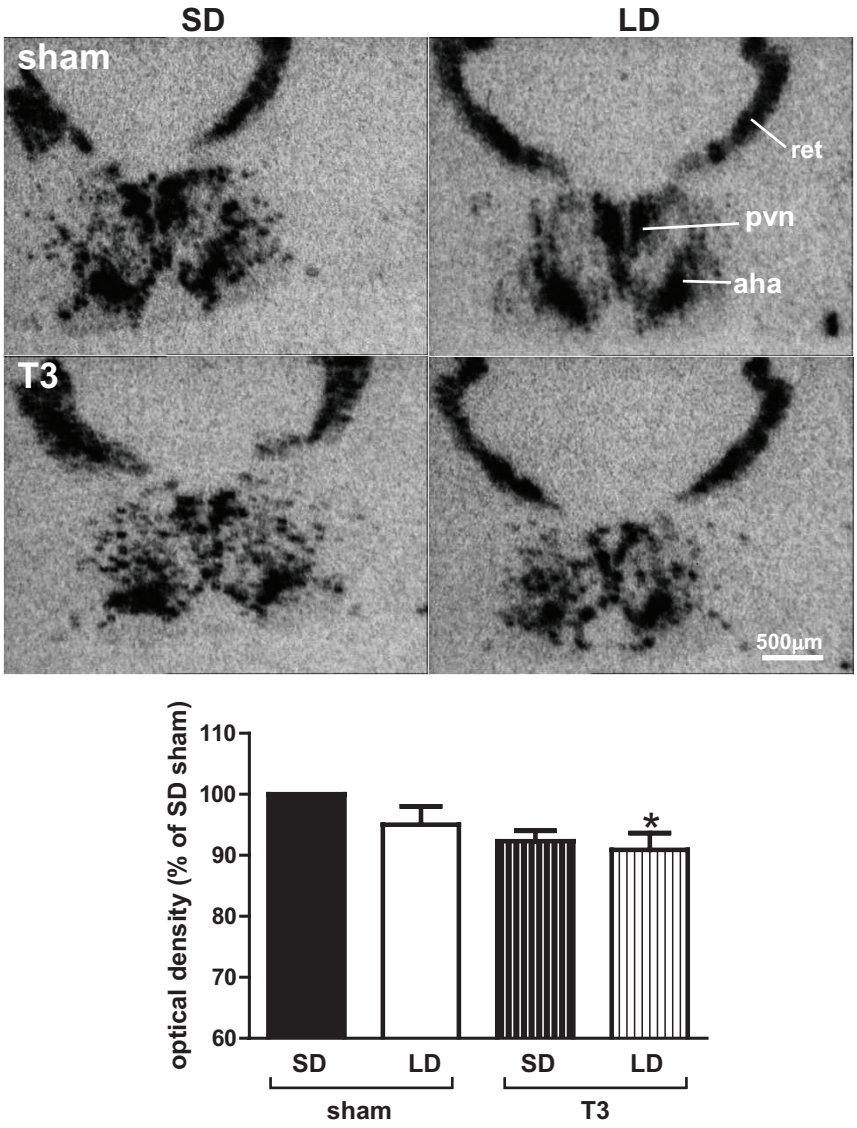

FIG. 5. *Top*, Prepro-TRH *in situ* hybridization in coronal sections through the hypothalamus of representative male hamsters in SD and LD with sham (*upper panels*) or T_3 implants (*lower panels*). *Scale bar*, 500 μm. aha, Anterior hypothalamic area; pvn, paraventricular nucleus; ret, reticular nucleus. *Bottom*, Relative density of prepro-TRH hybridization signal in the paraventricular nucleus for hamsters with sham implants in SD (n = 8) and LD (n = 8) or with T_3 implants in SD (n = 9) and LD (n = 10). Values are group mean ± SEM. *, $P < 0.05$ *vs.* SD-sham group.

tal Fig. 2), but no significant differences in total body fat had developed between the groups at this earlier time point.

