Peak duration of serum melatonin and short-day responses in adult Siberian hamsters

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BARTNESS, TIMOTHY J., AND BRUCE D. GOLDMAN. *Peak duration of serum melatonin and short-day responses in adult Siberian hamsters.* Am. J. Physiol. 255 (Regulatory Integrative Comp. Physiol. 24): R812–R822, 1988.—Long photoperiod-housed, adult Siberian hamsters were pinealectomized and given daily subcutaneous infusions of melatonin (MEL) to determine which characteristic of the MEL secretion profile is critical for short photoperiod-induced physiological responses. Long-duration MEL infusions (10 or 12 h) given for 5 wk elicited short-day-type responses [i.e., decreased body, testes, and epididymal white adipose tissue (EPIWAT) weights, EPIWAT lipoprotein lipase activity, carcass lipid content, and serum follicle-stimulating hormone and prolactin levels]. In contrast, short- or intermediate-duration (5 or 8 h) MEL infusions or saline infusions were without effect. Long-duration MEL infusions elicited short-day-type responses independently of both the time of day when MEL was administered and of the MEL dose if the latter was ≥6.25 ng MEL/daily infusion. The continuity of the 10-h MEL infusions was important for triggering short-day-type responses; 10-h MEL infusions interrupted at their midpoint by 2 h of no infusion were ineffective even though dose and total duration were held constant. The body and lipid mass decreases were independent of the gonads, since castrated and gonad-intact hamsters responded similarly to the daily 10-h MEL infusions. Decreased body weight resulting from long-duration MEL infusions were never accompanied by decreased food intake. We conclude that the peak nocturnal duration of MEL is the critical parameter of the MEL secretion profile for triggering short-day-induced responses in adult Siberian hamsters.

body weight; body fat; lipoprotein lipase; brown adipose tissue; testes; reproduction; follicle-stimulating hormone; prolactin; pineal gland; food intake; cytochrome oxidase; white adipose tissue

MANY MAMMALS exhibit an annual cycle of reproduction. Other physiological responses often accompany the changes in reproduction to optimize seasonal breeding and to enhance survival during periods of gonadal quiescence. These responses include alterations in body and lipid mass and thermogenic capacity. Daylength change is a major environmental cue for these and other seasonally adaptive responses in golden (Syrian; *Mesocricetus auratus*) and Siberian (*Phodopus sungorus sungorus*) hamsters (19, 38) and in other species (e.g., 9). After short-day exposure, golden and Siberian hamsters exhibit gonadal regression (6, 24, 25, 40) and increased thermogenic capacity (6, 23). However, short-day exposure elicits opposite changes in body and lipid mass in the two species (7); golden hamsters show increased body weight and body fat (6, 7), whereas Siberian hamsters exhibit decreases in these same parameters (25, 40).

The daylength changes that are responsible for these and other seasonal responses are transduced into an endocrine signal by the pineal gland and its hormone, melatonin (MEL). MEL synthesis and secretion occur at night, and in several species, the peak nocturnal duration of MEL secretion is inversely related to the daylength (10, 16, 26). Carter and Goldman (13, 14) tested the importance of the MEL peak duration for the photoperiodic control of testicular development in juvenile Siberian hamsters. The naturally occurring peak nocturnal duration of serum MEL exhibited by pineal-intact short- or long-day-housed hamsters was simulated in pinealectomized (PINX) animals by administering timed daily subcutaneous infusions of MEL. Long-duration MEL infusions ("short-day type") delayed testicular development (14) and short-duration infusions ("long-day type") stimulated testis growth (13). The ability of the long-duration MEL infusions to inhibit the pituitary-gonadal axis was not dependent on the time of day at which the hormone was infused. These results suggest that the peak nocturnal duration of serum MEL is the critical parameter of the MEL secretion profile responsible for triggering short photoperiod-induced gonadal regression (14).

The purposes of the present experiments were to examine *1*) whether a similar relationship exists between the peak duration of MEL secretion and reproduction in adult Siberian hamsters and *2*) whether this feature of the MEL secretion profile regulates changes in other photosensitive end points, such as body and lipid mass, lipid metabolism, and growth of the thermogenic component of brown adipose tissue (BAT).

METHODS

Animals. Adult male Siberian hamsters were obtained from our breeding colony. The colony was derived from stock generously supplied by Klaus Hoffmann (MPG Clinical Research Unit for Reproductive Medicine, Munster, FRG). Hamsters were given ad libitum access to Purina rodent chow (no. 5001, 3.4 kcal/g) and tap water. Body weight and food intake were measured weekly to the nearest 0.1 g, the latter corrected for spillage and pouching. The hamsters were housed singly in 22.25 × 12 × 20-cm polycarbonate infusion cages with Beta Chip bedding (Northeastern Products, Warrens-

bury, NY). A long photoperiod was employed [16 h light and 8 h dark (LD 16:8)] with lights on at 0200 h. Light was provided by fluorescent light bulbs located ~1.5 m from the top row of infusion cages (light intensity ~1,000 lux at this level).

Surgery. Pinealectomy was performed under pentobarbital sodium anesthesia (2.0–2.5 mg/animal). A trephine was used to make a hole in the skull above the confluence of the sinuses, and the sinus was penetrated with fine-toothed forceps to remove the pineal gland. Bleeding was stopped by inserting a gelatin pellet (Gelfoam, Upjohn, Kalamazoo, MI) into the trephined hole. Castrations were performed under methoxyflurane anesthesia via a unilateral incision offset from the midline to avoid urogenital damage.

Daily timed subcutaneous infusions. Hamsters were fitted with a subcutaneous catheter for chronic infusion of MEL according to a modification of the catheterization procedure of Carter and Goldman (14), as we recently described (8). The distal end of the catheter was attached to a flow-through, water-tight swivel (12). The tubing at the top of the swivel was connected to a syringe operated by an infusion pump (Razel Scientific Instruments, Stamford, CT) that was controlled by an electronic timer. The original MEL stock solution was prepared with synthetic MEL (Sigma Chemical, St. Louis) dissolved in 95% ethanol (final concentration 10 mg/ml). Aliquots of the final infusion solution were prepared by further dilution of the stock solution with 0.15 M NaCl. Frozen aliquots of the MEL infusion solutions were thawed, diluted, and used to replace the old MEL at 2- to 3-day intervals.

Carcass composition and tissue assays. Carcass composition was measured using a modification of the method of Leshner et al. (28), as we described previously (1, 6, 40), to determine which carcass components contributed to changes in body mass. The dehydrated delipidated tissue was termed fat-free dry mass (FFDM). To determine whether the changes in carcass lipid were uniformly exhibited in all fat pads, various white adipose tissue (WAT) and BAT pads were examined [i.e., sternal BAT (STERNBAT), inguinal subcutaneous WAT (INGSCWAT), and epididymal WAT (EPIWAT)]. During removal of the fat pads, the carcass was packed in ice, and the tissues of interest were bathed in ice-cold saline.

