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Starvation: Early Signals, Sensors, and Sequelae*

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ABSTRACT

To identify the sequences of changes in putative signals, reception of these and responses to starvation, we sampled fed and starved rats at 2- to 6-h intervals after removal of food 2 h before dark. Metabolites, hormones, hypothalamic neuropeptide expression, fat depots, and leptin expression were measured. At 2 h, insulin decreased, and FFA and corticosterone (B) increased; by 4 h, leptin and glucose levels decreased. Neuropeptide Y messenger RNA (mRNA) increased 6 h after food removal and thereafter. Adrenal and plasma B did not follow ACTH and were elevated throughout, with a nadir at the dark-light transition. Leptin correlated inversely with adrenal B. Fat stores decreased during the last 12 h. Leptin mRNA in perirenal and sc fat peaked during the dark period, resembling plasma leptin in fed rats. We conclude that 1) within the first 4 h, hormonal and metabolic signals relay starvation-induced information to the hypothalamus; 2) hypothalamic neuropeptide synthesis responds rapidly to the altered metabolic signals; 3) catabolic activity quickly predominates, reinforced by elevated B, not driven by ACTH, but possibly to a minor extent by leptin, and more by adrenal neural activity; and 4) leptin secretion decreases before leptin mRNA or fat depot weight, showing synthesis-independent regulation. (*Endocrinology* **140:** 4015–4023, 1999)

IN RAPIDLY GROWING, young male rats, starvation is an acute challenge to the systems that regulate energy balance. Starvation-induced changes in hormones and metabolites are perceived at the hypothalamus, and responses to starvation are mobilized by behavioral, autonomic, and neuroendocrine changes coordinated by the hypothalamus (1).

In rats fed *ad libitum*, there are diurnal rhythms in the amplitude of the stress response, the sensitivity of ACTH to corticosterone (B) feedback, and the presence of facilitated ACTH responses to prior stress; responses are of high amplitude at the beginning of the light period after rats feed in the dark and of low amplitude at the onset of dark, after they have voluntarily fasted during the light period (2). Overnight starvation markedly decreases the magnitude of ACTH responses to stress, corticosteroid feedback efficacy, and facilitated ACTH responses to acute stress in young male rats (3–5). These changes in the hypothalamo-pituitary-adrenal (HPA) axis induced by overnight starvation are not caused by corticosterone (B), because they occur in adrenalectomized rats (3) and the amplitude of ACTH responses to stress is increased by gavage feeding during the night of the fast (5). Removal of food for 14 h during the light period does not alter responses in the HPA axis when tested at the end of the light period, although gavage with food during the light (fasting) period does increase ACTH responses to restraint (5). Thus, a brief period of starvation beginning near the onset of dark markedly alters response properties in the HPA axis.

Marked changes in the activity of the HPA axis occur during the first 14 h of starvation in young rats (6). Activation occurs within 3.5 h after food removal before dark, and a temporal pattern of ACTH and B responses reflects patterns of food intake and insulin secretion in controls fed *ad libitum*. In the last sample collected in that study (at lights on), activity in the HPA axis had returned to or near the control level, suggesting, as in the studies of regulation of HPA axis responsivity by food, that not only fasting but also the cyclic circadian input importantly determine the responses.

We also compared responses to starvation 3, 15, and 48 h after food removal in intact rats and adrenalectomized rats provided with a constant B signal (7). In that study, we examined responses in the HPA axis, hypothalamic neuropeptide Y (NPY) messenger RNA (mRNA) and peptide, glucose, insulin, and leptin. One hour after dark and 3 h after removal of food, HPA variables were elevated; glucose, insulin, and leptin levels were decreased or unchanged; and NPY mRNA was not different in the fed and fasted rats. By 15 h, at lights on, HPA variables were near normal in intact rats, but ACTH was markedly elevated in the starved adrenalectomized B-replaced rats compared with normal ACTH in the fed group. This result suggests that lack of food drives the HPA axis but that the elevated B in intact rats tempers this drive. By 48 h, plasma B (or ACTH) levels were elevated in the starved groups. At 15 and 48 h, glucose, insulin, and leptin levels were low or undetectable. NPY mRNA was elevated in both groups of fasted rats at 15 h and was still higher at 48 h. Overall, there was little, if any, effect of preventing B responses on the other responses to starvation.

In this study we have exposed young male rats to starvation for 24 h. Our goals were to replicate the results of

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Akana *et al.* (6) and to characterize temporally the responses of plasma metabolite and hormone levels, hypothalamic neuropeptide expression, and fat depots with the prospect of identifying signals and hypothalamic responses important to the constellation of defense mechanisms provoked by starvation. Such data are essential for the design of future studies to determine the site(s) and mechanism(s) by which changes occur in response to starvation.

Materials and Methods

Young male rats (105–115 g), received from Bantin and Kingman suppliers (Fremont, CA) were placed two per cage in hanging wire baskets in an animal room with a controlled light cycle (12 h of light; lights on at 0600 h) and temperature (23 C). The animals were allowed to adapt to the diet (no. 5008, Ralston Purina, Inc., St. Louis, MO) and room for 3 days before the experiment began. All groups of rats were housed in one room that was used solely for this purpose. The experimental procedure was approved by the University of California San Francisco Committee on Animal Research.