Study 1: effects of hypothalamic T_3 implants on prepro-TRH mRNA in the paraventricular nucleus

Prepro-TRH mRNA expression was prominent not only in the paraventricular nucleus (Fig. 5, *top*) but also in the anterior hypothalamic area (Fig. 5, *top*) and reticular nucleus of the thalamus (Fig. 5, *top*), as previously described for the Siberian hamster (16). Image analysis of the autoradiographic films revealed that there was a small but significant ($P < 0.05$) decrease in mRNA in the paraventricular nucleus in hamsters treated with T_3 in LD but not in SD (Fig. 5, *bottom*) and no effect of photoperiod (Fig. 5, *bottom*). Prepro-TRH mRNA expression levels did not differ significantly in the other forebrain regions analyzed (Fig. 5, *top*; quantitative data not shown). Blood samples were not available for analysis of circulating thyroid hormone concentrations from this study, but analysis of free T_4 in samples from hamsters in comparable studies where blood samples were collected 9 wk after hypothalamic placement of T_3 implants in hamsters in long or short photoperiods revealed that hypothalamic T_3 implants increased body weight but did not significantly reduce systemic T_4 values (Supplemental Table 1).

Study 2: effects of hypothalamic T_3 implants on core body temperature and torpor

As in study 1, before treatment, all castrated hamsters transferred to SD showed a substantial spontaneous loss of body weight such that by wk 10 in SD, body weight had decreased by approximately 20% relative to the start of SD exposure. Also consistent with study 1, treatment with hypothalamic T_3 implants then induced a clear increase in body weight (Fig. 6A). ANOVA revealed a treatment × time interaction ($F = 3.15$; $P < 0.001$), and *post hoc* tests indicated that body weight was significantly elevated in the hamsters receiving hypothalamic T_3 by 2 wk after implantation (Fig. 6A). Food intake in the home cage of castrated hamsters did not differ significantly after treatment with T_3 or sham implants into the hypothalamus (Fig. 6B), but at wk 24 (14 wk after implantation), the cumulative food intake in the T_3-treated group was approximately 30 g greater than the sham-implant group. There were also no significant differences in pelage scores between the two groups (Fig. 6C). Both groups continued to molt to a winter pelage, reaching the lowest score at wk 14, and then both groups began to molt to gradually regain their summer coat.

The hypothalamic T_3 treatment had a marked effect on the occurrence of torpor bouts. All the hamsters with sham

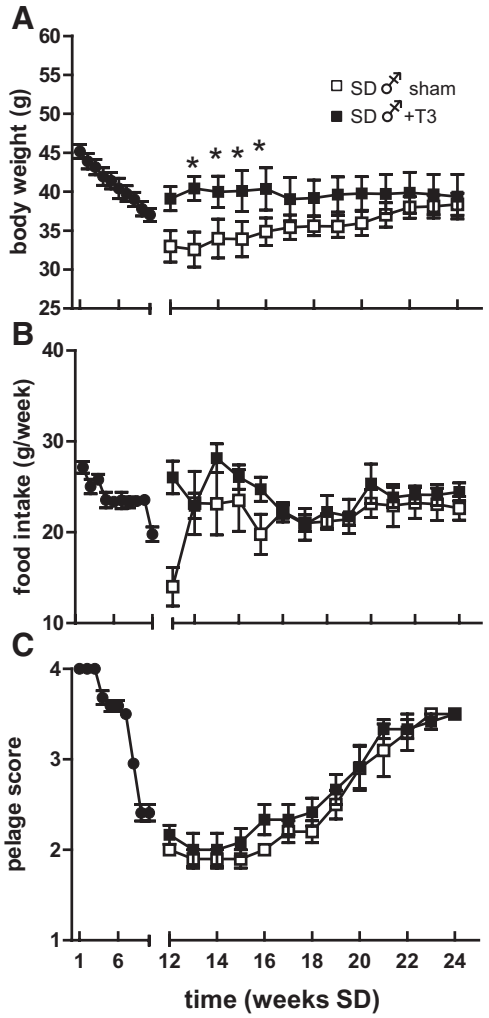

FIG. 6. Study 2: body weight (A), food intake in the home cage (B), and pelage score (C) in castrated male hamsters placed in SD at wk 0 and then receiving sham (□) or T_3 (■) implants in the hypothalamus at wk 11. Note that body weight is significantly higher after implantation of T_3. Values are group mean ± SEM; n = 5. *, $P < 0.05$ *vs.* SD-sham group.

