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Rats Stunted by High-Dose Glucocorticoid Treatment Are Capable of Undergoing Catch-Up Growth after Fasting

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ABSTRACT. Experiments were carried out to test the hypothesis that permanent growth retardation after glucocorticoid-induced growth suppression is due to an alteration of a central set point for target size rather than an inability of peripheral tissues to carry out catch-up growth. Rats were injected subcutaneously with saline, as controls, or with cortisone acetate, 1 mg/25 g body wt/d, for 4 d, beginning at 37 d of age. The treated animals were submitted to acute fasting for 48 h, beginning at 47 d of age, after which they were allowed to feed *ad libitum*. Cortisone treatment significantly stunted body wt, tail length, and tibia length. During recovery after fasting, both the cortisone-treated and the saline-injected rats exhibited catch-up growth in body wt and tibial length. In other rats killed at different time intervals during recovery after cortisone treatment, only, there was no pattern of catch-up growth in tibia length. There was no difference in tibial epiphyseal width between fasted and nonfasted rats within the saline- or cortisone-treated group. The findings demonstrate that rats that are permanently stunted by high-dose glucocorticoid treatment retain the capability for catch-up growth in both soft and skeletal tissues. The data support the hypothesis that catch-up growth is regulated by a central control with a mechanism (set point) for setting target size of the body. Stunting resulting from glucocorticoid treatment may be the result of a reset of the putative set point. (*Pediatr Res* 25:373–376, 1989)

Abbreviations

GH, growth hormone
s.c., subcutaneously

Treatment of young rats with injections of growth-suppressive doses of cortisone over a period of 4–8 d results in permanent stunting of body size (1). Permanently stunted growth has also been observed in the human after high-dose glucocorticoid treatment (2) or after Cushing's syndrome (3). The absence of catch-up growth in the recovery period has been attributed to a failure of response of target tissues to growth factors (4). That hypothesis is consistent with the existence in the rat of longstanding disturbances of cartilage ultrastructure (5), *in vitro* cartilage sulfation (6), and elevated serum GH secretion during the recovery period (7).

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We have recently observed that rats that were permanently stunted by neonatal head-irradiation are capable of catch-up growth of soft and skeletal tissues after a period of transient growth arrest produced by fasting (8). That result is compatible with the hypothesis that the stunting in the head-irradiated rat is the result of damage to a set point for body size (9). It also suggests the possibility that an alteration of the set point may be involved in stunting produced by exposure to glucocorticoids. In the present experiments, we test that possibility by observing growth responses during fasting and refeeding in rats that have been stunted previously by glucocorticoid treatment.

MATERIALS AND METHODS

Male rats of the Long-Evans strain were obtained at 31 d of age from Simonsen Laboratories, Inc. (Gilroy, CA) and were housed one to a cage in an environment of fresh filtered air, 22.2–25.6°C, and light/dark periods of 14/10 h. The diet was Purina Lab Chow (Ralston-Purina Co., St Louis, MO) and tap water *ad libitum*. Cages were stainless steel, the hanging type, 17.8 × 17.8 × 25.4 cm in size. The animals were handled by the same two attendants throughout the experiments. Routine handling consisted of once or twice weekly body wt and tail length measurements (10).

Experiment 1. This experiment was designed to show the effect of a fast superimposed on growth stunting produced by cortisone treatment. At 37 d of age, the animals were distributed according to body wt into two groups, each having approximately the same mean and variance of body wt. One group received cortisone acetate injected s.c. in a dose of 1 mg/25 g body wt/d for 4 d beginning at 37 d of age; the other group received injections of an equivalent vol of sterile saline. The d after the last injection was designated as recovery d 0. On recovery d 6, 47 d of age, the two groups were each subdivided into two subgroups matched as closely as possible for body wt. One of each pair of subgroups was fasted for 48 h. The other remained on the diet. The postfasting recovery period extended for 29 d to the d the animal was killed, 78 d of age. When the animal was killed, the right tibia was removed, stripped of soft tissue, and measured for its greatest length to the nearest 0.1 mm with a Vernier sliding jaw caliper. The width of the tibial epiphyseal growth plate was measured (11).

Experiment 2. This experiment was designed to show the pattern of tibial growth in length during and after treatment with cortisone. In this experiment, groups of rats similarly matched for body wt were treated with saline or cortisone acetate using the same dose schedule as in experiment 1. Subgroups of five animals were killed by ether euthanasia at the following d of age/recovery: 41/0, 48/7, 55/14, 62/21, and 69/28. Tibial length was determined.

Differences between means of experimental and control groups were tested for significance by t test.

