Manipulation of androgens causes different energetic responses to cold in 60- and 40-day-old male rats

FRANCISCA GOMEZ AND MARY F. DALLMAN
Department of Physiology, University of California, San Francisco, San Francisco, California 94143–0444

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Gomez, Francisca, and Mary F. Dallman. Manipulation of androgens causes different energetic responses to cold in 60- and 40-day-old male rats. Am J Physiol Regulatory Integrative Comp Physiol 280: R262–R273, 2001.—Previous studies suggested that adults respond differently than pubertal male rats to cold stress. To test the role of androgens in this difference, we adrenalectomized and replaced with corticosterone either 60- or 40-day-old male rats, then sham gonadectomized (Intact), gonadectomized (GDX), or GDX and replaced with testosterone (T; GDX+T) or dihydrotestosterone (DHT). One-half remained at room temperature (RT), and one-half lived in cold for 5 days. Cold reduced T in adult but not in pubertal Intacts. In 60-day-old rats, GDX with or without T replacement had minor effects on body weight (BW) and food intake (FI) at RT and cold. In 40-day-old rats at RT, androgens had slight effects; however, androgens affected almost all variables in cold. Separation of 40-day-old T-treated rats into two groups (moderate T levels, 1.4 ng/ml; high T levels, 1.9 ng/ml) revealed major differences between the groups. Moderate T (and DHT) prevented cold-induced loss of BW and increased FI. No T and high T induced decreased BW and FI in cold. We conclude that at 40 days of age, partial resistance to stress-induced reduction of T and high sensitivity to small changes in T have markedly positive effects on threatened energy balance.

Male rats have lower basal and stress-induced ACTH and glucocorticoid responses than females (46) due to inhibitory actions of circulating androgens at various levels of the HPA axis (18). Moreover, there are developmentally related differences in activity of basal and stress HPA (16) and the hypothalamic-pituitary-gonadal (HPG) axes (22). Therefore, the HPA and HPG axes may interact to determine stress responses.

It is common wisdom that gonadal steroid secretion is inhibited by activation of the HPA axis in response to stress (32), and several types of stressors have been shown to induce significant decreases in circulating testosterone (T) levels in adult male rats (4, 5, 23, 28, 38), but this does not occur in pubertal male rats exposed to the same type of stressor (4, 5). In one study of pubertal male rats, increased circulating T levels have been reported after stress (5). We have reported that exposure to chronic cold stress for 5 days induced significant decreases in median circulating T levels in adult but not in peripubertal male rats, although T levels in the cold were comparable between adult and peripubertal rats (4). There were also altered relationships between corticosterone (B), food intake, and fat stores in peripubertal compared with adult male rats.

Changes in gonadal steroid levels alter body weight and composition as well as related behaviors such as food intake (FI) and voluntary physical activity (41). In male rats, removal of androgens by gonadectomy decreases body weight gain and FI, and these effects are reversed by low doses of T (15). However, T effects appear to be dose related, because high concentrations of T induce body weight loss; it has been proposed that these inhibitory effects of T may be due to aromatization of T to estradiol (15, 19, 27, 40). Therefore, stress-induced differences in the changes in circulating levels of gonadal steroids between adult and pubertal male rats may also differentially affect food intake and metabolic responses to stress.

Because resistance to stress-induced decreases in T levels in young male rats occurs and because puberty is a period of major growth and sexual maturation, we tested here the hypothesis that adult (60 days old) male rats might have differential T-dependent re-
sponses to chronic cold stress compared with young male rats (40 days old). We designed studies to determine whether 1) circulating levels of androgens in gonadal intact (Intact), gonadectomized (GDX), and GDX+T replacement have different effects on FI and metabolism of 60- and 40-day-old male rats both under basal conditions at room temperature (RT) and after chronic cold exposure and 2) these effects were mediated by the androgenic effects of T. All studies were carried out in adrenalectomized (ADX) rats replaced with B to avoid any interference by adrenal activation in responses to stress and to discriminate between the metabolic effects of B and androgens.

**MATERIAL AND METHODS**

**Animals**

Male Sprague-Dawley rats delivered from Bantin and Kingman (Gilroy, CA) were used. Adult male rats (n = 6/group, 60–61 days old, 265 g body wt) were used in experiment 1. Young male rats (n = 5/group, 40–41 days old, 190 g body wt) were used in experiments 2a and 2b. Rats were individually housed in hanging wire cages. Before starting the experimental period, rats were maintained under standard conditions of light (lights on 6 AM, lights off 6 PM) and temperature (22 ± 2°C) for a minimum of 3 days to allow adaptation to the new environment. Animals were offered Purina Rodent Chow (diet 5008) and tap water (or 0.5% saline after ADX) ad libitum.