implants displayed torpor bouts. These were characterized by a decrease in T_b beginning at around the time of lights on, reaching a nadir just a few degrees centigrade above ambient room temperature, followed by spontaneous return to euthermia shortly before the onset of the dark phase (Fig. 7A). The frequency of torpor bouts increased to a maximum after 14 wk exposure to SD, at which point torpor bouts occurred on most days (Fig. 7C) such that the weekly duration of torpor was approximately 40 h (Fig. 7D). In contrast, torpor bouts were observed only in two of five T_3-implanted hamsters, and these were very infrequent, with no more than two bouts per week (Fig. 7C), and of short duration when they did occur (Fig. 7D). In the remaining three hamsters, no bouts were observed at all (Fig. 7B), and the hamsters remained euthermic throughout the study. Analysis of locomotor activity provided an independent assessment of torpidity in the two groups of hamsters. Although locomotor activity occurred at similar levels in the two groups during the dark phase across the whole study (Fig. 8A), the activity of the sham-treated hamsters in the light phase was significantly decreased compared with the T_3 group from wk 12–21 in SD (Fig. 8B), indicative of torpor.

Discussion

The major finding of these studies is that increasing hypothalamic T_3 availability in hamsters exposed to SD by means of implants placed bilaterally into the hypothalamus induced a metabolic phenotype characteristic of hamsters in the LD state. The implants produced a rapid and clear increase in body weight that reflected both an increased food intake and a slight initial decrease in energy expenditure. The implants also stimulated testicular recrudescence and the accumulation of intraabdominal fat pads, and in a separate study in castrated hamsters, T_3 prevented the occurrence of daily torpor bouts that are normally displayed by hamsters in SD. These findings build upon our previous studies in hamsters demonstrating that such implants could initially block the catabolic effects of transfer of hamsters from LD to SD (10) and also complement a number of recent studies in demonstrating direct actions of T_3 within the rodent hypothalamus on various aspects of energy metabolism. We observed that the weight gain in response to T_3 implants in hamsters maintained in SD was significantly more rapid than in hamsters transferred to LD. There are a number of reasons that might explain this discrepancy. First, it probably takes several cycles for the nocturnal melatonin rhythm in the sham-implanted hamsters moved to long days to adjust to the change in photoperiod, reflecting the inertia of the underlying circadian pacemaker in the suprachiasmatic nucleus, which drives the rhythm of nocturnal melatonin production in mammals (17). In contrast, our *in vitro* studies demonstrate that the implants very rapidly release T_3 (Murphy, M., unpublished). Second, one might also expect a time lag in the LD-sham hamsters due to the time taken for melatonin to alter TSH-β expression in the pars tuberalis, which appears to be the critical signal regulating deiodinase expression in the tanycyte cell layer (3, 18). Third, the implants release T_3 directly into the neuropil of the hypothalamus and thus may circumvent much of the degrading action of DIO3 on T_3, because *in situ* hybridization studies indicate that expression of this enzyme is much greater in the tanycyte cell population in Siberian hamsters than in neuronal and glial cells in the surrounding hypothalamus (10, 19).

The overarching anabolic effects demonstrated in the current study are consistent with the findings of Kong *et al.*