RESULTS

Body wt and tail length (experiment 1). Figure 1 shows growth curves for body wt. Wt was significantly stunted by cortisone treatment. During the period between cortisone injections and fasting, a resumption of growth was evident. Within the saline- and cortisone-treated groups, fasting resulted in significant decreases in body wt. However, in each of the subgroups there was no significant difference in body wt between fasted and nonfasted rats after 61 d of age. Tail length (not shown) demonstrated a significant retardation with cortisone treatment ($p < 0.005$). As the subgroups were initially matched for body wt, rather than tail length, it was not possible to demonstrate differences after fasting on the growth curves alone. Patterns of tail growth are more clearly shown in the tail velocity data, below.

Velocity of body wt and tail length growth (experiment 1). Body wt velocity dropped significantly ($p < 0.005$) during cortisone treatment. During recovery after cortisone treatment, velocity improved but was still slightly but significantly below the saline group velocity during the measurement period before the fast. During recovery after fasting, the velocity of body wt rose significantly in both the saline and cortisone groups. A significant increase in velocity was observed at most of the measurement intervals through 78 d of age in the fasted cortisone subgroup in comparison with the nonfasted cortisone subgroup (Fig. 2*A*).

Velocity of tail length decreased significantly during cortisone treatment. Velocity increased during recovery, but was still slightly below the saline-treated group in the period before the fast, as was the case with velocity of body wt. There was no immediate disturbance in velocity of tail length during the fast. During recovery after the fast, the subgroups tended to have decreased velocities, significant in the 52–54 d (age) interval in both the fasted saline and fasted cortisone subgroups in comparison with their respective nonfasted subgroups (Fig. 2*B*).

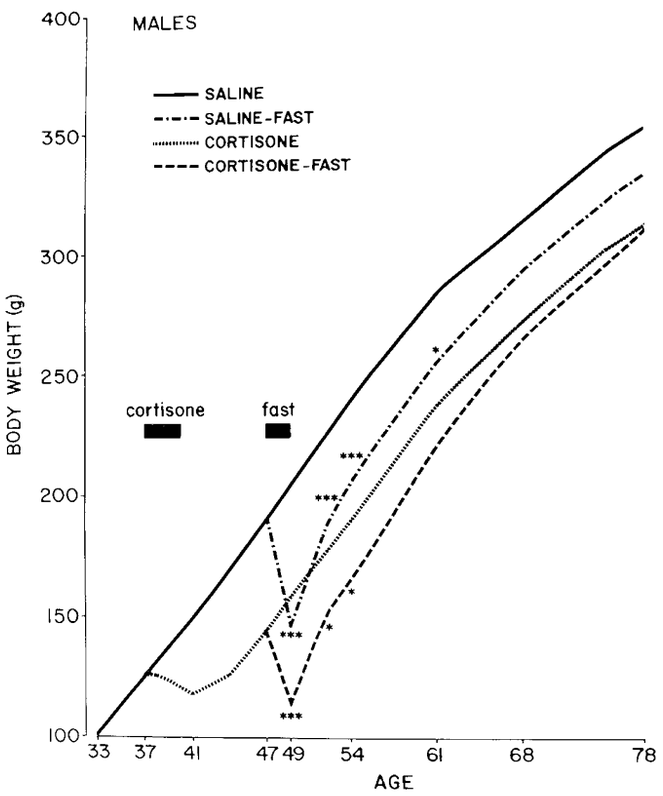

Fig. 1. Mean body wt of males injected with cortisone acetate s.c., 1 mg/25 g body wt/d, or with saline for 4 d starting at 37 d of age then fasted for 48 h starting at 47 d of age. Nonfasted controls were maintained in both saline and cortisone groups. Significance of differences for fasted *vs.* nonfasted rats within the saline-treated and cortisone-treated groups is as follows: * = $p < 0.05$, *** = $p < 0.005$.

Tibia length (experiments 1 and 2). In experiment 1, tibia length was compared between nonfasted cortisone-treated rats and nonfasted saline-treated rats and also between fasted cortisone-treated rats and the fasted saline-treated rats. The data indicate that cortisone treatment reduced tibia length, but that any decrease produced by fasting was fully compensated during the postfasting recovery period in both the saline- and cortisone-treated groups. p values by one-tailed t test for means of tibia length showed no significance between fasted and nonfasted groups in either the saline- or cortisone-treated rats. Means of tibia length were significantly different between the cortisone and saline groups and between the cortisone-fasted and saline-fasted groups at the level of $p < 0.005$ (Table 1).

In experiment 2, tibia length of the rats treated only with cortisone was stunted by the cortisone treatment and maintained approximately the same difference below tibia length of saline-treated rats throughout the 28-d period of observations (Table 2).