**Experimental Procedures**

All protocols were approved by the University of California, San Francisco (UCSF) Committee on Animal Research. Surgery and hormone replacement. All surgical procedures were performed in the morning under a rodent anesthetic cocktail of ketamine, xylazine, and acepromazine (77:1.5:1.5 mg/ml, respectively, 1 ml/kg ip). On experimental day 0, all rats were bilaterally ADX by the dorsal approach and replaced with one 100 mg B/cholesterol (wt/wt) pellet. Adult and young rats were replaced with 35% B/65% cholesterol (wt/wt) pellet. Adult male rats (40 days old). We designed studies to determine whether 1) circulating levels of androgens in gonadal intact (Intact), gonadectomized (GDX), and GDX+T replacement have different effects on FI and metabolism of 60- and 40-day-old male rats both under basal conditions at room temperature (RT) and after chronic cold exposure and 2) these effects were mediated by the androgenic effects of T. All studies were carried out in adrenalectomized (ADX) rats replaced with B to avoid any interference by adrenal activation in responses to stress and to discriminate between the metabolic effects of B and androgens.

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In experiment 1, gonadal manipulation resulted in three groups: Intact, GDX, or GDX + medium T (mT; GDX+mT). Two Silastic capsules filled with crystalline T were implanted to provide androgen levels within the physiological range (mT levels) seen in adult Intact rats.

In experiments 2a and 2b, rats were distributed into Intact, GDX, and GDX+T (experiment 2a) and GDX, GDX+T, and GDX+DHT (experiment 2b). A single Silastic capsule filled with crystalline T was implanted to provide androgen levels comparable with the physiological range seen in young gonadal-intact rats. One Silastic capsule filled with crystalline DHT was implanted in experiment 2b to compare the effects of similar doses of T and DHT.

**Chronic cold stress.** After surgery, all rats were supplied with fresh food and 0.5% NaCl to drink and were maintained at RT for at least 4 h for full recovery from anesthesia. Then, one-half of the rats of each group was placed in constant cold (5–7°C) (4), and the other one-half was maintained at RT until experimental day 5.

**Pooling and regrouping of data in experiment 2** (2a×2b). On the basis of the BW in cold observed between experiments 2a and 2b in the GDX+T groups (Fig. 1), it was clear that we had two sets of responses in the pubertal GDX+T groups. Analysis of the results showed that T levels between the GDX+T groups in the cold were slightly different in experiments 2a and 2b (1.46 ± 0.11 and 1.78 ± 0.16 ng/ml, respectively). Moreover, within the GDX+T group in experiment 2b, two subgroups could be differentiated based on chronic cold stress-induced changes in body weight: rats either grew similarly (mT) or achieved levels similar to those of GDX+DHT groups or lost BW similarly to the GDX groups. Analysis of circulating T concentration in experiment 2b revealed that T replacement provided two levels of circulating T at RT and in cold that we defined as medium and high T (hT) replacement, respectively [1.34 ± 0.08 (n = 4) and 1.91 ± 0.09 ng/ml (n = 7)]. We defined medium T concentrations to be those that supported body weight in cold and hT concentrations to be those that did not support body weight (as suggested by the literature (15, 19, 27)). No differences were found in initial body weight between rats in experiments 2a and 2b (192.7 ± 2.0 and 191.3 ± 0.7 g, respectively), and the rats were the same age; therefore, we pooled the results from experiments 2a and 2b and statistically analyzed them as one experiment (Fig. 1, bottom). We analyzed differences across five experimental groups: Intact, GDX, GDX+mt, GDX with hT replacement (GDX+hT), and GDX+DHT. In the combined experiment, the mT group included rats with testosterone values ≤1.7 ng/ml; the hT group included rats with testosterone values >1.7 ng/ml. Each T grouping comprised rats in basal (RT) and chronic cold conditions. The mT groups contained six rats from experiment 2a (2 at RT and 4 in cold) and four rats from experiment 2b (2 at RT and 2 in cold); the hT groups contained four rats from experiment 2a (3 at RT and 1 in cold) and six rats from experiment 2b (3 at RT and 3 in cold).

To be sure that the distribution of groups based on plasma T concentration was not arbitrary, regressions were done between both plasma T levels and body weight change (days 4–5) as well as FI on day 4. Because T appeared to be important only in the cold, we analyzed by regression against plasma T, Intact, GDX+mt, and GDX+hT groups in cold. As can be seen in Fig. 2, plasma T concentrations show significant inverted U-shaped relationships with both variables.