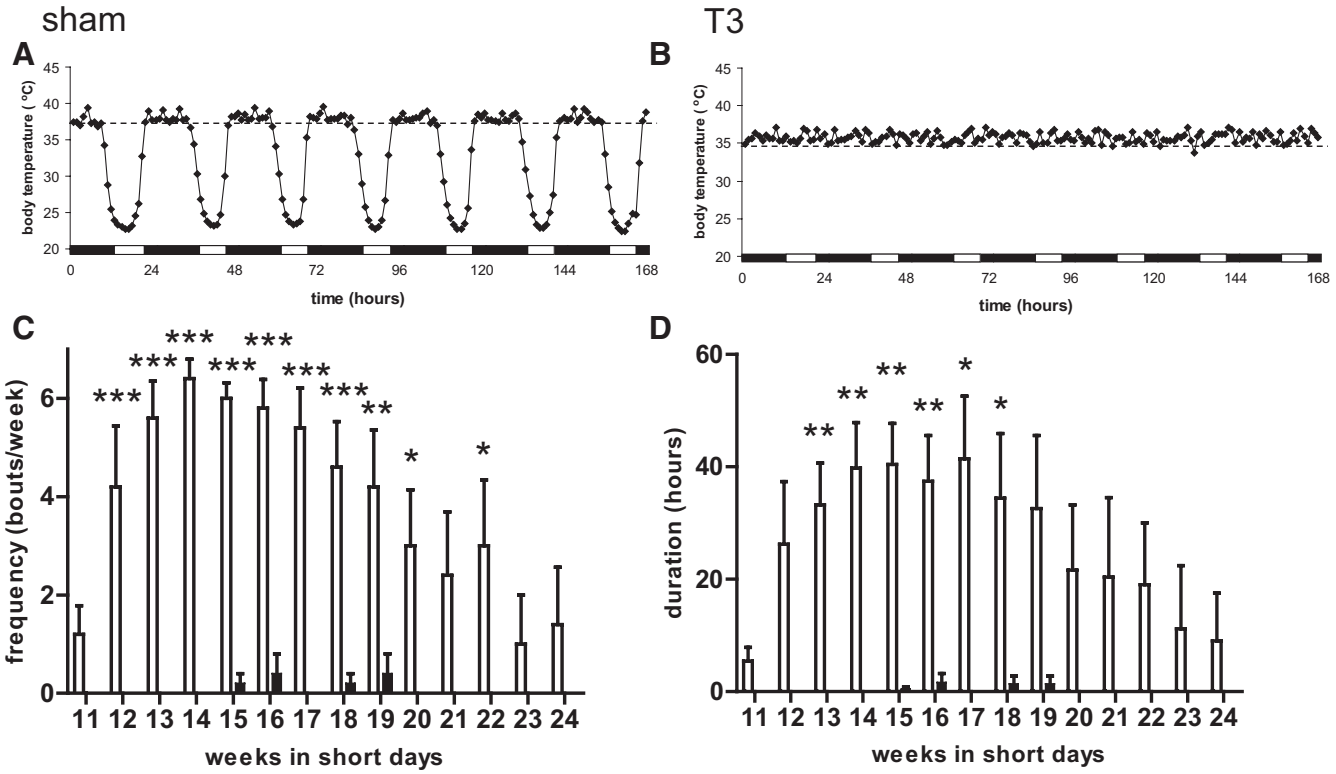

FIG. 7. Study 2: effects of central T_3 on torpor. A and B, Representative examples of torpor profiles from castrate male hamsters with sham (A) or T_3 (B) implants. Mean hourly T_b is shown in relation to the light-dark cycle for wk 17. The *dashed line* represents the animal's mean T_b-3SD calculated after 3 wk exposure to SD. C and D, Mean frequency (C) and duration (D) of torpor bouts in hamsters with sham (*white bars*, n = 5) or T_3 (*black bars*, n = 5) implants. Values are weekly mean ± SEM. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$ *vs.* SD-sham group.

(11), who demonstrated that 0.5–50 pmol T_3 injected directly into the ventromedial hypothalamus stimulated food intake in rats. Two recent studies in rats have also identified direct actions of T_3 within the hypothalamus, but the general trend in those studies was catabolic. First, Klieverik *et al.* (20) observed that infusion of nanomolar amounts of T_3 directly into the paraventricular nucleus increased hepatic glucose production and thus blood glucose concentrations, an effect likely mediated via stimulation of sympathetic activity (20). Second, López *et al.* (21) found that acute intracerebroventricular infusions of 4 ng T_3 increased sympathetic outflow to brown adipose tissue and thus thermogenesis, and infusion of this amount over 4 d decreased body weight without affecting food intake (21). In contrast, studies in transgenic mice found that dominant-negative expression of a mutant *TRα* receptor in the hypothalamus with a low affinity for T_3 increased VO_2 and produced a hypermetabolic state (22), implying that the physiological central action of T_3 in mice is to decrease metabolic rate as we observed in the current study in hamsters.

It is difficult to reconcile the precise intrahypothalamic actions of T_3 on energy metabolism revealed by these various studies in rats and hamsters because the studies differ in the exact sites of administration, the doses of thyroid hormone, and the time courses of hormone administration, but there are two common features. The first is that they point toward direct actions of thyroid hormone within the hypothalamus, because the doses of thyroid hormone infused would not be expected to leach out of the hypothalamus and exert peripheral effects. Second, it is very unlikely that the metabolic actions are secondary consequences of central treatment with thyroid hormone increasing negative feedback suppression of the endogenous hypothalamo-pituitary-thyroid axis, such that the metabolic effects results from a reduction in peripheral thyroid hormone production (hypothyroidism). In our own studies, we found only a very slight suppression of TRH mRNA in the paraventricular nucleus, which would largely reflect the hypophysiotropic population at the end of the 9-wk treatment period, and this was only significant in the hamsters transferred to LD. Although blood samples were not available from the main study for measurement of peripheral thyroid hormone concentrations, data from pilot studies using the same experimental approach showed that circulating free T_4 concentrations were not decreased by intrahypothalamic T_3 implants (Supplemental Table 1). It should be noted that our SILASTIC implants probably released far less T_3 than the pure crystalline hypothalamic T_3 implants that were originally used to