Total adipose tissue lipoprotein lipase (LPL) activity was measured according to the method of Heitanen and Greenwood (23) and Schotz et al. (35), as we described previously (2, 5, 8). LPL activity was assayed using [^{14}C]triolein as the substrate. Specific LPL activity was calculated as LPL activity per milligram postmitochondrial infranatant protein content, the latter measured according to the method of Lowry et al. (29). LPL is responsible for the clearance of fatty acids from circulating triglycerides into adipose tissue (15). Decreases in LPL activity are normally associated with decreases in fat pad growth.

Thermogenesis increases after short-day exposure in Siberian hamsters, and this is reflected, in part, by an increase in the growth of the metabolic component of BAT. This response was assessed by measuring the protein content and cytochrome oxidase activity (COA) of the tissue. COA is a marker enzyme for mitochondria, the thermogenic organelles of BAT (31). COA was measured according to a modification of the method of Wharton and Tzagoloff (41), as described previously (2, 22). BAT protein content was measured according to the method of Lowry et al. (29).

When applicable, testicular status was determined by the weight of the paired testes at the termination of the experiment.

Hormone assays. The animals were killed by decapitation and trunk blood was collected. Serum was obtained by centrifugation (2,500 rpm for 20 min) after 24 h of refrigeration at 4°C.

Prolactin (PRL) was measured by a homologous radioimmunoassay (RIA) validated for use in golden hamsters (11). The RIA employed an antiserum raised against golden hamster PRL (antiserum supplied by Katerina T. Borer, University of Michigan). Purified golden hamster PRL was used both to prepare the labeled trace and as the standard (PRL supplied by Frank Talamantes, University of California, Santa Cruz). The purified PRL produced standard curves parallel to those from serial dilutions of pooled sera from lactating and nonlactating Siberian hamsters (17).

Follicle-stimulating hormone (FSH) was measured by a RIA method that was previously validated for use in Siberian hamsters (42). The assay employed a RIA kit obtained from National Institute of Arthritis Metabolic and Digestive Diseases, with anti-rat FSH-7 as the primary antibody and rat FSH-RP-1 as the standard.

Undetectable concentrations of the hormones were assigned the value for the lower limit of sensitivity of the RIA, determined according to the method of Rodbard (34). Sensitivities for FSH and PRL were 2.12 and 6.87 ng/ml serum, respectively. All hormone measurements were carried out in a single assay for each respective hormone.

Experiment 1: mimicking effects of short-day exposure with melatonin infusions. Hamsters were PINX and given a 2-wk recovery period. Only animals that had postsurgery body weight losses of 5% or less continued in the experiment. The animals were divided into two groups ($n = 10$) balanced for their body weights. At this time the animals were implanted with subcutaneous catheters for timed 12-h infusions of either *1*) MEL (100 ng/daily infusion; $n = 9$) or *2*) the saline vehicle ($n = 10$). All infusions were centered at the midpoint of the light phase of the light-dark cycle. The 12-h MEL infusion duration approximates the peak nocturnal duration of serum and pineal MEL in short-day housed (LD 10:14) male Siberian hamsters (16, 20). Body weight and food intake were measured weekly. After 5 wk, the animals were killed by decapitation, and trunk blood was collected for serum hormone assay. The WAT and BAT pads of interest were rapidly dissected, blotted dry, weighed, and homogenized for the assay of LPL activity. The rest of the shaved eviscerated carcass was processed for carcass composition.

Experiment 2: dose-effect range for melatonin infusions. Hamsters were PINX, given a 2-wk postsurgery recovery

TABLE 1. *Effect of melatonin infusions on carcass composition in experiments 1 and 2*

	Saline		Melatonin				
	10 h	12 h	10 h				12 h
			1.56 ng	6.25 ng	25 ng	100 ng	100 ng
			Experiment 1				
Paired testes wt, mg		774.9±37.8					96.5±6.7*
			Experiment 2				
Paired testes wt, mg	740.5±36.3		755.1±43.3	167.0±47.3*	142.0±33.3	68.4±5.4*	

Values are means ± SE. All infusions in both experiments were centered on midpoint of light cycle.

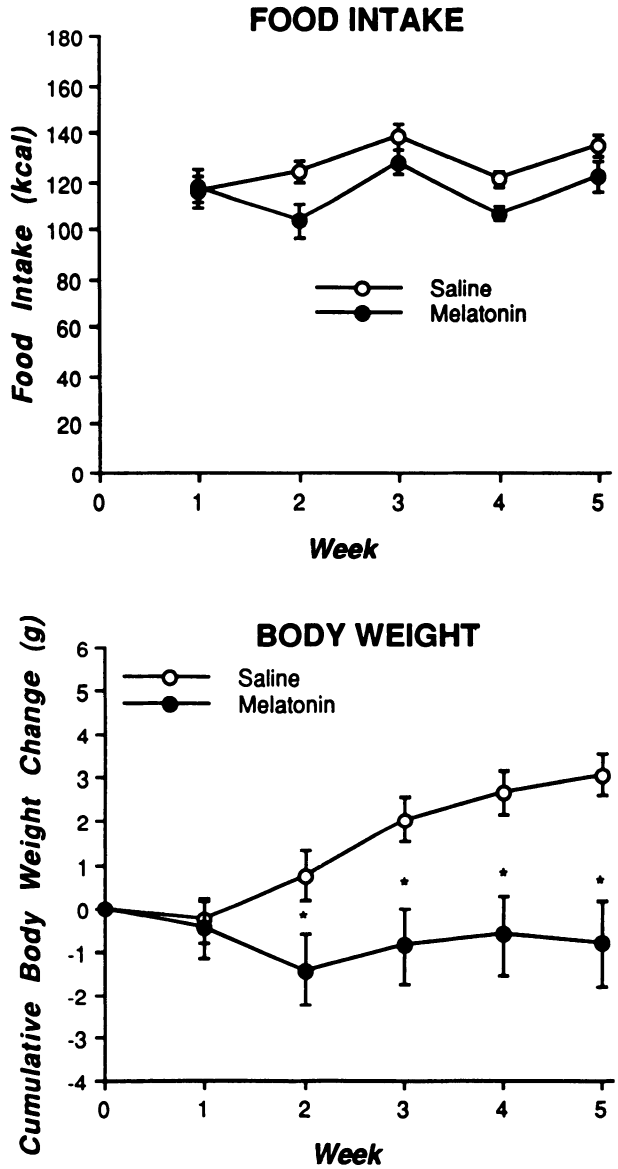

FIG. 1. Mean ± SE food intake (*top*) and cumulative body weight change (*bottom*) of adult male Siberian hamsters in *experiment 1* infused daily for 12 h with melatonin (MEL) or saline for 5 wk. Mean base-line body weights ± SE: MEL 12 h, 41.0 ± 1.1 g; saline 12 h, 41 ± 1.5 g. * Body weight values different from saline controls.

period, and divided into five groups (n = 7–8) based on the body weight criteria of *experiment 1*. At this time the animals were implanted with subcutaneous catheters for timed 10-h infusions of the saline vehicle or MEL (1.56, 6.25, 25, or 100 ng/daily infusion). Infusions were centered on the midpoint of the light phase. The MEL infusion duration was set at 10 h in this experiment to yield a duration that was close to the minimum required to cause testicular regression in immature Siberian hamsters (14, 16, 20). The protocol of *experiment 1* was then followed.