Width of the tibial epiphyseal growth plate (experiment 1). Cortisone treatment resulted in a wider epiphyseal width when the animal was killed whether the rats were fasted or not ($p < 0.01$, 2-tailed). There were no differences between the fasted and nonfasted subgroups within the saline- or cortisone-treated groups (Table 1).

DISCUSSION

The results in the present experiments demonstrate that catch-up growth of both body wt and tibia length occurred during refeeding after a total fast for 48 h in young rats that had been permanently stunted through growth suppressive doses of a glucocorticoid. In previous studies, we have shown that tibia length, tail length, and tibial epiphyseal width are only transiently reduced by a fast in rats (12). The absence when animals were killed of any difference in these parameters after a similar fast in the present experiments indicates that catch-up growth occurred in the skeleton, as well as in body wt, in the fasted-refed cortisone-treated rats.

It has been proposed that the changes in cartilage produced by growth suppressive levels of glucocorticoids in young animals and humans account for the failure of catch-up growth during recovery (2–5). However, the results of the present experiments show that both soft tissues and skeletal tissues maintain the capability of supporting catch-up growth after cortisone-induced stunting.

Recent findings support the likelihood that tissues have an inherent capacity for growth stimulation. IGF-I has been demonstrated in various tissues, suggesting that it is synthesized locally (13). GH (14) and IGF-I (15) receptors are present in cartilage. After local administration of GH into cartilage, the number of IGF-I-like immunoreactive cells increase in the injection site (16). In cartilage, IGF-I immunoreactivity has been localized in the endoplasmic reticulum and the Golgi apparatus (17), and binding proteins for IGF-I have been demonstrated (18, 19). Thus, the growth capacity of peripheral tissues may depend on their response to GH and their ability to synthesize IGF-I. As the glucocorticoid-treated rat is capable of catch-up growth, it is likely that these putative growth mechanisms are intact.

The finding that catch-up growth in the stunted glucocorticoid rat returns the animal only to the stunted size appropriate for the glucocorticoid model supports the hypothesis that catch-up growth involves a central mechanism for setting target size (20–22). The present results are compatible with resetting of the putative set point by the glucocorticoid treatment.

Various pharmacologic and functional factors appear to influence the set point. Perinatal administration of thyroxine to rats