Experiment

All rats were weighed at 0800 h on the morning of the experiment. At 1600 h, a group of 12 rats was killed to determine initial values. Food was then removed from the hoppers, and preweighed food was provided to half of the remaining rats. Groups of 6 fed and 6 starved rats were sampled at 2-h intervals until 1600 h on the following day. The 1800 h (lights out) and 0600 h (lights on) samples were both collected during the light period; during the rest of the dark period, dim red light was used. Alternate pairs of fed and fasted rats were collected at each time interval. All rats were killed by decapitation within 15 sec of touching their cages. Blood (5 ml) was collected from the trunk into chilled plastic centrifuge tubes and was kept on ice until centrifuged, and the plasma was aliquoted. Bodies and heads were weighed; 5 g, representing the weight of blood known to have been removed, were added to the weights. Brains and pituitaries were removed from the skull and were blocked and frozen immediately for subsequent *in situ* hybridization measurement. Thymus and adrenal glands and various single fat depots were removed and kept in closed, saline-saturated dishes for subsequent cleaning and weighing. At 6-h intervals, samples of the contralateral sc and perirenal fat depots were snap frozen in liquid N_2 for subsequent measurement of leptin mRNA. To prevent individual experimenter differences in technique, each task of the experiment was assigned to and carried out by one individual, from performing the collection to cleaning and weighing of tissues.

In a separate experiment, groups of six rats, either fed or with food removed at 1600 h, were anesthetized with a rodent cocktail containing acepromazine-ketamine-xylazine (77:1.5:1.5 mg/ml; 1 mg/kg, sc) and perfused with saline followed by 4% formaldehyde 14 and 27 h after the onset of the fast. Brains from these rats were sliced at 30 μm, and free floating sections were stained with an antibody to Fos protein as described previously (8). Fos-immunoreactive cells were counted in the parvocellular region of the paraventricular nuclei (PVN) and the arcuate nuclei as detailed previously (8).

Measurements

Food intake was calculated by subtracting the weight of the remaining food in the hopper plus the amount spilled from the weight of the food provided initially. At each time there were three values for each group. To estimate food eaten per two rats per 2-h interval, the mean weight of food eaten at a given time was subtracted from the mean of the preceding period.

Adrenal and thymus glands were cleaned and weighed. Adrenals were then homogenized in 20% ethanol-80% normal saline for subsequent measurement of B content. Perirenal, sc (inguinal), and epididymal white fat depots were cleaned of extraneous tissue and weighed. Interscapular brown fat was cleaned of white fat and muscle, weighed, and homogenized for subsequent measurement of uncoupling protein.

Plasma glucose and FFA were measured using the glucose oxidase reaction in a Beckman Coulter, Inc. Glucose Analyzer II (Palo Alto, CA) and a kit based on colorimetric assay (9), respectively.

Because there were more samples than could be measured in a single assay, each measurement was performed over two or three assays that contained equal numbers of samples from fed and fasted rats, usually distributed across the entire experiment. ACTH, B, insulin, and leptin were measured by previously reported RIAs (7). Uncoupling protein was measured in a single assay in interscapular brown adipose tissue at 12-h intervals by a previously reported method (10, 11) with reagents provided by Dr. Jean Himms-Hagen (University of Ontario, Ottawa, Canada).

In situ hybridization

Brains were sliced at 20 μm from the optic chiasm to the ventromedial nuclei, and one in six sections at the appropriate level were reacted for CRF, vasopressin (AVP), POMC, and NPY. The CRF and AVP probes used have been reported previously (12); the NPY probe was provided by Dr. M. W. Schwartz, University of Washington (Seattle, WA) (13), and the POMC probe used was a 45-base complementary DNA (cDNA) oligomer: 5′-CTTCTTGCCCACCGGCTTGCCCCAGCGGAAGTGCTCCATGGAGTAGGA-3′ (14). All hybridizations for a given mRNA were performed in a single lot, and x-ray films of three sections of each of three brains from each group were examined after hybridization. Semiquantitative analyses were performed by subtracting a set threshold value in each of the four analyses and determining the optical density of a standard area in the site of interest using NIH Image (Rasband version 1.4).

Leptin mRNA in perirenal and sc depots

RNA was purified from white adipose tissue samples using Trizol (Life Technologies, Inc., Grand Island, NY), and cDNA was transcribed from each RNA sample using Superscript II RNase H^- reverse transcriptase (Life Technologies, Inc.), following protocols supplied by the manufacturers. Quantitative PCR was performed to quantify leptin and α-tubulin mRNA using the TaqManPCR reagent kit and ABI Prism sequence detection system (PE Applied Biosystems, Foster City, CA) (15). The two rat leptin primers used were (forward) CACACACGCAGTCGGTATCC and (reverse) TGAAGCCCGGGAATGAAGT, and the two rat α-tubulin primers used were (forward) GCTGTGGTTGAGCCCTACAAT and (reverse) CATTGTCTACCATGAAGGCACAA. A hybridization probe that binds to each PCR product was labeled with a reporter dye, FAM, on the 5′-nucleotide and a quenching dye, TAMRA, on the 3′-nucleotide. Fifty-microliter PCR reactions contained 2 ng of the cDNA sample and 1.25 U TaqGold DNA polymerase (Perkin Elmer Corp., Norwalk, CT). Cycling parameters were 2 min at 50 C, 20 min at 95 C, followed by 40 cycles of 60 sec at 60 C, and 15 sec at 95 C. Serial dilutions of plasmid DNA were analyzed in parallel for each target cDNA. These served as standard curves from which the number of copies of leptin and α-tubulin in each sample were determined.