Experiment 3: time of day and continuity of the melatonin infusion. Hamsters were PINX, given a 2-wk postsurgery recovery period, and divided into six groups (n = 8–11) based on the body weight criteria of *experiment 1*. At this time the animals were implanted with subcutaneous catheters for timed infusions of the saline vehicle or MEL at a dose of 6.25 ng/daily infusion, which was the lowest effective dose of MEL determined in *experiment 2*. The following infusion regimens were used: *1*) the standard 10-h MEL infusions centered on the midpoint of the light phase, *2*) 10-h MEL infusions centered on the midpoint of the dark phase, *3*) 5-h MEL infusions given during the first 5 h of the dark phase, *4*) 5-h MEL infusions during the last 5 h of the dark phase, *5*) two 5-h MEL infusions separated from each other by 2 h and centered on the midpoint of the dark phase, and *6*) 10-h infusions of the saline vehicle centered on the midpoint of the light phase. The 5-h MEL infusion duration was chosen because it approximates the peak nocturnal duration of serum and pineal MEL in long-day housed (LD 16:8) male Siberian hamsters (16, 20). The protocol of *experiment 1* was then continued.

Experiment 4: dependence of body and lipid mass effects on gonads. Hamsters were divided into two initial groups based on body weight. Half the animals were castrated (CAST), and all were housed in the long photoperiod. Nineteen weeks later, both CAST and gonad-intact hamsters were PINX and given a 2-wk postsurgery recovery period. The animals were then implanted with a subcutaneous catheter for timed infusions that were centered on the midpoint of the light phase. The hamsters were divided into the following groups based on the body weight criteria of *experiment 1* (n = 9–11): *1*) castrated hamsters given saline (10 h) or 10-, 8-, or 5-h MEL infusions (designated CAST saline 10 h, CAST MEL 10 h, CAST MEL 8 h, and CAST MEL 5 h, respectively) and *2*) gonad-intact hamsters given saline (10 h) or 10- or 5-h MEL infusions. The 10- and 8-h MEL infusion durations were selected in an attempt to determine the minimum MEL infusion duration that would result in short-day responses. For all MEL infusions the dose was 6.25 ng/daily infusion. The protocol of *experiment 1* was then followed.

TABLE 2. *Effect of melatonin infusions on carcass composition in experiments 1–3*

Treatment	n	Carcass Component						
		Total CW, g	Total FFDM, g	Total BF, g	Carcass, g	% CW	% FFDM	% BF
Experiment 1: mimicking short-day exposure								
Saline 12 h	10	19.29±0.34	7.28±0.13	9.76±0.68	35.90±0.80	52.74±1.7	20.23±0.37	27.01±1.37
MEL 12 h 100 ng	9	17.09±0.46	6.68±0.36	6.48±0.57*	30.25±0.95*	56.71±1.47	21.96±0.78	21.34±1.57*
Experiment 2: effect of melatonin dose								
Saline 10 h	7	17.62±0.63	6.88±0.19	9.70±0.98	34.20±1.42	51.79±1.57	20.26±0.76	28.07±1.92
MEL 10 h								
1.56 ng	7	18.29±0.44	6.69±0.29	8.36±0.42	33.91±0.67	53.95±1.03	19.72±0.71	26.33±1.12
6.25 ng	8	16.23±0.75	6.14±0.44	5.19±0.54*	27.56±1.72*	59.17±0.81*	22.27±0.97	18.56±1.01*
25 ng	7	17.00±0.73	6.89±0.35	6.26±0.59*	30.16±1.55*	56.55±0.80*	22.91±0.53	20.51±1.22*
100 ng	7	15.44±0.24	6.17±0.10	5.38±0.55*	27.14±0.75*	57.6±1.33*	22.82±0.57	19.55±1.68*
Experiment 3: effect of time of daily infusion								
Saline 10 h	10	19.41±0.57	7.73±0.21	8.17±0.54	35.31±1.07	55.06±0.92	21.95±0.37	23.00±1.10
MEL 6.25 ng								
10 h light	8	18.29±0.73	7.16±0.35	5.92±0.86*	31.25±1.68	58.92±1.68	21.78±0.67	18.15±1.97*
10 h D	10	16.36±0.44	6.6 ±0.18	6.01±0.48*	28.97±0.88*	56.61±0.97	22.82±0.36	20.57±1.26*
5 h × 2 D	12	18.28±0.53	7.54±0.27	8.27±0.60	34.10±1.22	53.85±0.98	22.16±0.36	24.00±1.05
5 h E D	10	18.95±0.55	7.34±0.24	8.09±0.80	34.38±1.27	55.40±1.36	21.45±0.52	23.15±1.76
5 h L D	11	19.29±0.46	7.71±0.15	9.48±1.34	35.57±1.73	54.47±1.23	21.75±0.33	23.75±1.46

Values are means ± SE. CW, carcass water; FFDM, fat-free dry mass; BF, body fat; carcass, wet weight of shaved eviscerated carcass; MEL, melatonin. Percent carcass components computed as [(carcass component/carcass wet weight) × 100]. Saline 12 h or 10 h and MEL 12 h or 10 h, 12- or 10-h saline or MEL infusions centered on midpoint of light cycle, respectively. MEL 10 h light, 10-h MEL infusion centered on midpoint of light cycle. MEL 10 h, D, MEL 10-h infusion centered on midpoint of dark (D) cycle. MEL 5 h E D and MEL 5 h L D, 5-h MEL infusions given at either beginning ("early"; E) phase of dark cycle or at end ("late"; L) phase of dark cycle, respectively. MEL 5 h × 2, two 5-h MEL infusions separated by 2 h centered on midpoint of dark cycle. * $P < 0.05$ vs. saline controls.