**Measures.** Body weight was recorded at surgery and daily in the morning (8:00–9:30 AM). FI was recorded twice daily: in the morning (8:30–9:30 AM) and in the evening (5:00–6:00 PM). In experiments 2a and 2b, a total of four rats died in the cold before day 5; two rats from the GDX group and two from the GDX+hT group. All other rats were killed on the morning of day 5 by decapitation within 10 s after they had been taken from their cages. Trunk blood was collected in ice-cold tubes containing EDTA, and the plasma obtained after centrifugation at 4°C was stored at −20°C until analysis. Mesenteric, perirenal, and subcutaneous white adipose tissues (WAT) as well as thymuses were dissected, cleaned, and weighed. In Intact rats, a testis was also dissected, cleaned, and weighed.
Hormone Measurements

Plasma B was measured using an RIA kit (ICN Biomedicals) with [125I]B as tracer [intra- and interassay coefficients of variation (CV; CV_intra and CV_inter, respectively) were 2.6 and 5.6%, respectively]. Plasma T levels were measured using a double-antibody RIA (ICN, Costa Mesa, CA) with [125I]T as tracer (CV_intra = 3.2%, CV_inter = 10.0%). Plasma DHT was measured using an RIA kit (Diagnostic Systems Laboratories) with [125I]DHT as tracer after hexane extraction of the plasma (CV_intra = 3.6%, CV_inter = 14.8%). Plasma concentrations of insulin (CV_intra = 3.8%, CV_inter = 5.3%) and leptin (CV_intra = 3.6%, CV_inter = 7.6%) were measured using RIA kits from Linco (St. Charles, MO) with [125I]insulin and [125I]leptin as tracers, respectively.

Statistical Analysis

Two-way ANOVAs with androgen status and chronic cold as main factors were used to assess statistical significance. The degrees of freedom and F and P values are shown in Table 1. When the effects of the main factors or the interaction were found to be significant (P < 0.05) or marginally significant (P < 0.1), further comparisons were made using one-way ANOVA followed by post hoc comparisons with the Student Newman-Keuls test (P < 0.05) to determine effects of androgen status or Student’s t-test to compare effects of stress.

RESULTS

Endocrinectomies and Hormone Replacement

ADX and B replacement (Table 2). At RT, B pellets provided plasma B levels ranging from 3 to 6 μg/dl. These levels are comparable with the mean achieved over the diurnal cycle (12). This range of B concentrations is also known to sustain occupancy of glucocorticoid and mineralocorticoid receptors (31). Plasma B
concentrations in the cold groups were higher than those observed at RT. B levels ranged from 4 to 10 μg/dl and mimicked the mean achieved over the diurnal cycle in mild chronic stress (12).

In adult rats, plasma B concentrations differed significantly as a function of androgen status and cold with a significant interactive effect. At RT, the GDX rats had lower plasma B levels than Intact rats. After chronic cold stress, the GDX had lower B levels than the GDX+T group. Chronic cold induced significantly higher plasma B concentrations in GDX and GDX+T but not in Intact rats.

In pubertal rats, plasma B concentrations differed significantly as a function of androgen status and cold. Plasma B was higher in Intact rats compared with the other groups at RT. There was no effect of androgen status on B after chronic cold exposure. Plasma B levels were significantly higher in the cold in all groups except in GDX+mT.

Plasma T and DHT levels (Table 2). As expected, in all experiments, GDX resulted in T concentrations that were undetectable. In adult rats, chronic cold induced a significant decrease in T concentrations in Intact rats. T replacement provided significantly higher plasma T concentrations than those observed in Intact rats both at RT and after chronic cold. In pubertal rats, although mean T values were lower, chronic cold did not significantly decrease plasma T in Intact rats. T replacement provided two significantly different concentrations of T (medium and high; see MATERIAL AND METHODS and Figs. 1 and 2) that were both significantly higher than those observed in Intact rats at RT and after chronic cold. In the GDX+DHT group, plasma T levels were undetectable. Plasma DHT levels were analyzed only in the DHT-replaced groups in experiment 2b. DHT replacement provided mean plasma DHT levels that were

<table>
<thead>
<tr>
<th>Table 1. Two-way ANOVA results</th>
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<tr>
<td><strong>Main Factors</strong></td>
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<td>Plasma Hormones</td>
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<td>B</td>
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<td>Insulin</td>
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<td>Leptin</td>
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<td>Energy balance over days 2–5</td>
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<td>Body weight</td>
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<td>FI dark period</td>
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<td>FI light period</td>
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<td>Mean total daily FI</td>
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<tr>
<td>Mean caloric efficiency</td>
</tr>
<tr>
<td>White adipose tissue</td>
</tr>
<tr>
<td>Mesenteric</td>
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<tr>
<td>Perirenal</td>
</tr>
<tr>
<td>Subcutaneous</td>
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<tr>
<td>Other organs</td>
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<tr>
<td>Thymus</td>
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</table>

B, corticosterone; FI, food intake.
significant relationships were detected between T and tact, mT, and hT in cold are shown in Fig. 2. No Individual responses of 40-day-old rats that were In-

compared with those of T observed in GDX+mT groups in pubertal male rats. Plasma DHT levels tended to be lower in the rats exposed to chronic cold (t-test, P = 0.09).