Statistics

Two-way ANOVA was used to compare differences between fed and starved rats; overall results of the two-way ANOVAs on plasma and tissue variables are shown in Table 1. *Post-hoc* differences between groups were determined using Fischer's protected least significant difference test (PLSD), and $P \leq 0.05$ was considered significant. Regression analyses were used to determine the relationships between leptin and fat, insulin, or B. All statistical manipulations were performed using the StatView program (16).

Results

Body weight, food intake, and metabolites (Fig. 1)

Similar initially, body weights had diverged in the fed and starved groups by 2000 h and differed significantly at 2400 h and thereafter (Fig. 1, *top left*). By 2000 h, the weight of the fed rats was increased as a consequence of the considerable first bout of food consumption that occurred between 1600–2000 h (Fig. 1, *top right*). Plasma glucose began to decrease in

TABLE 1. Statistical results for some measured variables (two-way ANOVA)

Variable	Time of day			Fed/starved			Interaction		
	df	F	*P*	df	F	*P*	df	F	*P*
Glucose	12	21.4	<0.0001	1	1097	<0.0001	12	16.0	<0.0001
FFA	12	12.6	<0.0001	1	67	<0.0001	12	4.0	<0.0001
Insulin	12	6.08	<0.0001	1	311	<0.0001	12	8.94	<0.0001
Leptin	12	3.17	<0.0001	1	217	<0.0001	12	6.14	<0.0001
ACTH	12	15.5	<0.0001	1	65	<0.0001	12	14.3	<0.0001
Adrenal corticosterone	12	6.79	<0.0001	1	14.5	<0.0001	12	16.0	<0.0001
Corticosterone (B)	12	5.1	<0.0001	1	82	<0.0001	12	1.4	NS
Adrenal wt	12	11.5	<0.0001	1	17.4	<0.0001	12	1.1	NS
Thymus wt	12	3.9	<0.0001	1	2.9	0.09	12	1.8	0.053
Perirenal WAT	12	3.9	<0.0001	1	10.4	0.0016	12	2.7	0.0028
Epididymal WAT	12	1.9	0.0367	1	20.8	<0.0001	12	3.3	0.004
sc WAT	12	3.3	0.0004	1	13.9	0.0003	12	2.4	0.0068

WAT, White adipose tissue weight.

the starved groups at 1800 h and had declined significantly by 2000 h and thereafter (Fig. 1, *middle left*). Plasma insulin had declined significantly in the starved groups at 1800 h, and by 2000 h and thereafter remained just above the limit of detection (Fig. 1, *middle right*). The significant decrease in insulin at 1800 h, when glucose had not yet decreased significantly, suggests that within 2 h of its onset, signals of starvation had been received. Plasma FFA levels were significantly increased at 1800 h, the first time that samples were collected after the onset of starvation; levels remained high for the subsequent 22 h (Fig. 1, *bottom left*). By contrast, plasma FFA in fed rats was low throughout the dark period, but increased to the levels observed in starved rats between 0800–1200 h; the values began to decline again between 1400–1600 h (Fig. 1, *bottom left*), perhaps as a consequence of the meal taken between 1200–1400 h (Fig. 1, *top right*). Plasma leptin began to decrease at 1800 h in the fasted rats and was significantly lower than that in the fed rats from 2200 h and thereafter when many samples were below the limit of detectability (Fig. 1, *bottom right*). In the fed rats, there was a nocturnal excursion in leptin that peaked at 2200 h and maintained a plateau until 0600 h; thereafter, leptin levels fell throughout the light period (Fig. 1, *bottom right*).

There were significant negative regressions of FFA on insulin that were stronger in starved than in fed rats (fed: $F = 8.07$; $P = 0.0058$; $r = -0.310$; starved: $F = 68.6$; $P < 0.0001$; $r = -0.689$). Similarly, there were significant regressions of leptin on insulin that were stronger in starved than fed rats (fed: $F = 4.28$; $P = 0.04$; $r = 0.234$; starved: $F = 30.0$; $P < 0.001$; $r = 0.542$), suggesting that changes in insulin may be important for the changes in both FFA and leptin that occur with starvation (not shown).

Hypothalamic neuropeptide expression (Fig. 2)

Although not significantly different from the fed rats, the starved rats had higher CRF mRNA expression in the PVN 6 h after the removal of food and lower levels thereafter (Fig. 2A); overall, there was a significant difference in CRF mRNA between fed and starved rats ($P < 0.05$). Vasopressin mRNA measured in the same parvocellular region of PVN as CRF mRNA did not change as a function of starvation, but in both groups there was a slight decline in levels at 0400 h (Fig. 2B). In the arcuate nuclei, NPY expression increased significantly ($P < 0.01$) within 12 h ($P < 0.05$) of the onset of starvation (Fig. 2C). POMC mRNA exhibited a rhythm as a function of time of day ($P < 0.05$), but there was no effect of the first 24 h of starvation (Fig. 2D).