TABLE 3. *Effect of melatonin infusions on brown adipose tissue measures in experiments 1 and 2*

Treatment	STERNBAT				
	Wet wt	Total LPL	Specific LPL	Total protein	Total COA
Experiment 1					
Saline 12 h	228.0±20.4	1.72±0.31	0.34±0.06	10.20±1.84	2.86±0.61
MEL 12 h (100 ng)	209.2±18.2	1.46±0.27	0.19±0.07	15.79±2.63*	4.08±0.60*
Experiment 2					
Saline 10 h	214.1±27.3	1.37±0.22	0.20±0.03	7.46±0.38	
MEL 10 h (1.56 ng)	233.9±28.5	1.30±0.15	0.30±0.05	7.53±1.53	
MEL 10 h (6.25 ng)	163.3±31.1	1.63±0.09	0.30±0.04	8.21±1.20	
MEL 10 h (25 ng)	206.3±22.9	1.54±0.26	0.26±0.01	11.06±1.63*	
MEL 10 h (100 ng)	183.7±28.5	1.60±0.18	0.21±0.02	12.63±1.98*	

Values are means ± SE. STERNBAT, sternal brown adipose tissue; COA, cytochrome oxidase activity in μmol cytochrome *c* oxidized·min^{-1}·pad^{-1}; total LPL activity, lipoprotein lipase activity in μmol FFA released·h^{-1}·pad; specific LPL activity, lipoprotein lipase activity in μmol FFA released·h^{-1}·mg^{-1} protein; see Table 2 for treatment group abbreviations. * $P < 0.05$ vs. saline controls.

Statistical analyses. Repeated measures data and/or data from experiments with more than two experimental groups were analyzed by analysis of variance (ANOVA) and, where applicable, Duncan's new multiple-range tests were used for post hoc analyses. Nonrepeated measures data from two groups were analyzed by *t* tests. Results for all statistical tests were considered statistically significant if $P < 0.05$. Note that formal statistical analysis is theoretically not appropriate for the serum hormone data, since a number of the samples had undetectable concentrations of hormone and were assigned the value associated with the sensitivity of the assay. Despite these concerns, the formal ANOVAs were performed. The number of outlying values for each group relative to the total number of samples in the group is included in the graphic or tabular form of the data.

RESULTS

Experiment 1: mimicking effects of short-day exposure with melatonin infusions. Despite being housed in a long photoperiod, MEL-infused hamsters exhibited a number of short-day-type responses, including regressed testes (Table 1; $P < 0.05$), decreased body weight (Fig. 1; *weeks 2–5*, $Ps < 0.05$), and decreased EPIWAT and ING-SCWAT weights (Table 4; $Ps < 0.05$) relative to the saline-infused hamsters. The decrease in body weight was not accompanied by a decrease in food intake (Fig.

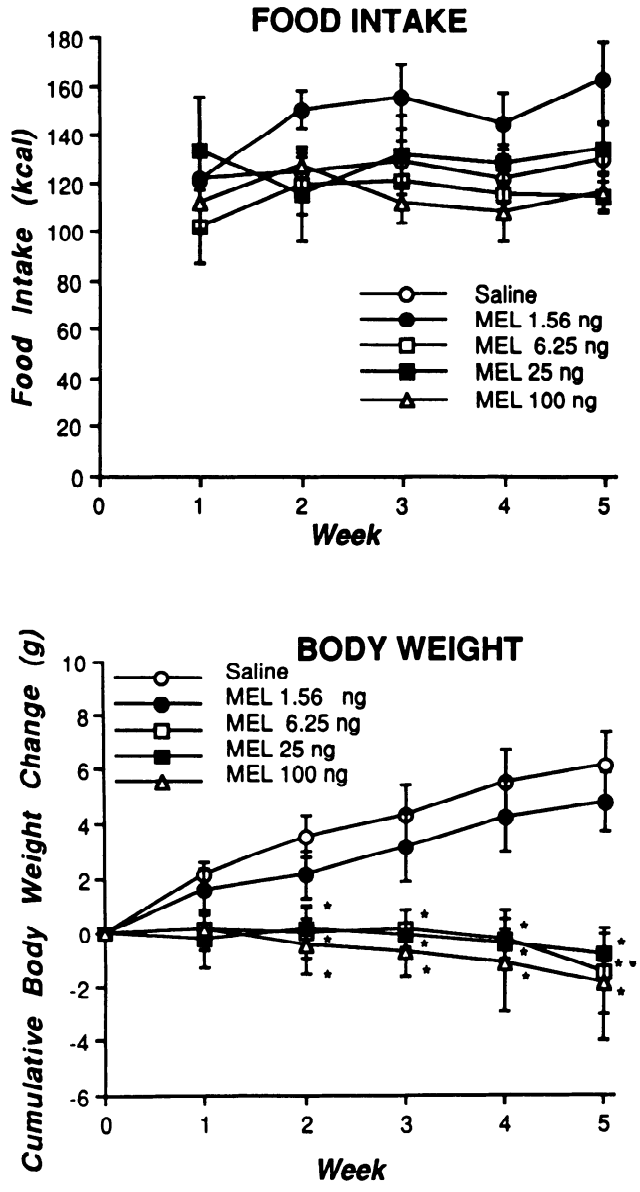

FIG. 2. Mean ± SE food intake (*top*) and cumulative body weight change (*bottom*) of adult male Siberian hamsters in *experiment 2* infused for 10 h daily with melatonin (MEL; 1.56, 6.25, 25, or 100 ng/infusion) or saline daily for 5 wk. Mean base-line body weights ± SE were: saline, 38.3 ± 2.1 g; MEL 1.56 ng, 40.7 ± 1.8 g; MEL 6.24 ng, 37.69 ± 1.9 g; MEL 25.0 ng, 41.6 ± 1.5 g; MEL 100 ng, 37.6 ± 0.9 g. * Body weight values different from saline controls.

1) and was reflected exclusively as a decrease in carcass lipid whether the data are expressed absolutely or as a percent of the carcass weight (Table 2; Ps < 0.05). Although both EPIWAT and INGSCWAT masses were decreased, total and specific LPL activities were decreased only in EPIWAT.

MEL infusions increased the growth of the thermogenic compartment of STERNBAT (i.e., increased COA and protein content, Table 3, Ps < 0.05), effects that normally accompany short-day exposure in pineal-intact hamsters (31, 40). STERNBAT mass and LPL activities were not affected by the MEL infusions (Table 3).

Experiment 2: dose-effect range for melatonin infusions. Only hamsters that received MEL doses of 6.25 ng/day or larger exhibited short-day-type responses, including regressed testes (Table 1, Ps < 0.05), decreased body weight (Fig. 2; *weeks 2–5*, Ps < 0.05), and decreased EPIWAT pad weight and total LPL activity. The decreases in body weight were not accompanied by decreases in food intake (Fig. 2) and were reflected exclusively as decreases in carcass lipid content. These decreases were statistically significant regardless of whether the results were expressed absolutely or relative to carcass weight (Table 2; Ps < 0.05). The decreases in testes, body, and EPIWAT weights, total EPIWAT LPL activity, and carcass lipid content were not different for each measure among the hamsters infused with 6.25 ng MEL/day or larger doses. The effects of MEL were fat pad specific. Thus the EPIWAT pads were affected by the MEL infusions but the INGSCWAT pads were unresponsive (Table 4).

Short-day-type increases in STERNBAT protein content were observed in hamsters receiving the 100 and 25 ng doses of MEL (Table 3; Ps < 0.05). However, unlike the other MEL sensitive end points measured in this experiment, the 6.25-ng dose of MEL did not affect this BAT measure (COA was not measured in *experiment 2*). BAT weight and LPL activity were not affected by any of the infusions (Table 3).