**Energy Balance**

**Body weight** (Fig. 1). The first three rows of Fig. 1 show results from the three experiments. The bottom row with the shaded background shows the results of experiments 2a and 2b combined. The GDX+T groups are subdivided into those with mT (T ≤ 1.7 ng/ml) and hT (T > 1.7 ng/ml). Examination of the figure shows that there was a marked effect of surgery on body weight between days 0 and 1. Thereafter, the rats entered a new growth rate that appeared to be quite stable for each group. Because of the effect of surgery, we analyzed all treatment effects on energy balance only on days 2-5 of the experiments.

**Mean changes in body weight over days 2–5 (Fig. 3).** In adult (60 days old) rats (Fig. 3, left), GDX slightly decreased BW gain, and T replacement partially reversed this effect. Chronic cold stress decreased body weight gain in all groups. In pubertal (40 days old) rats (Fig. 3, right) at RT, GDX+mT rats showed a greater mean daily increase in body weight than Intact rats. Chronic cold decreased body weight gain in all groups, but the magnitude of this effect depended on androgen levels. The effect of cold on body weight gain was not statistically different between Intact and GDX rats. However, unlike the hT group, mT and DHT replacement improved body weight gain in the cold. The strongest effect of cold was observed in GDX+hT group, which showed the maximal decrease in body weight. Individual responses of 40-day-old rats that were Intact, mT, and hT in cold are shown in Fig. 2. No significant relationships were detected between T and FI or body weight in 60-day-old rats in RT or cold or in 40-day-old rats in RT.

**Mean FI over days 2–5. DARK PERIOD.** In 60-day-old rats, chronic cold decreased FI during the dark independently of gonadal status. In 40-day-old rats at RT, GDX+mT rats exhibited higher FI during the dark period than Intact rats. Chronic cold significantly decreased FI in GDX, GDX+hT, and GDX+DHT groups but not in Intact or mT rats. Under chronic cold conditions during the dark period, GDX+mT rats ate more and GDX+hT rats ate less than all the other groups.

**Light period.** In adults, chronic cold increased FI during lights on similarly across all groups. In pubertal rats, there were no differences among the groups in RT. Chronic cold increased FI during the light period in all groups. However, this effect was larger in GDX+DHT rats. GDX+hT rats in the cold had significantly lower FI than any other GDX group.

**Mean total daily FI over days 2–5.** In 60-day-old rats, chronic cold increased total FI. In pubertal rats at RT, neither GDX nor androgen replacement affected total FI. Chronic cold increased total FI in GDX+hT rats (P < 0.0001). GDX+mT rats ate more food than Intact and GDX groups. GDX+DHT rats ate more than GDX but did not differ from Intact rats.

**Mean caloric efficiency over days 2–5 (Fig. 3).** Caloric efficiency (CE) was calculated as the total g of BW gain/total Kcal eaten. In 60-day-old rats, there were minor effects of T, although GDX reduced CE compared with Intact rats; however, chronic cold significantly decreased total CE in all groups. In 40-day-old rats at RT, again there were minor effects of T, although GDX+mT rats had higher CE than Intact rats. Again, chronic cold significantly reduced CE in all groups; however, this effect was greater in GDX and