The number of Fos-immunoreactive neurons was used, in a separate experiment, as an index of neuronal activity in rats 15 and 27 h after the onset of starvation. At both times, compared with fed rats, fewer parvocellular PVN cells were stained with Fos in the starved rats (at 15 h: fed, 93 ± 14; starved, 54 ± 7 cells; at 27 h: fed, 165 ± 19; starved, 92 ± 19 cells; condition: $F = 14.0$; $P < 0.001$; time: $F = 6.99$; $P = 0.0145$; interaction: $P =$ NS). By contrast, the numbers of Fos immunoreactivity-positive cells in the arcuate nuclei were similar in the two groups at both times of day (not shown).

HPA axis hormones and targets (Fig. 3)

As previously (6), there were marked increases in plasma ACTH in the starved rats during the dark (Fig. 3, *top left*) that mirrored the bouts of feeding in the control rats (Fig. 1, *top right*) and that returned to the level in fed rats at the onset of light and thereafter. Significant differences in plasma ACTH between fed and starved rats occurred at 2000 and 0200–0400 h. In starved rats, ACTH levels were higher during the hours of dark than light (174 ± 15 *vs.* 82 ± 6 pg/ml, respectively; $P < 0.0001$; Fig. 3, *top right*). Adrenal B content did not follow ACTH in either fed or starved rats, and although higher in starved rats, the B content in the two groups did not differ throughout the dark period (Fig. 3, *middle, left*). The large, sustained peak in ACTH that occurred between 0200–0400 h was not reflected by increased adrenal B in the starved rats. During the light period, adrenal B content in starved rats was always higher than that in the fed animals (Fig. 3, *middle left*), possibly because adrenal weight was increased during the light period (Fig. 3, *middle right*).

Plasma B was remarkably elevated in starved rats during the first hours of dark (1800–0000 h); it then decreased toward the levels observed in fed rats during the second half of the dark period. Particularly during the second half of the dark and during the light periods, plasma B reflected adrenal B rather than ACTH (Fig. 3, *bottom left*). The regression of plasma on adrenal B was highly significant

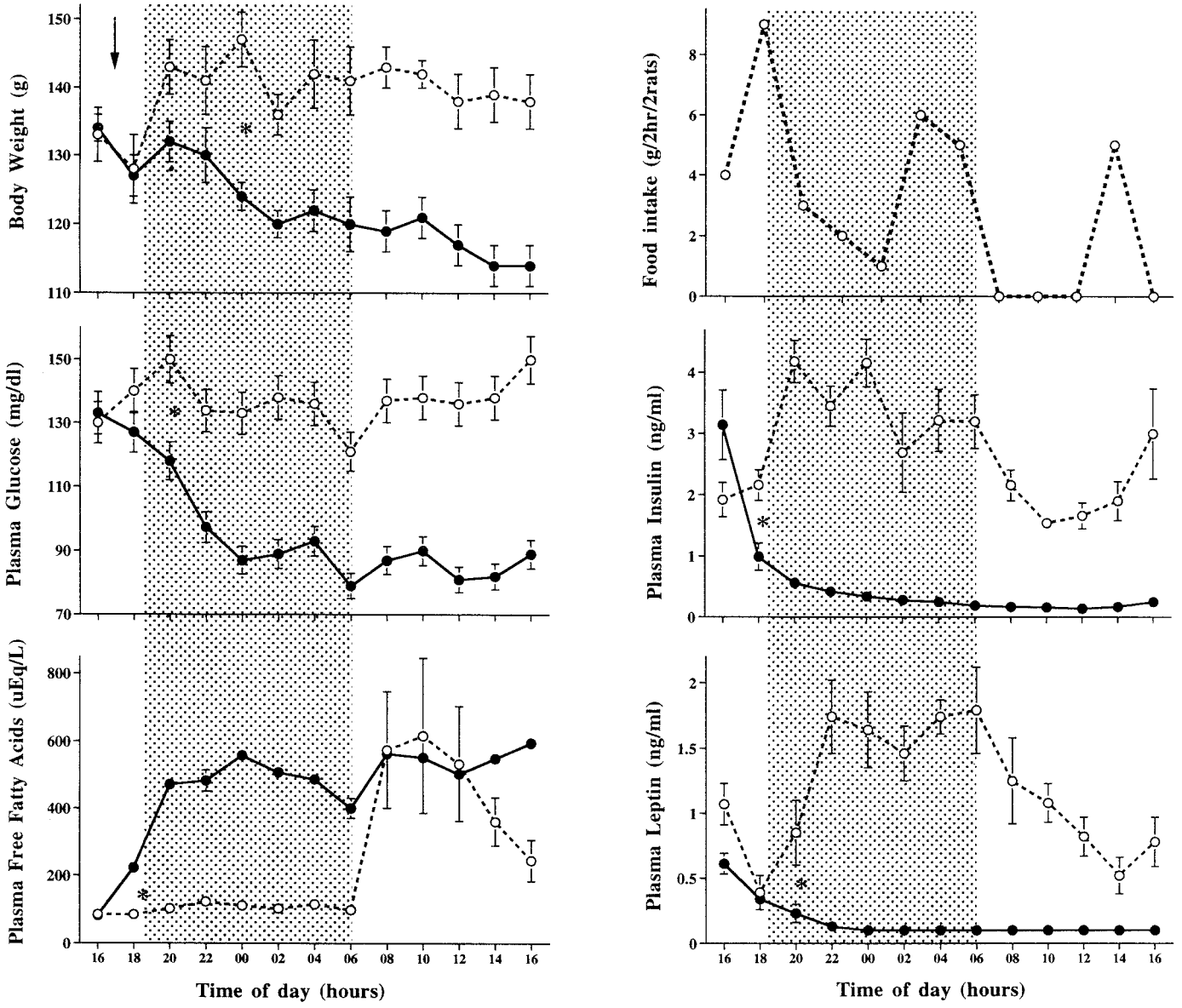

FIG. 1. Changes with time in body weight, food intake, hormones, and metabolites in young male rats fed *ad libitum* or starved. *Open symbols* and *dashed lines* represent fed rats; *closed symbols* and *solid lines* represent starved rats (n = 6/group ± SEM). The *arrow* represents the time of onset of starvation. The *shaded vertical bar* represents the 12 h of dark. Analyses of the data are shown in Table 1 and in the text. *, The first significant difference in fed and starved rats (by Fischer's PLSD).