Experiment 3: time of day and continuity of melatonin infusion. Long-duration infusions of MEL (10 h) resulted in short-day-type decreases in testes weight, serum FSH, and PRL concentrations (Table 5; Ps < 0.05), body weight (Fig. 3, *weeks 3–5*; Ps < 0.05), carcass lipid content (Table 2; $P < 0.05$), EPIWAT weight, and total EPIWAT and INGSCWAT LPL activities (Table 6; Ps < 0.05). These short-day-type effects were the same whether the 10-h MEL infusion was administered primarily during the dark phase or during the light phase of the photocycle. The decreases in body weight in hamsters receiving the 10-h uninterrupted MEL infusions were not accompanied by corresponding decreases in food intake (Fig. 3). BAT wet weight was decreased in the 10-h MEL group infused during the dark relative to the saline-infused controls ($P < 0.05$); none of the other measures of BAT were affected by any of the infusions (Table 7).

Experiment 4: dependence of body and lipid mass effects on gonads. Gonad-intact hamsters receiving 10-h MEL infusions exhibited short-day-type decreases in testis weight accompanied by decreases in serum FSH and PRL (Table 5; Ps < 0.05) and decreases in body weight (Fig. 4, *bottom*; Ps < 0.05) and carcass lipid content (Table 8; $P < 0.05$). These animals also exhibited decreases in the weight and total LPL activity of the EPIWAT pads (Table 6; Ps < 0.05); however, the INGSCWAT pad was not affected (Table 6). Gonad-intact hamsters receiving daily 8-h MEL infusions did not exhibit any of the short-day-type responses.

Ten-hour MEL-infused CAST hamsters exhibited responses that were comparable to those of the gonad-intact animals receiving the same MEL treatment; specifically, both groups showed decreased body weight (Fig. 4, *bottom*; Ps < 0.05), carcass lipid content (Table 8; Ps < 0.05), and the fat pad-specific decreases in EPIWAT mass and LPL activity (Table 6, Ps < 0.05). However,

TABLE 4. *Effect of melatonin infusions on white adipose tissue measures in experiments 1 and 2*

Treatment	INGSCWAT			EPIWAT		
	Wet wt	Total LPL	Specific LPL	Wet wt	Total LPL	Specific LPL
			Experiment 1			
Saline 12 h	948.7±66.3	1.69±0.44	0.37±0.06	473.5±25.2	4.55±0.77	0.71±0.09
MEL 12 h (100 ng)	671.3±92.4*	1.96±0.66	0.33±0.05	307.6±32.7*	1.22±0.35*	0.36±0.05*
			Experiment 2			
Saline 10 h	897.5±103.5	2.48±0.21	1.85±0.23	958.2±96.5	9.99±1.06	1.15±0.11
MEL 10 h (1.56 ng)	985.9± 83.6	2.00±0.25	1.32±0.25	899.3±57.4	8.73±0.58	0.95±0.06
MEL 10 h (6.25 ng)	687.6±112.4	2.00±0.42	1.01±0.11	526.7±85.6*	2.72±0.5*	0.71±0.05
MEL 10 h (25 ng)	890.5±113.9	2.25±0.22	1.39±0.11	727.0±79.5*	4.17±0.62*	1.04±0.11
MEL 10 h (100 ng)	812.8± 36.8	1.52±0.26	0.94±0.17*	485.6±65.2*	1.78±0.25*	0.98±0.18

Values are means ± SE. EPIWAT, epididymal white adipose tissue; INGSCWAT, inguinal subcutaneous white adipose tissue; total LPL activity, lipoprotein lipase activity in μmol FFA released·h^{-1}·pad^{-1}; specific LPL activity, lipoprotein lipase activity in μmol FFA released·h^{-1} mg $protein^{-1}$. See Table 2 for description of treatment group abbreviations. * $P < 0.05$ vs. saline controls.

with 8-h MEL infusions, the body weight and fat pad responses were different between CAST and gonad-intact males. The CAST MEL 8-h animals exhibited short-day-type responses for these parameters, but the gonad-intact MEL 8-h hamsters failed to show decreases in body weight or EPIWAT measures.

Some effects of castration alone were observed. Reflective of the castration-induced decrease in body mass were decreases in total EPIWAT LPL activity (Table 6; Ps < 0.05). The typical hypersecretion of FSH that follows castration was observed in castrated hamsters in this experiment (Table 5, Ps < 0.05). Food intake was not affected by any of the treatments (Fig. 4).

DISCUSSION

Carter and Goldman (14) reported that the duration of the nocturnal MEL peak is the most important parameter of pineal activity for regulating FSH and PRL secretion and testis growth in juvenile Siberian hamsters. The results of the present experiments suggest that the MEL peak duration is the critical feature of the nighttime MEL secretion profile for mediating the effects of short days on testicular status in adult Siberian hamsters and also for other short-day-type responses such as decreases in body and lipid mass and some associated lipid metabolic responses. There was little indication of "intermediate" levels of response with the various doses of MEL used in this study. Thus, with the 10-h infusions, the lowest dose of MEL (1.56 ng/day) failed to elicit a measurable response, whereas the next highest dose (6.25 ng/day) was equally as effective as the largest doses tested (25 and 100 ng/day). The time of day during which the MEL was infused was not important, since long-duration infusions were equally effective when given during the light phase as when given during the nocturnal phase.

After exposure to short days, Siberian hamsters decrease their body weight before any measurable decrease in food consumption (40). Food consumption eventually decreases by ~30% after 5–7 wk of exposure to short days (4, 40). In the present experiments, hamsters receiving long-duration MEL infusions during the 5-wk infusion period also exhibited weight loss without a decrease in food intake. Collectively, these results suggest that the weight loss during the first few weeks of short-day exposure, or in response to long-duration MEL infusions, is due to an increase in energy expenditure. One means by which increased energy expenditure might contribute to the decrease in body weight could be through an increase in BAT thermogenesis. However, body weight loss was not always accompanied by increases in the indexes of BAT thermogenesis. This suggests that the weight loss observed in these animals was not linked to increases in BAT thermogenesis, although measures more directly related to BAT mitochondrial heat production, such as GDP-binding (30), need to be assessed.