**Table 2. Endocrinectomies and plasma hormone concentrations in 60- and 40-day-old male rats**

<table>
<thead>
<tr>
<th>60 days</th>
<th>Intact</th>
<th>GDX</th>
<th>GDX+mT</th>
<th>GDX+hT</th>
<th>GDX+DHT</th>
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</thead>
<tbody>
<tr>
<td>B, μg/dl</td>
<td>6.95±0.20a</td>
<td>4.77±0.46b</td>
<td>5.83±0.70</td>
<td>10.06±1.11Ba</td>
<td>10.06±1.11Ba</td>
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<tr>
<td>T, ng/ml</td>
<td>7.13±0.80</td>
<td>7.20±0.51Aa</td>
<td>10.06±1.11Ba</td>
<td>10.06±1.11Ba</td>
<td>10.06±1.11Ba</td>
</tr>
<tr>
<td>RT</td>
<td>1.62±0.23a</td>
<td>NDb</td>
<td>2.51±0.21c</td>
<td>2.83±0.40c</td>
<td>2.83±0.40c</td>
</tr>
<tr>
<td>Cold</td>
<td>0.75±0.12Aa</td>
<td>NDb</td>
<td>2.51±0.21c</td>
<td>2.83±0.40c</td>
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<table>
<thead>
<tr>
<th>40 days</th>
<th>Intact</th>
<th>GDX</th>
<th>GDX+mT</th>
<th>GDX+hT</th>
<th>GDX+DHT</th>
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<tbody>
<tr>
<td>B, μg/dl</td>
<td>6.23±0.76a</td>
<td>2.83±0.48</td>
<td>3.70±0.93</td>
<td>3.70±0.63</td>
<td>2.58±0.36</td>
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<tr>
<td>T, ng/ml</td>
<td>8.51±0.36a</td>
<td>7.15±1.06a</td>
<td>7.15±1.59</td>
<td>8.72±0.97a</td>
<td>4.79±0.61a</td>
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<tr>
<td>RT</td>
<td>1.05±0.12a</td>
<td>NDb</td>
<td>1.49±0.13c</td>
<td>1.81±0.04d</td>
<td>NDb</td>
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<tr>
<td>Cold</td>
<td>0.68±0.22Aa</td>
<td>NDb</td>
<td>1.43±0.07C</td>
<td>1.97±0.12D</td>
<td>NDb</td>
</tr>
</tbody>
</table>

Results are expressed as means ± SE (n = 4–12/group). *P < 0.05 or †0.05 < 0.1 indicate effect of chronic cold stress vs. respective control groups at room temperature (RT) (t-test); within the same stress condition (*RT, †Cold), groups labeled with different letters are statistically different (Student Newman-Keuls test, P < 0.05). Intact, gonads intact; GDX, gonadectomized; GDX+mT, GDX+moderate testosterone (T); GDX+hT, GDX+high T; GDX+DHT, GDX+dihydrotestosterone; ND, nondetectable; NM, not measured.
GDX+hT rats. In the cold, the GDX+hT group showed the lowest CE of all groups.

**WAT Depots (Fig. 4)**

In 60-day-old rats, GDX ± androgen replacement had no effect on WAT weights at RT. Chronic cold decreased mesenteric, perirenal, and subcutaneous WAT depot weights to a similar extent in all groups. In the 40-day-old groups, GDX ± androgen replacement did not have any effect on WAT weights at RT. Chronic cold significantly reduced mesenteric, perirenal, and subcutaneous depot weights. After chronic cold stress, the GDX+mT group had higher mesenteric WAT depot weights than all other GDX groups. Chronic cold stress reduced perirenal WAT depot weight in all groups, but again GDX+mT rats had higher levels than Intact, GDX, and GDX+hT groups. Chronic cold stress decreased subcutaneous WAT depot weight in GDX and GDX+hT, and there was a tendency toward a decrease in Intact ($P = 0.08$) and GDX+DHT ($P = 0.09$) groups; no significant decrease was observed in GDX+mT rats. After chronic cold stress, GDX+mT had a greater subcutaneous WAT depot weight than GDX+hT rats.

**Other Organs (Table 3)**

**Thymus.** In the 60-day-old rats, there were no differences in thymus weight among groups at RT. However, chronic cold induced a significant decrease in thymus weight in GDX+T rats but not in Intact or GDX rats. In the 40-day-old rats, there were no differences among
groups at RT. Chronic cold induced a significant decrease in thymus weight only in GDX and GDX+hT rats; thymus weight was significantly lower in GDX+mT compared with Intact rats.

**Testis.** The testes of 60-day-old rats were heavier than those of 40-day-old rats \(F(1,14) = 44.4; P < 0.001\), suggesting that advancement of puberty had occurred between 40 and 60 days of age. Furthermore, histological examination of the testes showed that sperm were present in the tubules at both ages (not shown). Although cold tended to decrease testicular size, the effect was not significant, and there was no significant interaction between age and stress on testicular size.

**Plasma Insulin and Leptin Concentrations.**

**Plasma insulin (Table 4).** Independently of stress treatment, Intact adult rats had higher plasma insulin concentrations than GDX rats. Chronic cold significantly decreased plasma insulin levels in all groups. In pubertal rats at RT, the GDX+mT group showed higher plasma insulin levels than Intact groups. Chronic cold significantly reduced plasma insulin concentrations in all groups. Nonetheless, 40-day-old GDX+mT rats had higher plasma insulin concentrations than GDX+hT rats in cold.
Table 3. Effects of androgen status and chronic cold stress on plasma concentrations of insulin and leptin in 60- and 40-day old male rats