(F = 85; $P < 0.0001$) as expected from examination of the curves. Fed rats exhibited a normal rhythm in plasma B throughout the dark with a peak of about 20 μg/dl at 1800 h and a nadir of less than 1 μg/dl at 0400–0600 h. During the light period, plasma (and adrenal) B in the fed rats increased unusually rapidly toward an evening peak, suggesting that there may have been some loss of control in the animal room during the latter part of the experiment. Just as adrenal weight of fasted rats increased during the light, probably as a consequence of the excess ACTH secreted, thymus weight was significantly reduced in the last sample collected, at 1600 h, probably as a consequence of the prolonged and marked elevation in plasma B (Fig. 3, *bottom right*).

Multiple regression analysis of adrenal B on insulin, leptin, FFA, glucose, and log ACTH showed only leptin to be significantly negatively correlated [fed: r = 0.426; $P = 0.151$; leptin (t) −2.895, $P = 0.0051$; starved: r = 0.324; $P = 0.0061$; leptin (t) −3.275, $P = 0.0013$].

Fat depot weights and leptin expression (Fig. 4)

Starved rats lost weight and fed rats gained weight in all fat stores during the 24 h; however, the dynamics differed. The weight of the dynamic, small perirenal fat depot decreased in both groups at 0200 h, 6 h after the first major feeding bout had ended in the fed rats. Perirenal fat weight increased in the fed groups between 0600–1200 h; after a

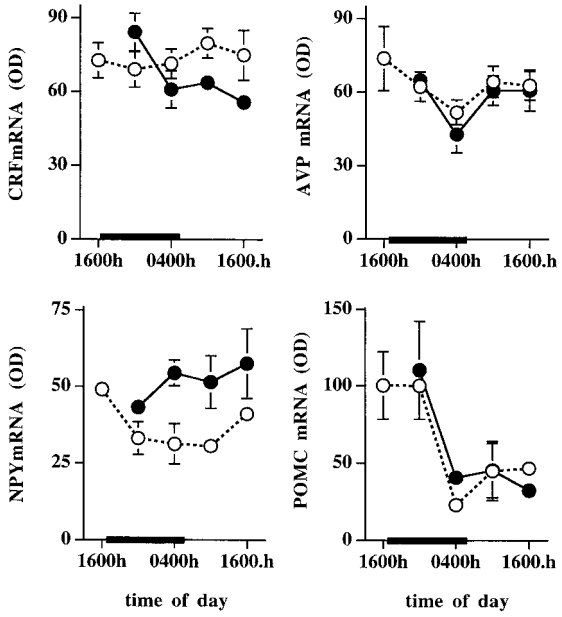

FIG. 2. mRNA in the hypothalamus of fed (*open symbols, dashed lines*) and starved (*closed symbols, solid lines*) rats with time after the onset of the experiment. CRF and AVP mRNAs (*top*) were determined in the parvocellular portion of the PVN, and NPY and POMC mRNAs were determined in the rostral, medial, and caudal portions of the arcuate nuclei. Data represent the mean values for three rats per group, and significance is discussed in the text. *Black bars* represent the 12 h of dark.

transient trough at 1400 h, coincident with the bout of food intake in fed rats (Fig. 1, *top right*), perirenal fat stores increased again at 1600 h (Fig. 4, *top left*). Compared with fed rats, perirenal fat weight was decreased at 1000, 1200, and 1600 h in starved rats. (The marked changes in perirenal fat weights were apparent to the individual doing the dissection, before obtaining measured weights.) Epididymal fat weights did not differ through the dark period in fed and starved rats; however, again during the light period, weight increased in the fed rats (Fig. 4, *middle*). The weight of this depot in starved rats was significantly decreased at 1000 h and thereafter. The sc fat weight increased in fed rats at 1000–1200 h and then decreased slightly thereafter (Fig. 4, *bottom*). In starved rats, the weight of sc fat fell slowly throughout the day. Leptin mRNA levels were similar in fed and starved rats in both perirenal and sc depots during the hours of darkness (Fig. 4, *top* and *bottom right*). There were nocturnal increases in leptin expression in the sc depots of both groups ($P < 0.05$) that temporally mimicked the excursions in circulating leptin in fed rats (Fig. 1, *bottom right*). With the marked loss in perirenal fat weight, leptin expression in this depot appeared to decrease at the end of the dark period and during the light hours.

Uncoupling protein content in interscapular brown adipose tissue was not different in starved and fed rats at 12 h, but was decreased at 24 h after the onset of starvation, indicating reduction of sympathetic outflow to this organ in food-deprived rats (fed, 61 ± 7 μg/depot; starved, 38 ± 7 μg/depot; $P < 0.05$).