The dissociation of the growth of the metabolic component of BAT from other responses resulting from MEL treatment has been previously reported. Pineal-intact Siberian hamsters given timed daily subcutaneous MEL injections either 4 h after lights-on or 4 h before lights-off both exhibited short-day-type increases in nonshivering thermogenesis, a response attributable to BAT. However, only hamsters injected with MEL in the afternoon had regressed gonads, a white winter pelage, and decreased body weight (27). In the present experiments, BAT protein content and COA were increased only when long-duration infusions (10 and 12 h) were combined with the largest doses of MEL (25 and 100 ng). By use of the timed MEL injection paradigm, the dosage and timing of MEL administration necessary to increase nonshivering thermogenesis and induce gonadal regression were found to differ in white-footed mice; morning or afternoon injections of MEL elicited a maximal increase in thermogenic capacity, but reproductive tract weight was not affected or only slightly affected, respectively (21). These results, coupled with the MEL injec-

TABLE 5. *Effect of melatonin infusions on paired testes weight, FSH, and PRL in experiments 3 and 4*

Treatment	Paired Testes Wt, mg	Serum FSH, ng/ml	Serum PRL, ng/ml
	Experiment 3		
Saline 10 h	693.8 ±55.2	4.11 ±0.61	42.80 ±0.50
MEL (6.25 ng)			
10 h light	174.6* ±32.9	2.12* (7/7<)	5.91* ±0.66
10 h D	186.0* ±51.4	2.06* (9/10<)	6.06* ±0.68
5 h × 2 D	693.9 ±49.3	4.23 ±0.72	29.67 ±0.25
5 h E D	766.2 ±56.0	5.18 ±0.05	36.49 ±0.20
5 h L D	782.8 ±30.2	3.50 ±0.30	33.63 ±0.27
	Experiment 4		
Gonad intact			
Saline 10 h	709.6 ±39.7	5.26 ±0.83	38.47 ±4.36
MEL (6.25 ng)			
10 h	178.0* ±35.1	2.36* (6/8<)	6.89* ±1.99
8 h	637.4* ±90.0	2.77* (4/8<)	23.10 ±8.59
Castrates			
Saline 10 h		43.01† ±1.71	27.10 ±7.85
MEL (6.25 ng)			
10 h		47.45† ±2.33	5.17* (5/11<)
8 h (6.25 ng)		46.59† ±2.39	11.53* ±2.65
5 h (6.25 ng)		40.95† ±2.45	30.02 ±3.60

Value are means ± SE. FSH, follicle-stimulating hormone; PRL, prolactin. Ratios in parentheses indicate number of assay values for each group relative to total number of samples assayed that were less than (<) sensitivities of each hormone assay (2.12 and 6.87 ng/ml for FSH and PRL, respectively). See text and Table 2 for description of treatment group abbreviations. * $P < 0.05$ vs. saline controls; † $P < 0.05$ vs. gonad-intact counterparts.

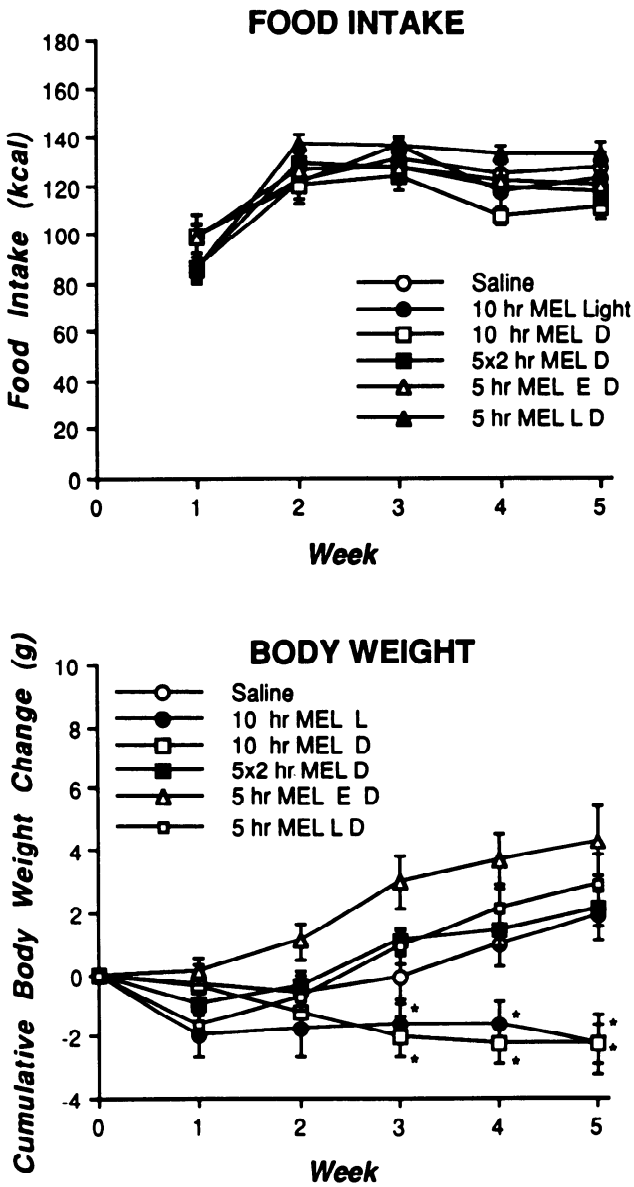

FIG. 3. Mean ± SE food intake (*top*) and cumulative body weight change (*bottom*) of adult male Siberian hamsters in *experiment 3* infused with melatonin (MEL; 6.25 ng/infusion) or saline daily for 5 wk. Light, light portion of photocycle; D, dark portion of photocycle; ED, early dark (beginning at lights-out + next 5 h of darkness; LD, late dark (beginning at lights-on + previous 5 h of darkness); 5 × 2, 5-h MEL infusion followed by 2 h without infusion followed by 5 additional h of MEL infusion. Mean base-line body weights ± SE: saline 10 h, 44.8 ± 1.0 g; MEL 10 h light, 43.4 ± 1.7 g; MEL 10 h D, 41.33 ± 1.2 g; MEL 5 h ED, 41.7 ± 1.1 g; MEL 5 h LD, 44.65 ± 0.8 g; MEL 5 h × 2, 42.4 ± 1.3 g. * $P < 0.05$ body weight values vs. saline controls.

tion data described above, suggest that the principles underlying the regulation of BAT by MEL may be somewhat different from those that apply for the other short-day-type responses examined in this study. Further experimentation is needed to understand which feature(s) of the nighttime secretion of MEL is most important for BAT growth and thermogenesis after short-day exposure.

Ten-hour infusions of threshold or greater doses of MEL led to decreased WAT pad mass and total LPL activity, but this response was not uniform for all WAT pads. For example, EPIWAT weight and total LPL activity were decreased relative to the saline-infused controls; however, these parameters were not changed in INGSCWAT. These data are consistent with our recent findings in intact adult male Siberian hamsters transferred from long to short days for 6 or 12 wk. At both time points, INGSCWAT and the dorsal subcutaneous fat pads had smaller relative decreases in weight, LPL activity, and in vivo lipogenesis compared with two more internally localized WAT depots, EPIWAT, and retroperitoneal fat pads (Bartness, Wade, and Goldman, unpublished observations). The mechanism underlying the unresponsiveness of the INGSCWAT pad to the long-duration MEL infusions is not known; however, this relative insensitivity might be adaptive in retaining a layer of fat for insulation during the harsh winter conditions.