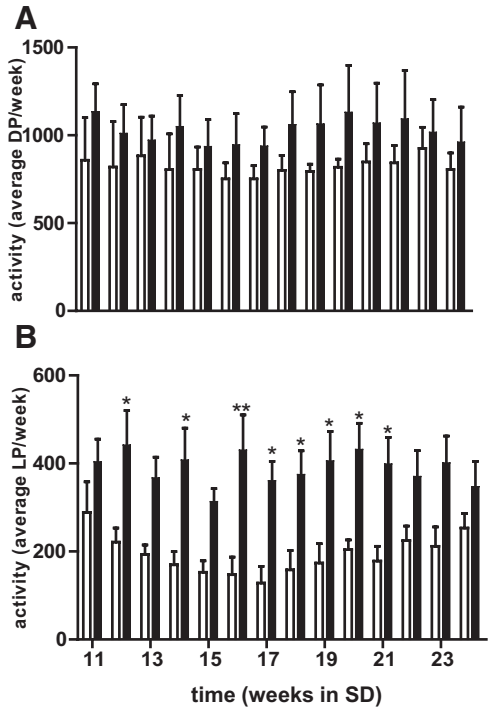

FIG. 8. Study 2: effects of central T_3 on locomotor activity measured by radiotelemetry during the dark phase (DP, A) and light phase (LP, B) in hamsters with sham (*white bars*, n = 5) or T_3 (*black bars*, n = 5) implants. Values are group mean total counts per week ± SEM. *, $P < 0.05$; **, $P < 0.01$ *vs.* SD-sham group. Note the significant increase in locomotor activity during the light phase in T_3-implanted hamsters.

demonstrate suppression of TRH gene expression in hypothyroid rats (23). A limitation of our own studies is that we have been able to assess TRH mRNA abundance and systemic T_4 concentrations only at the end of the treatment periods. Our *in vivo* data suggest that the effects of T_3 may be beginning to wane at this point, although our *in vitro* tests suggest that there is still significant release of T_3 at this time (Murphy, M., unpublished). Importantly, a more detailed analysis of the acute effects of intrahypothalamic T_3 administration in the rat did not find significantly lower peripheral free thyroid hormone concentrations compared with vehicle-infused controls and concluded that the effects of central T_3 on hepatic glucose metabolism were independent of plasma T_3 concentrations (20).

The current findings provide considerable support for the hypothesis that changes in hypothalamic thyroid hormone availability underlie the seasonal changes in food intake and energy expenditure that result in annual cycles of fat storage and utilization. Treatment of hamsters in the lean SD state produced a strikingly rapid gain in body weight, which was significantly advanced compared with the weight gain in a positive control group bearing sham implants that was placed on LD. This difference in timing likely reflects the fact that the signaling of the LD photoperiod requires a change in the nocturnal pattern of melatonin production by the pineal gland. Past studies in the hamster have found that it takes several weeks for an abrupt change in photoperiod to be translated completely into a change in the nocturnal duration of melatonin secretion (17), so a delay in the downstream physiological and behavioral responses would be expected. It was noticeable that the effects of LD and intrahypothalamic T_3 implants on body weight were not additive, which is also consistent with the hypothesis that LD ultimately induce an anabolic state by increasing hypothalamic T_3 availability. It is also clear in both studies that the T_3 treatments had no impact on the pelage cycle. In study 1, both groups of hamsters transferred to LD began the molt to summer pelage, whereas the sham- and T_3-treated hamsters remaining in SD maintained their winter pelage. Likewise in study 2, T_3 did not affect the molt cycle, although after prolonged SD exposure, both sham-implanted and T_3-implanted hamsters began a molt back to summer pelage. This separation of the pelage cycle from the seasonal metabolic cycle demonstrates that the control of hair follicle function is independent of the control of metabolism and is consistent with several lines of experimental evidence from hamsters (13, 24, 25) and sheep (25, 26) that it is actions of melatonin via the pars tuberalis on anterior pituitary prolactin secretion that are the primary determinant of molting.