Discussion

Starvation initiated just before the onset of the daily feeding period is a profound stimulus to the energy economy of prepubescent, growing male rats. These results show compensatory responses in metabolites and hormones that begin within 2 h of food deprivation. In addition to direct peripheral effects, changes observed in insulin, FFA, leptin, glucose, and B may provide the afferent signals that organize hypothalamic responses, as these variables change significantly over the first 2–6 h after food removal. Changes in expression of the four hypothalamic neuropeptides show that starvation-induced changes in CRF and NPY, but not AVP or POMC, occur within the first 6–24 h, suggesting that neurons containing these neuropeptides are early sensors that mediate responses to starvation. As reported previously, activity in the HPA axis is stimulated rapidly by fasting and is markedly affected by the circadian cycle. However, control of adrenal and plasma B by ACTH is greatly reduced during the second half of the dark period and thereafter. These results suggest strongly that starvation shifts adrenal regulation of B synthesis and secretion away from hypothalamic control, through CRF and AVP, of ACTH secretion. Fat depots release their stores sequentially: perirenal = epididymal > subcutaneous. However, during the first day of starvation, expression of leptin in sc fat stores is not affected and continues its normal daily variation. By contrast, leptin expression in perirenal fat is decreased.

Systemic effects of starvation

Because food was removed toward the end of the light period, it is unlikely that there were large stores of glycogen available for immediate energy, and the rapid increase in FFA suggests that energy requirements during the early dark were met by mobilization of fat, initially from liver and then from adipose stores and probably muscle. Fat mobilization would be fostered by the immediate and marked reduction in insulin. The plateau of glucose that occurred by 2200 h was maintained at low but constant levels thereafter; this strongly suggests that by this time hepatic gluconeogenesis was sufficient to maintain output of this substrate. Again, the reduction in insulin would foster increased glucagon secretion and glucose synthesis and secretion. Although systemic plasma glucagon levels were not elevated during the dark in Akana's study (6) (not shown), it is likely that increased glucagon stimulated the liver. Additionally, the nearly immediate increase in B secretion in starved rats would be expected to increase hepatic gluconeogenesis and gluconeogenetic enzymes, thus acting to sustain glucose production throughout the starvation period (17, 18). In the absence of insulin and the presence of high B, increased muscle breakdown and amino acid availability for gluconeogenesis would also be anticipated.

Signals and sensors

Changes in either FFAs or in insulin, leptin, or glucose serve as precise afferent signals to the hypothalamus about decreasing energy stores. Insulin was decreased, and FFA were increased at the first time tested after removal of food.