Ten-hour MEL-infused hamsters had decreased body and lipid mass regardless of whether they were castrated or gonad-intact. These data corroborate our previous report where castrated and gonad-intact Siberian ham-

TABLE 6. *Effect of melatonin infusions on white adipose tissue measures in experiments 3 and 4*

Treatment	INGSCWAT			EPIWAT		
	Wet wt	Total LPL	Specific LPL	Wet wt	Total LPL	Specific LPL
			Experiment 3			
Saline 10 h	945.6±71.9	3.27±0.26	1.41±0.17	896.2±70.5	7.16±0.93	0.68±0.07
MEL (6.25 ng)						
10 h light	680.6±126.3	1.82±0.17*	1.23±0.22	582.4±86.3*	3.14±0.12	0.52±0.06
10 h D	708.3±82.1	2.40±0.34*	1.13±0.15	604.6±44.2*	3.83±0.51*	0.86±0.17
5 h × 2 D	906.7±60.2	3.51±0.21	1.66±0.35	1042.1±115.1	7.89±1.04	0.76±0.09
5 h E D	936.7±112.8	3.55±0.61	1.77±0.22	901.9±127.4	10.37±1.20	1.04±0.11*
5 h L D	1029.4±104.2	3.01±0.32	1.60±0.20	983.9±58.6	13.23±1.57 *	1.10±0.15*
			Experiment 4			
Gonad intact						
Saline 10 h	782.8±78.2	1.50±0.22	0.66±0.05	792.8±74.9	8.42±1.12	1.95±1.04
MEL (6.25 ng)						
10 h	783.0±90.0	1.73±0.16	0.91±0.12	635.4±46.4	5.54±0.50	0.89±0.12
8 h	995.7±135.4	1.85±0.35	0.67±0.08	673.0±64.4	6.99±0.87	0.70±0.06*
Castrates						
Saline 10 h	812.5±85.2	1.64±0.21	0.84±0.12	908.7±164.8	5.22±1.21†	1.33±0.30
MEL (6.25 ng)						
10 h	591.5±110.2	1.31±0.18	0.87±0.09	533.5±78.6*	3.25±0.53*†	1.14±0.11
8 h	542.7±85.1*†	1.28±0.23	0.94±0.07†	483.5±94.2*†	4.24±0.74*†	1.63±0.26†
5 h	804.9±112.4	1.49±0.15	0.89±0.18	650.3±104.6*	3.54±0.63*	1.28±0.18

Values are means ± SE. See Table 4 for description of measurement abbreviations and text and Table 2 for treatment group abbreviations. * $P < 0.05$ vs. saline controls; † $P < 0.05$ vs. gonadal-intact counterparts.

TABLE 7. *Effect of melatonin infusions on brown adipose tissue measures in experiments 3 and 4*

Treatment	STERNBAT				
	Wet wt	Total LPL	Specific LPL	Total protein	Total COA
		Experiment 3			
Saline 10 h	212.6±10.4	1.15±0.15	0.40±0.06	6.28±0.94	
MEL (6.25 ng)					
10 h light	170.9±25.1	1.36±0.17	0.46±0.04	6.78±0.62	
10 h D	156.3±8.2*	1.42±0.10	0.34±0.03	7.83±0.51	
5 h × 2 D	214.5±19.8	1.67±0.19	0.58±0.16	6.89±0.80	
5 h E D	183.0±15.2	1.23±0.12	1.33±0.56	4.46±0.77	
5 h L D	234.6±16.7	1.62±0.15	0.57±0.16	6.97±0.76	
		Experiment 4			
Gonad intact					
Saline 10 h	202.0±15.6	1.08±0.23	0.31±0.06	8.59±0.68	3.54±0.44
MEL (6.25 ng)					
10 h	168.9±14.6	1.03±0.11	0.23±0.02	9.30±0.93	4.52±0.61
8 h	213.6±37.4	0.87±0.09	0.48±0.18	9.20±0.95	3.41±0.69
Castrates					
Saline 10 h	174.9±13.2	0.80±0.08	0.16±0.02	11.62±1.48	3.32±0.44
MEL (6.25 ng)					
10 h	126.6±15.2	0.92±0.38	0.28±0.04	7.86±0.59	4.54±0.33
8 h	153.6±18.2	1.28±0.18	0.19±0.01	12.01±2.09	3.55±0.30
5 h	163.0±13.7	1.01±0.11	0.21±0.03	10.36±0.99	3.57±0.21

Values are means ± SE. See Table 3 for abbreviations for measurements listed and text and Table 2 for treatment group abbreviations. * $P <$ 0.05 vs. saline controls.

sters had similar, albeit slightly diminished, decreases in body weight and carcass lipid content after short-day exposure (40). Together these results indicate that seasonal body weight fluctuations are at least partly independent of changes in gonadal status in this species (40).

Castrated hamsters receiving 8-h MEL infusions had decreased body weight, EPIWAT weight, and LPL activity, but gonad-intact males receiving identical MEL treatment did not exhibit these responses. One possible explanation for these results is based on the anabolic action of testosterone in this species (3, 39). Thus the effects of 8-h MEL infusions on the body and lipid mass of castrated hamsters might have been opposed by the growth-promoting effects of testosterone in the testis-intact males. This might result in a masking of potential MEL effects on body mass. However, when the infusion duration was increased to 10 h, testicular regression occurred and this presumably was accompanied by decreased testosterone and therefore an absence of the stimulatory effects of the testes on body and lipid mass.