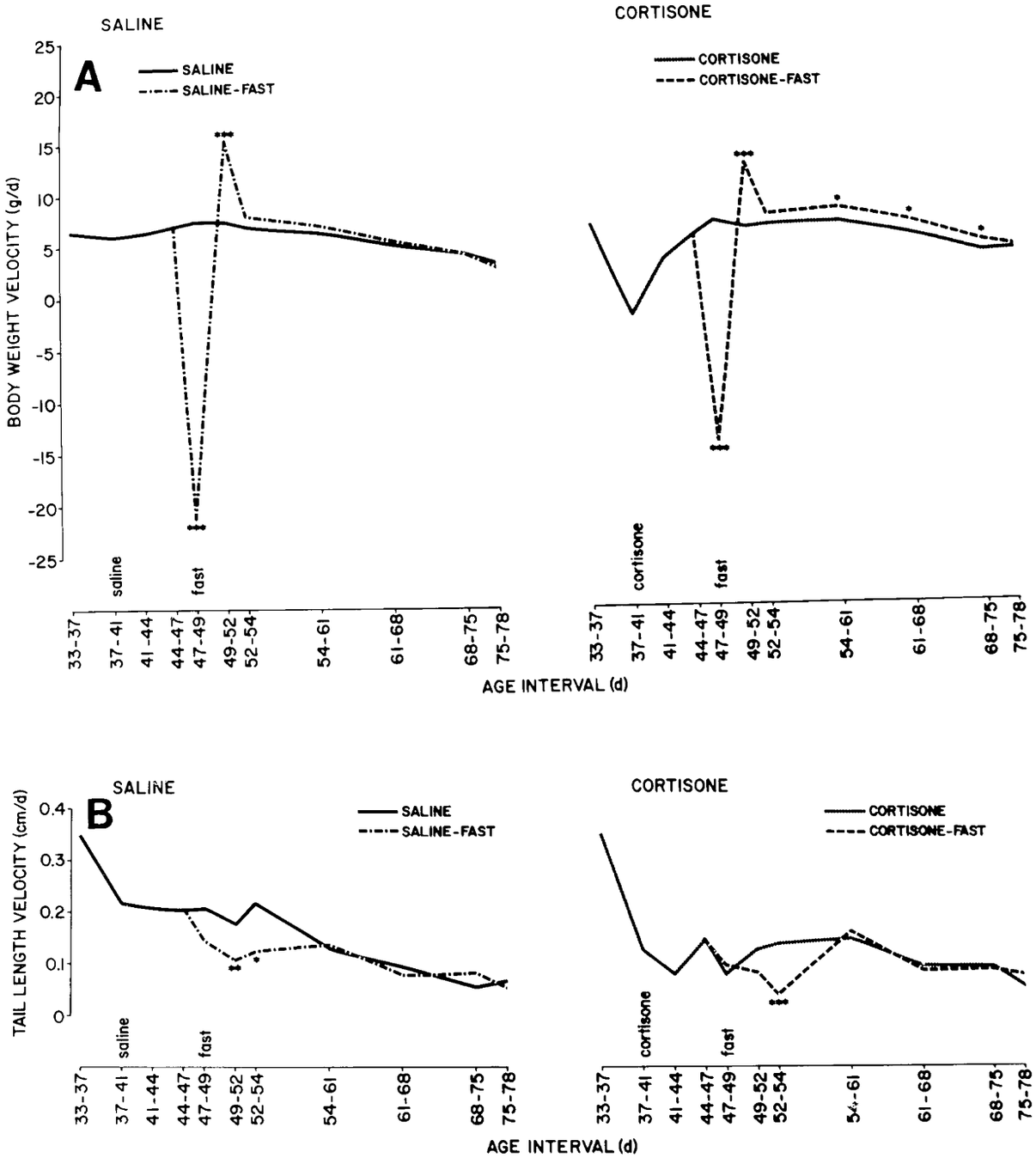

Fig. 2. *A*, velocity of body wt of the rats represented by Figure 1. *B*, velocity of tail length of the same rats. Significance of differences between mean values of fasted and nonfasted rats within the saline- and cortisone-treated groups is shown as follows: * = $p < 0.05$, *** = $p < 0.005$.

has been shown to produce permanent growth retardation (23). Recent evidence in rats suggests that imprinting by neonatal androgen secretion contributes to sex differentiation of body growth (24). Permanent resetting to a lower body wt may occur after treatment with the environmental toxin, 2,3,4,7-tetrachlorodibenzo-*p*-dioxin in rats (25) and in guinea pigs (26). Fenfluramine, an anorectic, may reduce body wt in rats independent of its effect in reducing food intake (27). Ground squirrels vary body wt over a circannual cycle; efforts to change their cyclic body wt set points evoke adjustments in energy intake or expenditure that tend to maintain the current wt (28). Dormice, with shorter body wt cycles, undergo cyclic changes in feeding efficiency corresponding to the wt cycle (29). Body wt set point changes in female rats have been shown to correlate with the estrus cycle (30). Body size is also influenced in rats by the *in utero* location of the fetus (31).

Evidence from experimental brain lesions support location of the set point in the brain. Dose-related stunting results from irradiation of the head of the neonatal rat (32). This stunting is unassociated with disturbances of known endocrine or metabolic functions and is unresponsive to treatment of the animals with GH and/or thyroxine (33). We have demonstrated that the stunted head-irradiated rats also display catch-up growth to their stunted body size after a fast (8). Chronically reduced body size with similar patterns of catch-up growth occur in rats with lesions of the dorsomedial hypothalamic nucleus (34), the lateral hy-

Table 1. *Length of tibia and width of tibial epiphyseal growth plate in rats at termination of experiment 1**

Groups	*n*	Tibia (cm)	*p*†	Tibial epiphysis (μ)	*p*‡
Saline	8	3.92 ± 0.03	<0.005	156 ± 1.0	<0.01
Cortisone	8	3.74 ± 0.02		175 ± 4.9	
Saline-fasted	9	3.90 ± 0.02		160 ± 1.7	
Cortisone-fasted	9	3.77 ± 0.03	<0.005	180 ± 3.8	<0.01

* Data are mean ± SEM. *p* values are reported for differences between saline- and cortisone-treated rats within the fasted and nonfasted groups. There was no significant difference between means of fasted and nonfasted rats within the saline- and cortisone-treated groups.

† *p* values by 1-tailed *t* test.

‡ *p* values by 2-tailed *t* test.

Table 2. *Tibia length of rats of experiment 2**

Age/recovery (d)	*n*	Saline (cm)	*n*	Cortisone (cm)	*p*†
41/0	5	3.25 ± 0.04	5	3.22 ± 0.04	
48/7	5	3.49 ± 0.04	5	3.36 ± 0.05	<0.05
55/14	5	3.74 ± 0.02	5	3.50 ± 0.02	<0.005
62/21	5	3.83 ± 0.04	5	3.68 ± 0.02	<0.005
69/28	5	3.96 ± 0.02	4	3.82 ± 0.03	<0.01

* Data are mean ± SEM.

† *p* values by 1-tailed *t* test.

pothalamic area (35), the area postrema/caudal medial nucleus of the solitary tract (36), and the striatum (37). These separate findings support the existence in the brain of a reference for body size (set point) that influences the action of growth controls.

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