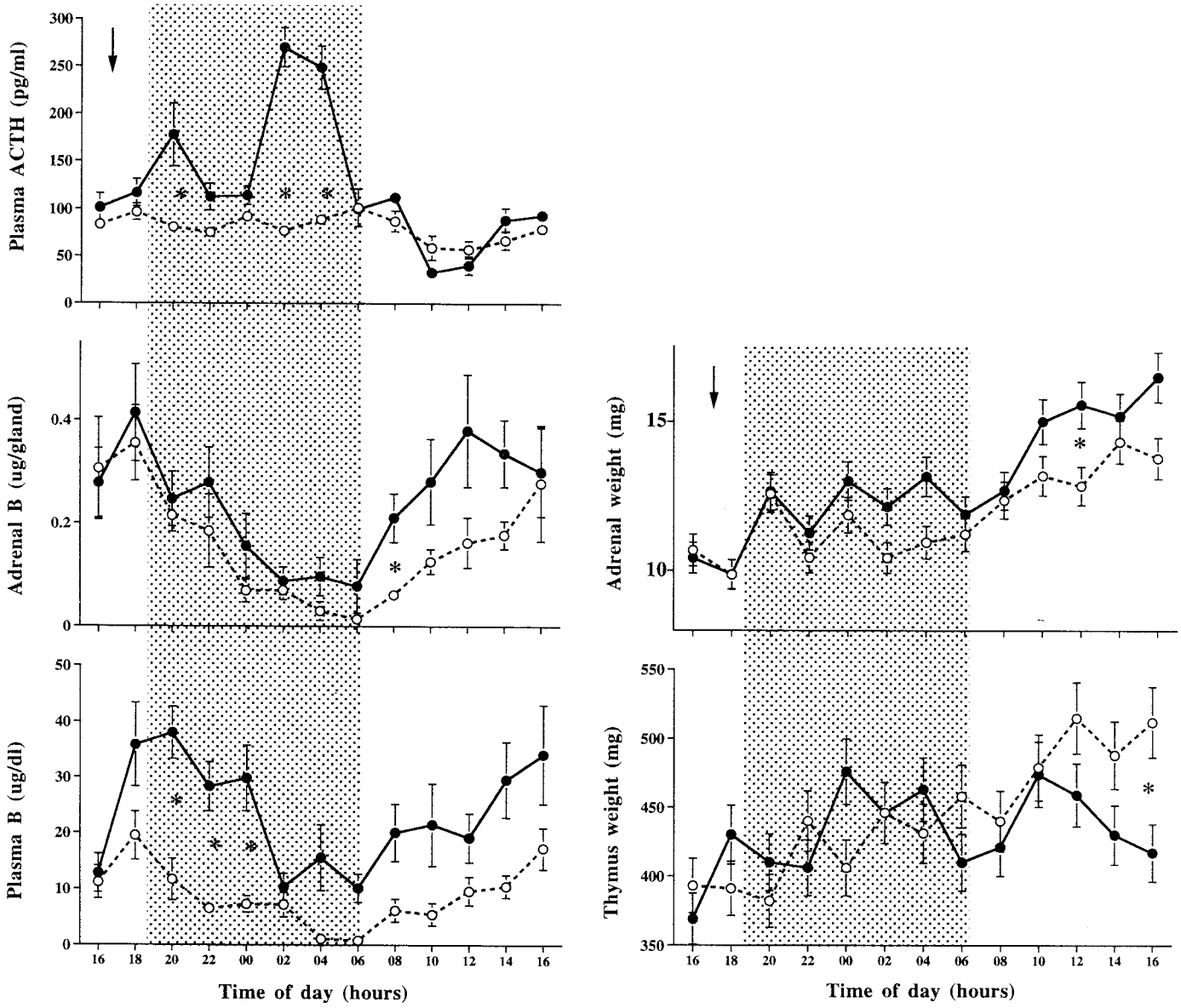

FIG. 3. Changes with time in activity of the HPA axis and a target for ACTH (adrenal weight) and for glucocorticoids (B). Symbols and shading are explained in Fig. 1; the analysis is reported in Table 1 and in the text. *, Significant *post-hoc* differences between fed and starved rats (by Fischer's PLSD).

The medial and lateral hypothalamus contains neurons that are directly sensitive to insulin, glucose, and FFA (19, 20); thus, changes in these would be directly perceived. Fatty acids also stimulate afferent nerves (21, 22) and act at the level of both ACTH secretion and directly at the adrenal to alter synthesis and secretion (23–25). The decrease in plasma leptin that occurs 2 h after the decrease in insulin and the increase in FFAs and B would also be registered by receptors in hypothalamic cell groups (26–28). The decreases in insulin and leptin would be expected to result in increased NPY synthesis in the arcuate nuclei (29–33) as would the increase in B (34, 35). Finally, by 2200 h, glucose levels have declined by approximately 30% to a new plateau, which is maintained for the duration of the experiment. This decrease in circulating glucose would be expected to act at the hypothalamus to reinforce the other peripheral signals of energy need. Infusion of glucose into the ventromedial nuclei of the hypothalamus prevents normal sympathetic and endocrine responses to severe insulin-induced hypoglycemia (36), and the sensitivity of these glucoreceptors may be sufficient to respond to the relatively small changes in glucose concentrations observed here. Thus, these studies identify rapid changes in at least five signals known to alter the hypothalamic control of energy balance that are apparent within 2–6 h of the onset of the fast.

The initial small increase in CRF mRNA at 6 h may reflect housekeeping chores in CRF neurons after the pronounced initial ACTH (and presumably CRF) secretion that occurred with the onset of the fast. However, the overall decrease in CRF mRNA during the subsequent hours surprised us, as