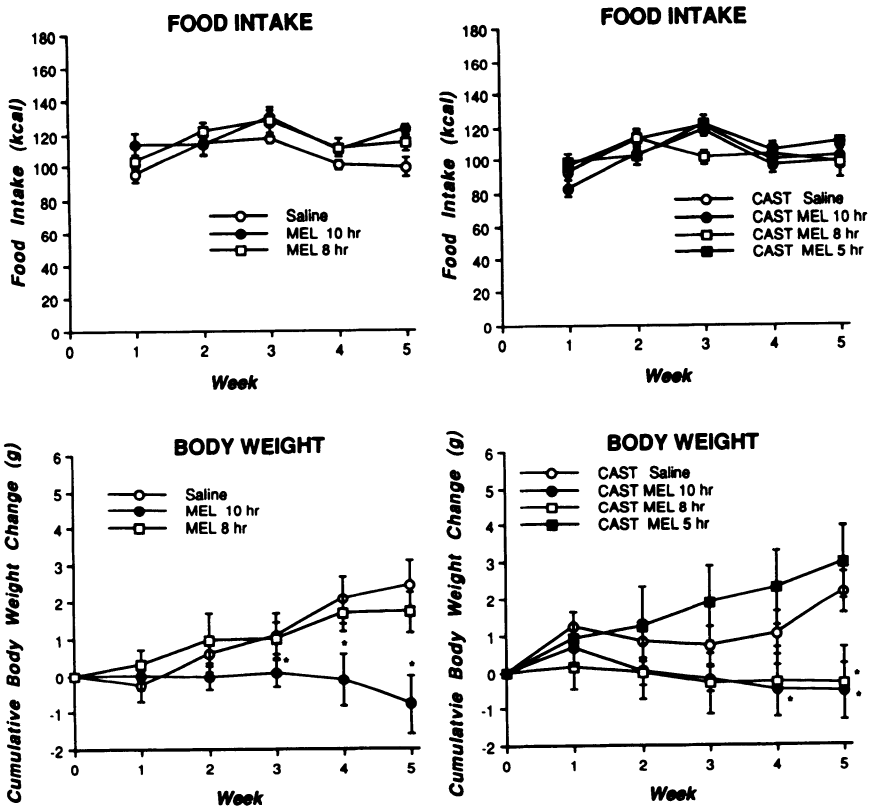

FIG. 4. Mean ± SE food intake (*top*) and cumulative body weight change (*bottom*) of adult male Siberian hamsters in *experiment 4* infused daily with melatonin (MEL; 6.25 ng/infusion) or saline for 5 wk. Hamsters were initially castrated (CAST) or left gonad intact and housed in long days for 19 wk. Mean base-line body weights ± SE for gonadal-intact hamsters: Saline 10 h, 43.1 ± 1.5 g; MEL 10 h, 43.7 ± 1.2 g; MEL 8 h, 42.3 ± 1.1 g; for castrated hamsters: CAST Saline 10 h, 38.7 ± 1.0 g; CAST MEL 10 h, 38.3 ± 1.7 g; CAST MEL 5 h, 37.4 ± 1.8 g. Body weight values different from saline controls.

TABLE 8. *Effect of melatonin infusions on carcass composition in experiment 4*

Treatment	*n*	Carcass component						
		Total CW, g	Total FFDM, g	Total BF, g	Carcass, g	% CW	% FFDM	% BF
Experiment 4: dependence of melatonin infusion-induced decrease in body mass on gonads								
Castrates								
Saline 10 h	10	16.07±0.28	6.81±0.15	6.93±0.78	29.81±0.98	54.34±1.49	22.98±0.54	22.68±2.01
MEL (6.25 ng)								
10 h	11	15.86±0.47	6.51±0.23	4.78±0.92*	27.16±1.45	59.15±1.58	24.24±0.62	16.61±2.15*
8 h	10	15.21±0.33	6.23±0.34	4.52±0.42*	26.15±1.06	58.65±0.16	26.57±1.09	17.05±1.10*
5 h	10	16.08±0.48	6.68±0.42	6.27±0.73	29.58±1.61	55.91±1.57	22.96±0.89	21.42±1.76
Gonad intact								
Saline 10 h	9	17.12±0.42	7.30±0.19	7.54±0.75	31.97±1.11	53.79±1.12	22.95±0.59	23.30±1.62
MEL (6.25 ng)								
10 h	8	19.03±0.72	7.78±0.24	5.63±0.35*	32.44±1.12	58.64±0.64*	24.02±0.40	17.34±0.88*
8 h	8	17.53±0.78	7.21±0.32	7.52±1.05	32.26±2.02	54.80±1.46	22.50±0.63	22.65±2.00

See Table 2 for description of measurement abbreviations and text for treatment group abbreviations. * $P < 0.05$ vs. saline controls.

An alternative interpretation of the data is that castration and the accompanying reduction in gonadal hormone levels directly affect the photoperiod control system per se. We are unaware of any evidence that changes in gonadal hormones alter the photoperiodic mechanism in mammals. Therefore we prefer the first interpretation of these results.

The continuity of each daily MEL infusion was found to be critical in triggering the short-day-type decreases in testis weight, body and lipid mass, and lipid metabolism in *experiment 3*. Thus splitting a 10-h MEL infusion into two 5-h infusions separated by 2 h without infusion resulted in a failure to elicit any of the short-day-type responses measured in these experiments, even though the total duration and dose of MEL was the same as with the standard 10-h MEL infusion. These results support previous findings that utilize a similar paradigm and measure testicular growth in PINX juvenile hamsters (20).

A circadian sensitivity of target sites to the secretion of MEL has been proposed to explain differences in the sensitivity of the reproductive system to injections of microgram amounts of MEL in pineal-intact adult Siberian (36), golden (37), and Turkish hamsters (S. M. Hong and M. H. Stetson, unpublished observations). In all three species, daily MEL injections are most effective in causing gonadal regression if administered during a period extending from early afternoon until about the

time of lights-off or during a brief period just before lights-on. The results of the present study do not exclude the possibility of a circadian basis for the effects of MEL on reproduction; however, they provide added support for the earlier observation of Carter and Goldman (14), where short day-type responses were elicited if the duration of the MEL infusion was long enough, irrespective of the time of day the hormone was administered. Clearly, short-duration MEL infusions are not effective in eliciting short-day-type responses, regardless of when they are administered during the light-dark cycle. In the present study, 5-h MEL-infused hamsters showed a trend toward modestly increased testis and body weights and EPIWAT LPL activity, although only the LPL activity measures were significant, whereas the other increases narrowly missed statistical significance. Similar stimulation of testes and body weight was reported for PINX juvenile Siberian hamsters receiving short-duration MEL infusions. In this case, the infusions began when the animals had immature testes as a result of having been raised in a short photoperiod, and the degree of testicular stimulation produced by MEL treatment was more pronounced (14).

Photoperiodic history can influence photoperiodic responsiveness. In Siberian hamsters and sheep, the response to a MEL peak of a given duration depends in part on photoperiodic history and presumably on the prior pattern of MEL secretion (for review see 31). Thus the response to MEL peaks of a given duration may depend on whether the animal had previously been exposed to peaks of longer or shorter durations (26, 33). In the present experiments, the directional change in the peak duration of MEL could not have been the only important factor. For example, all groups were deprived of MEL peaks for the 2 wk of postoperative care after PINX. However, once the hormone infusions were begun short-day-type responses were observed only when the duration of the MEL infusion reached 10 h or longer; these responses were not seen after 5- or 8-h MEL infusions. Thus, although the directional change in the duration of MEL is undoubtedly important, the absolute length of the peak must also be significant.

Finally, in a recent pilot study we assayed serum MEL content by RIA in adult PINX hamsters infused for 2 wk with saline or MEL (6.25, 25, 100, and 400 ng/daily infusion). Relative to its nocturnal peak in long-day housed pineal-intact hamsters, the 6.25-ng dose of MEL produced serum hormone concentrations that were within the lower portion of the physiological range of the hormone. The 25-ng dose of MEL produced serum concentrations of the hormone somewhat greater than the normal peak levels. These data should be considered preliminary because a fully validated RIA for MEL in this species does not exist. However, these preliminary results, coupled with the results of the present experiments, suggest that MEL may be able to trigger changes in body and lipid mass and reproductive status when the serum levels of the hormone are less than the maximum concentrations achieved in normal animals. This implies that the duration of the peak circulating levels of MEL may be an important parameter to monitor in clinical studies in addition to the typically measured peak concentrations of the hormone.

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