<table>
<thead>
<tr>
<th></th>
<th>Intact</th>
<th>GDX</th>
<th>GDX+mT</th>
<th>GDX+hT</th>
<th>GDX+DHT</th>
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<tr>
<td></td>
<td>Insulin, ng/ml</td>
<td></td>
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<tr>
<td>60 days</td>
<td>RT 3.60 ± 0.37*</td>
<td>2.81 ± 0.44b</td>
<td>3.03 ± 0.29</td>
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<tr>
<td></td>
<td>Cold 2.51 ± 0.34 OR</td>
<td>1.48 ± 0.31Rsb</td>
<td>1.56 ± 0.36*</td>
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<td>Leptin, ng/ml</td>
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<tr>
<td>60 days</td>
<td>RT 2.89 ± 0.56</td>
<td>2.20 ± 0.47</td>
<td>2.97 ± 0.67</td>
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<td></td>
<td>Cold 0.64 ± 0.15b</td>
<td>0.82 ± 0.24*</td>
<td>0.28 ± 0.09*</td>
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<td>40 days</td>
<td>Insulin, ng/ml</td>
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<tr>
<td></td>
<td>RT 2.87 ± 0.09a</td>
<td>3.14 ± 0.24</td>
<td>4.70 ± 1.09b</td>
<td>3.39 ± 0.31</td>
<td>4.44 ± 0.50</td>
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<td></td>
<td>Cold 1.50 ± 0.24*</td>
<td>1.31 ± 0.20As</td>
<td>2.24 ± 0.26Rs</td>
<td>0.81 ± 0.16As</td>
<td>2.18 ± 0.17Rs</td>
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<td></td>
<td>Leptin, ng/ml</td>
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<tr>
<td>40 days</td>
<td>RT 1.85 ± 0.35</td>
<td>1.76 ± 0.13</td>
<td>2.27 ± 0.11</td>
<td>1.76 ± 0.14</td>
<td>2.12 ± 0.04</td>
</tr>
<tr>
<td></td>
<td>Cold 0.55 ± 0.13a</td>
<td>0.53 ± 0.13*</td>
<td>1.00 ± 0.17As</td>
<td>0.29 ± 0.09Rs</td>
<td>0.73 ± 0.09*</td>
</tr>
</tbody>
</table>

Results are expressed as means ± SE (n = 4–12/group). *P < 0.05 or †0.05 < P < 0.1 indicate effect of chronic cold stress vs. respective control groups at RT (t-test); within the same stress condition (*RT, †Cold), groups labeled with different letters are statistically different (Student Newman-Keuls test, P < 0.05).

DISCUSSION

Manipulation of circulating testosterone concentrations had only minor effects on energy balance in adult (60 days old) rats either at room temperature or in the cold; most effects appeared to result from the surgical stress of gonadectomy. There were also no marked effects of manipulating androgens in pubertal (40 days old) rats at room temperature. There were, however, remarkable effects of androgen treatment in pubertal rats challenged by cold. In the presence of low mT or DHT concentrations, pubertal rats flourished in the cold, and a few were unable to sustain life. The “pubertal” and “adult” rats used in this study differed in age by 3 wk, and their weights ranged between 190 and 265 g at the beginning of the experiment. Frequently, male rats spanning this weight range are considered adult, and distinctions are not made between the use of animals in the bottom and top of the age range. Our results show clearly that there is an age-dependent, major effect of T revealed by cold stress on FI and metabolism that occurs over this weight range.

It must be remembered that all rats in these studies were bilaterally adrenalectomized and treated with a pellet of B meant to maintain plasma B concentrations at a fixed, physiological replacement concentration. Examination of the data shows a consistent elevation of plasma B concentrations of rats in cold compared with those at RT, a finding previously reported and probably a consequence of reduced hepatic clearance rate. Normally, there is a ~2.5-fold increase in daily B excretion in the cold in rats with adrenals (12). Because of the profound effects of B on energy balance, had the rats in these studies been free to hypersecrete B in the cold, we might not have observed the marked androgenic effects in cold-exposed pubertal rats.

However, a major purpose of this study was to decipher the reasons for the marked differences observed in adrenalectomized peripubertal and adult rats in the...
The effects of various doses of B on feeding behavior and metabolism (4). In that study, there was a strong effect of increasing B concentrations on FI in the cold in pubertal but not adult rats that was complemented by the different metabolic effects of cold between the two groups (4). In that study, as in this, cold induced a significant decrease in T concentrations in adults but not in pubertal rats. Both studies together suggest that, in the presence of low-moderate androgen concentrations, B stimulates FI in pubertal rats.

Chronic stress is consistently associated with decreased plasma T concentrations in adult male rats (Refs. 4, 5, 38, and present data), a finding that has been interpreted as a physiological mechanism that puts reproduction on hold in the face of an immediate emergency. By contrast, pubertal rats do not significantly decrease T (Ref. 4 and present data) in response to cold stress, suggesting that at this age and stage of development, different physiological considerations apply. Throughout puberty there is a progressive increase in basal T secretion that continues until adulthood is achieved (22). During this period many changes related to the maturation of reproductive function occur, and it is likely that gonadal regulation during this developmental period could be affected differentially by stress.