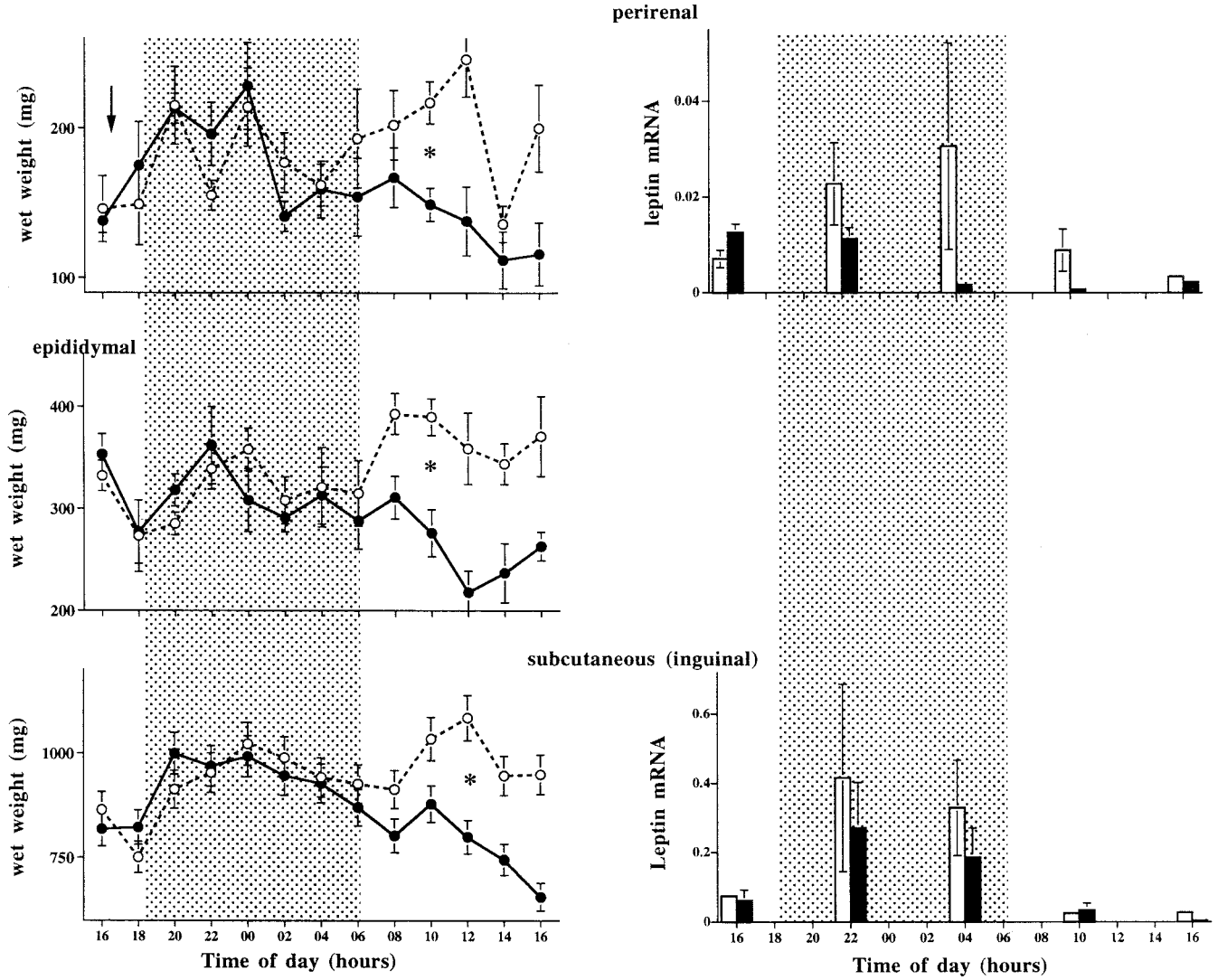

FIG. 4. Changes with time in fat mass and leptin expression. Symbols on the *left* are explained in Figs. 1 and 3; analysis is reported in Table 1 and the text. *, The first significant difference in fed and starved rats (by Fischer's PLSD). *Open* and *solid bars* on the *right* represent results from fed and starved rats, respectively (n = 6/group). Results are discussed in the text.

NPY is stimulatory to CRF (37, 38), and NPY production was clearly elevated from 12 h on. The significantly decreased numbers of c-Fos-immunoreactive cells in the parvocellular PVN supports the idea that input to this region was reduced. However, it may be that the marked and sustained increase in B that occurred at the beginning of the fast curtailed CRF synthesis, as NPY infused intracerebroventricularly for 5 days results in inhibited ACTH and B responses to acute stress (9).

ACTH-adrenal uncoupling

Even during the first part of the dark, adrenal B content did not track ACTH well. During the rest of dark and throughout the light period, adrenal B did not appear to be regulated by ACTH. There was, however, a negative regression of adrenal B on leptin, and this result fits well with the previously reported inhibition of plasma B by leptin in starved rats (39). Thus, changes in leptin concentrations in both fed and starved rats explain at least part of the decoupling between adrenal B and ACTH in this study and where a similar dissociation between ACTH and adrenal B was found (but not shown) (6). Leptin receptors are found in adrenal (40, 41) as well as hypothalamus, and it is not clear whether the relationship we observed between leptin and B is direct or indirect. In addition to the fairly small correlation between B and leptin, there is clearly a strong effect, particularly evident at the dark-light transition, that may be a consequence of altered activity in adrenal nerves (42, 43) directed by changes in hypothalamic activity (44–46). The combination of adrenal regulatory effects resulted in a marked elevation in mean plasma B in starved *vs.* fed rats (24.3 *vs.* 8.7 μg/dl, respectively). This major increase in glucocorticoid levels is sufficient to act on glucocorticoid receptors in targets throughout the animal (47, 48). Although older male rats with fixed, low B levels survive starvation for 48 h, and elevated B is not essential for responses in NPY mRNA (7),

it is probable that elevated B levels abet many of the responses determined by other hormones, neural action, and metabolites.

Adipose tissue stores

White adipose tissue depots were mobilized during the light period of the day after the onset of starvation. It is unlikely that changes in sympathetic outflow to white adipose tissue (49) were increased, both because starvation is known to decrease sympathetic outflow (50) and because sympathetic stimulation decreases leptin biosynthesis (51), which was not observed until the later parts of the study. It is also likely that overall sympathetic neural outflow to fat depots was decreased, as shown by the significant decrease in uncoupling protein content in interscapular brown adipose tissue by 24 h. This raises the question of what caused the abrupt decrease in leptin secretion in the absence of a concurrent change in leptin mRNA. Again, it may be that insulin is the effector, as the decrease in insulin precedes the drop in leptin secretion, and insulin is clearly related to both leptin synthesis and secretion (52–54).

Sequelae

Within several hours of the onset of starvation, young male rats exhibit alterations in metabolism, hormones, and neuropeptides that equip them for survival, if food is to be obtained. The increased NPY mRNA, will act, when the peptide is secreted, to increase the drive for food (55), and thus food-seeking behavior. The late decrease in CRF mRNA should both decrease central sympathetic drive (56, 57) and decrease the anorexogenic effect of CRF (1). Decreases in circulating leptin and insulin, in addition to the peripheral effects they exert, should also bolster the increases in NPY synthesis and secretion (58). The uncoupling of adrenal B regulation from ACTH allows (CRF and) ACTH levels to fall after a pronounced secretory burst, which may have a trophic effect on the adrenals. The persistently increased B level would be expected to stimulate NPY mRNA (59) and decrease CRF mRNA (60), again abetting changes in other hormones and metabolites. Finally, although there is relatively little fat in the young male rats that were studied, mobilization of fat stores provides sufficient metabolic fuel for foraging.

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