One aspect of the current study that does fit well with the recent findings alluded to above that T_3 has direct actions on sympathetic outflow from the hypothalamus is that the T_3 implants were almost completely effective in blocking the occurrence of torpor bouts in SD. The endocrine conditions that are permissive for torpor, including low circulating androgen, prolactin, and leptin concentrations, and the role of the circadian system in timing individual bouts of torpor are well established (27–29). We therefore carried out studies in previously castrated hamsters to avoid the possibility that hypothalamic T_3 implants could indirectly block torpor by inducing a LD stimulation of the reproductive axis. The actual cellular mechanisms by which animals become hypometabolic and enter into the torpor bout are not well understood, but it is clear that the recovery from torpor involves sympathetic activation of thermogenesis in brown fat. It therefore seems plausible that the effect of T_3 within the hypothalamus is to maintain a level of sympathetic tone that would maintain hamsters in a euthermic state despite the photoperiodic signals and endocrine milieu being permissive for torpor. This is certainly consistent with the hypothesis that T_3 directly regulates lipid metabolism within the hypothalamic ventromedial nucleus and consequently activates sympathetic control of energy expenditure in brown fat (21). In addition, it has recently been observed

that treatment with the thyroid hormone derivative 3-iodothyronamine induces hypometabolism and torpor in Siberian hamsters (30), which raises the question as to whether T_3 may also be blocking torpor by counteracting the production or actions of endogenous thyronamines.

The broader significance of our studies is that it provides concrete experimental evidence to support the conjecture that seasonal cycles in energy balance and reproduction are driven by changes in hypothalamic thyroid hormone availability, which in turn reflects changes in deiodinase gene expression in tanycytes. Photoperiodic regulation of DIO2 and/or DIO3 gene expression has been robustly documented in a variety of seasonal mammals and birds (see Ref. 31 for review), and the elegant studies of Yoshimura and colleagues (18) and Hanon *et al.* (3) identify mechanistic pathways by which photoperiod and melatonin act via TSH-β secretion from the pars tuberalis to signal to the tanycyte cells in the mediobasal hypothalamus. What has been lacking is evidence that such changes in deiodinase gene expression have biological significance; thus, our findings that local manipulation of T_3 can induce a LD phenotype provide important support for a role for the deiodinases in seasonal regulation. Of course we remain ignorant of the sites and mechanisms of action whereby T_3 regulates seasonal cyclicity. Nuclear TRα is expressed almost ubiquitously in the adult brain, and TRβ1 and TRβ2 are abundant in the mediobasal hypothalamus (10), so a wide range of genomic actions could be envisaged, but the recent study from López *et al.* (21) also identified acute actions of intracerebroventricular T_3 administration that included decreased phosphorylation of AMP-activated protein kinase and of acetyl-coenzyme A carboxylase in the ventromedial hypothalamus and thus increased lipogenesis in this region. The direct hypothalamic effects of intracerebroventricular T_3 on sympathetic control of hepatic glucoregulation reported by Klieverik *et al.* (20) are also very rapid, which the authors therefore consider to reflect nontranscriptional mechanisms. An alternative view based on the knowledge that thyroid hormone plays a crucial role in the initial development of the brain (32–34) is that seasonal cycles of metabolism and reproduction represent additional plastic changes in adult hypothalamic structure and function. This is clearly a speculative hypothesis at present, but it is worth noting that there is ultrastructural evidence for seasonal plasticity in glial ensheathment of synaptic inputs to GnRH neurons in sheep (35) and that recent studies in rodents have identified markers of neurogenesis in the hypothalamus (36, 37) and have indicated that the chronic actions of certain growth factors on caloric intake are dependent upon the induction of neurogenesis (38). Moreover, recent studies have demonstrated that sonic hedgehog and other components of its signaling cascade, which are an important gene pathway in development of the forebrain and other tissues, are regulated by thyroid hormone (39, 40). Importantly, this continues to be the case into adulthood and thus may be a mechanism for neural plasticity in the mature brain (40).

In summary, we have found that intrahypothalamic implants releasing T_3 induce an anabolic state that resembles the LD seasonal metabolic phenotype. These findings add to the recent body of evidence that hypothalamic thyroid availability plays a direct role in regulation of appetite, energy expenditure, and thus body weight and support the hypothesis that changes in central regulation of thyroid availability by deiodinase enzymes underlie long-term seasonal cycles of energy metabolism.

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