Chronic stress applied to either pubertal or adult rats (2) causes plastic changes in the brain such that neuronal pathways leading to stimulation of corticotropin-releasing factor and ACTH secretion differ in response to acute, novel stress (2, 6, 7). In addition to changes in the regulation of HPA activity, chronic stress also alters feeding patterns (3, 7), weight gain, and the amplitude of core body temperature rhythms (7). Because treatment with T in pubertal rats at RT has few effects, whereas in the cold, androgens have marked effects in 40-day-old rats, this result again suggests that the interface between circulating androgens and metabolism is revealed by the reemphasis of brain pathways used in chronically stressed rats. There is clearly an interface between circulating androgens and at least autonomic, stress, and FI circuits in pubertal rats exposed to cold. Our results show that the differential response of T to chronic stress between adult and pubertal male rats has differential effects on T-induced changes in metabolism.

In most male adult mammals, withdrawal of androgens by GDX decreases body weight gain and FI (41). These effects can be reversed by treatment with physiological doses of T, but this is strongly dose related, because supraphysiological doses induce decreased BW gain and FI (15, 19). The effects of physiological levels of T on body weight gain are due to its anabolic effect on the lean mass of somatic tissues, whereas the body weight loss induced by high doses of T results from loss of body fat content (15, 19). It has been suggested that the reduction in body weight gain during treatment with high doses of T is due to the aromatization of T to estradiol by the T-sensitive enzyme cytochrome P-450 aromatase (15, 19, 27).

In general, our results support these previous data. In adults, we found few, if any, effects of T at the doses used, although, compared with the intact group, these were 55% higher at RT and 280% higher in cold. The major effect of our manipulations in adult rats appeared to be caused by the surgery of GDX that had marked effects on both body weight, FI, and CE on day I; those effects were not reversed by treatment with androgen. By contrast, marked effects of androgens were observed in 40-day-old rats only after chronic cold but not at RT. In pubertal rats, T replacement resulted in distinct plasma T levels differing by ~0.5 ng/dl. Both groups had higher T levels than intact, both at RT (42% and 72%, respectively) and in cold (110% and 272%, respectively). This small difference in concentration did not have differential effects at RT. These results suggest that in chronic cold, the sensitivity of pubertal male rats to small differences in plasma T is increased, possibly by stress-induced inclusion of neuronal pathways containing higher densities of androgen and/or estrogen receptors.

The effects of T on FI are not so well explored in rats. T may act directly on the brain, because androgen and estrogen receptors as well as the enzyme aromatase are located in some hypothalamic nuclei. These are not only related to reproductive behavior but also to feeding behavior (35, 42) and/or autonomic outflow that produces metabolic changes that could act as signals of energy stores for the brain.

In 60-day-old rats, cold but not androgen status affected FI with a characteristic stress-induced increase in feeding during the light and decrease during the dark period. In 40-day-old male rats, there were no effects of androgen on FI in rats at RT but FI was significantly decreased by cold in both GDX and GDX+hT rats. This result supports the hypothesis that low concentrations of T or DHT have positive effects on FI but that hT concentrations decrease FI, an effect possibly mediated by aromatization of the steroid to estrogen. Furthermore, our results suggest that under chronic cold, aromatase activity (or the number of active, aromatase-containing neurons) may be increased in pubertal male rats.

Aromatase activity is regulated by androgens (1, 33, 34, 42). Prepubertal GDX reduces aromatase activity in adult brain, and it has been suggested that gonadal hormones secreted during puberty may enhance the expression of aromatase activity in adulthood (33). It has also been shown that there is a decrease in hypothalamic aromatase activity that occurs during puberty, between 48 and 68 days of age (24). However, although some reports have studied the effects of prenatal stress on T metabolism and sexual differentiation (21, 49), as far as we know, the effects of stress on aromatase activity in adult and in pubertal male rats remain undescribed. Therefore, whether or not aromatase activity is increased by stress in pubertal male rats and how these changes might regulate FI deserves further study.

Chronic cold caused changes in the daily feeding rhythm in all groups and was androgen independent.
FI during the light period increased, whereas it decreased during the dark period in all groups exposed to chronic cold. These effects on feeding pattern have been consistently observed in ADX rats with B replacement in the cold (3, 4). Increased light period FI is characteristic of rats with lesions of the ventromedial (10, 36), but not dorsal, paraventricular or arcuate nuclei of the hypothalamus (10) and also of rats with lesions of the paraventricular thalamic nuclei (7). It is possible that the effects of chronic cold exposure on rhythms in FI are mediated by altered activity in the hypothalamic ventromedial and thalamic paraventricular nuclei that have both been shown to be involved in the neural circuits responding to chronic cold (6).

Cold activates sympathetic neural outflow. Increased activity of brown adipose tissue provides essential thermogenesis to sustain the demand for increased heat generation by rats in cold (20). Sympathetic stimulation of WAT is reflected by its weight decrease in the cold. In adult male rats, chronic cold significantly reduced fat depot weights, but this effect was androgen independent. However, in pubertal rats, there were differential effects of androgen status in cold. In vitro as well as in vivo, androgens stimulate lipolysis in rat adipocytes by increasing the number of β-adrenoreceptors, an effect mediated only by T, and by enhancing the activity of the adenylate cyclase, an effect mediated both by T and DHT (8, 44, 45).

Although changes in body weight can be caused by coordinated shifts in a variety of metabolic substrates, the strong effects of cold on WAT depots and the possible interaction between cold and androgen effects on WAT are interesting. In pubertal rats, the final body weight was tightly related to WAT depot weight in Intact rats ($P = 0.002, r^2 = 0.923$). However, although the GDX+MT and DHT groups tended to have similar relationships, there were no significant relationships in the other groups. These results suggest that in the cold, body weight loss could be partially explained by decreases in WAT depot weight in male rats maintaining physiological levels of T or DHT; this agrees with previous work showing the effects of androgens on lipolysis mediated by changes in β-adrenoreceptors and adenylate cyclase.

Insulin concentrations were decreased by cold probably as a consequence of increased sympathetic drive to the pancreas and the known inhibitory effects of norepinephrine, acting through α-adrenergic receptors on insulin-secreting B cells (14). In pubertal rats, this effect was greater in GDX+HT than in MT- and DHT-replaced groups. However, the effect appears to be related to the effects of androgens on FI and not directly on insulin secretion, because a significant linear relationship was found between insulin and total FI ($r^2 = 0.623; P = 0.0003$) but not between insulin and T.

Leptin, a hormone secreted from adipocytes, also plays an important role in regulation of body weight and metabolism (17, 29). Plasma leptin levels correlate with the degree of adiposity and are regulated by feeding and fasting (25, 39). In adult and pubertal male rats at RT, no effects of androgen status were observed on plasma leptin levels. Chronic cold significantly reduced leptin. Leptin secretion from fat is known to be inhibited by increased sympathetic outflow to WAT (11, 26), and catecholamines added in vitro inhibit leptin output (25, 30). Effects of cold on plasma leptin levels reflected cold-induced changes in total fat depot weights, and no significant relationships were found between leptin and T levels. Therefore, our results do not support a direct relationship between T and leptin.

**Perspectives**

The results of these studies show major, age-dependent interactions between androgens, their circulating concentrations, and stress and sound some major warnings. First, the use of male rats across body weights ranging between 190 and 300 g in a single experimental paradigm is dangerous at best, although it occurs frequently. Even if variables other than those associated with the gonadal system are being examined, it is clear that this system markedly affects responses in other physiological systems to (probably) a variety of maneuvers. The fact that male rats in this weight range are undergoing the pronounced changes in their HPG axes associated with puberty suggests using serious caution about choosing this age or weight range to study. There is, of course, no guarantee from the results in this study that the rats studied, starting at 60–61 days of age, are in a stable, mature state. The tendency by investigators to use the youngest and lightest rats possible for studies is natural; however, this should be tempered by the understanding that male rats in this age range are a moving target, with their physiology changing daily.

Second, very small apparent differences in circulating hormone concentrations characteristically cause different responses. This, after all, is what hormones are all about and why their secretion rates are so tightly controlled. The difference between the effects of circulating T concentrations of 1.4 and ~1.9 ng/ml (4.9 and 6.6 nM) represents a moderately large difference in the occupancy of the androgen receptor with its $K_D$ of 2.9–3.9 nM (9). Although we were very conscious of the marked effects of very small changes in circulating B concentrations on B-dependent variables (12), we did not take seriously enough the same phenomenon in the gonadal system.

Third, it required a provocative maneuver to reveal the marked effects of pubertal changes on energy balance in male rats. Again, this is more typical than not and is characteristic of physiological findings: to see what a system can do, don’t study it under basal conditions, provoke it. Without the provocation of cold, we would not understand the marked and ferocious effects of appropriate androgen concentrations on helping to immunize the pubertal organism against the effects of stress on energy balance. It seems likely that having spent such energy to become pubescent, there is considerable heuristic...
value in providing a mechanism to ensure that adulthood and procreative capacity occurs. This may explain, in the long view, the lack of marked suppression of androgens by stress during puberty. The hormones are required to thrust the individual into adulthood.

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