Role of testosterone in regulating the growth of mice from lines selected for low vs high plasma insulin-like growth factor-I concentrations

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Abstract. A study was undertaken to investigate the role of testosterone in regulating growth and circulating levels of insulin-like growth factor-I in male mice from lines divergently selected on the basis of plasma IGF-I. Controls of each lines were sham-operated at 10 days of age and treated with peanut oil from day 14 to day 70. A second group, which was castrated at 10 days and treated with testosterone enanthate (0.5 μg · (g body weight)−1 · day−1) from day 14 to 70, did not differ from controls in body weight but had higher plasma IGF-I concentrations. Delaying testosterone therapy until day 42 in a third group retarded growth, with body weights being significantly lower than those of other two groups from days 35 to 56. However, plasma IGF-I levels in this group were not different from those of controls. Effects of line and treatment were additive. It is concluded that the greater pubertal growth of high-line compared to low-line males is not due to greater stimulation of circulating IGF-I by testosterone. Furthermore, testosterone does not appear to influence pubertal growth by acting on circulating levels of IGF-I.

In normal humans the adolescent growth spurt (1) is accompanied by a rise in circulating IGF-I concentrations (2). Plasma IGF-I concentrations are more strongly related to pubertal development than to chronological age (3–5) and appear to correlate positively with rising plasma levels of gonadal steroids in females and males (4). However, male pygmies, despite their normal levels of testosterone, do not show an increase in plasma IGF-I concentrations or a growth acceleration during adolescence (6). It has therefore been hypothesised that circulating IGF-I is the principal factor responsible for normal pubertal growth and that testosterone does not accelerate growth appreciably in the absence of increased plasma levels of IGF-I (6).

Divergent selection of mice on the basis of plasma IGF-I concentrations at 42 days of age has led to the development of lines differing markedly in plasma IGF-I concentrations and body growth (7–9). Although mice from the low IGF-I and high IGF-I lines both show an adolescent growth acceleration, circulating IGF-I levels and the rate of growth are greater in high-line animals at this time (R. A. Siddiqui, unpublished observations). The present study was undertaken to test the hypothesis that the greater pubertal growth of high-line males reflects an enhanced stimulation of circulating IGF-I levels by gonadal androgens in high-line compared with low-line males.

Materials and Methods

Experimental design
The experiment was a factorial design involving 2 selection lines by 3 treatments (combinations of castration
and testosterone therapy). Selection pressure in the low and high IGF-I lines described by Blair et al. (9) ceased after 7 generations and the mice were randomly bred within lines for two further generations. Ten litters each of low- and high-lines were selected from the original 20 litters of each line produced in generation 9 on the basis that they were born within a 4-day period. Litter size was not standardised and male mice from each litter were assigned at random (within litters) to one of the three following treatments:

Castrated/replacement therapy from day 14 (CRT14). Animals were castrated at 10 days of age and treated with testosterone (in peanut oil) from day 14 until day 70.

Castrated/replacement therapy from day 42 (CRT42). Animals were castrated at day 10, treated with peanut oil from day 14 until day 42, and with testosterone from day 42 until day 70.

Sham-operated control (SOC). Animals were sham-operated at day 10 and treated with peanut oil from day 14 until day 70 of age.

Surgical procedures
The neonatal mice were anaesthetised using hypothermia (10) produced by placing them on insulating material in a refrigerator at 1–2°C for about 15–20 min until all movement ceased. Castration was performed by removing both testes through a single transverse low abdominal incision with sterile forceps and cutting away the testes and epididymides. For the sham operation, both testes were pulled through the incision and then carefully placed back in the abdominal cavity. The wound was closed with a drop of acrylate glue (Supa glu, Selley, NSW, Australia). On completion of the operation, the animals were warmed under a 60 W electric lamp for 3–5 min until normal colour and movement were regained. They were returned to their natural mother in the same cage and rubbed in the original bedding to acquire the dam’s scent. The health of all animals was monitored throughout the experiment. Those animals which became moribund and had abnormal color were excluded from the study. Survival rate to 48 h after the operation was 82% with no marked differences between castrated and sham-operated animals.

The animals were weaned at a mean 21 days of age and allowed ad libitum access to water and laboratory pellets. All mice were maintained in a constant ambient temperature (20°C) and photoperiod (14 h light: 10 h dark). At the end of the experiment mice were euthanased with ether and the presence (SOC) or absence (CRT14 or CRT42) of testes confirmed by mid-ventral laparotomy and examination of the reproductive tract.

Testosterone therapy
Testosterone enanthate (Primoteston®, 250 g/l, Schering AG, Berlin/Bergkamen) was diluted to a concentration of 5 g/l in sterile peanut oil. It was injected sc in the nape of the neck via a 21G hypodermic needle at a dose rate of 0.5 μg · (g body weight)⁻¹. Injections were carried out at two weekly intervals commencing on day 14 of age. The sham-operated animals, and the CRT42 group during days 14–42, were injected with equal amounts of sterile peanut oil on the same days.

Live weights and plasma IGF-I concentrations
Live weights of the animals were recorded three times a week (between 09.00 and 11.00 h). Blood samples for plasma IGF-I determination were collected from the orbital sinus as described by Riley (11). At 21 days of age, blood from 3 mice (200 μl per animal) was pooled within treatment (because of the small volume of blood obtained from each mouse). At later ages, 500 μl of blood was obtained from each individual. Blood was collected in heparinised polypropylene microfuge tubes (9 IU sodium heparin per tube). Plasma was harvested immediately by centrifugation at 2000 × g for 20 min at 4°C. Plasma samples were stored at −20°C until assayed. IGF-I concentrations in plasma were determined by radioimmunoassay as described previously (12) and are expressed in terms of recombinant human met-IGF-I (batch #742–44; Dr B. D. Burleigh, International Minerals and Chemicals, Pitman-Moore, Northbrook, IL). The intra- and inter-assay coefficients of variation were 5.0 and 9.8%, respectively.

Statistical procedures
Analysis of covariance (ANCOVA) was performed using the ordinary least squares method. The least squares means for plasma IGF-I concentrations were obtained as described by Urquhart (13). The live weights and plasma IGF-I concentrations were adjusted to account for the effects of number of pups born and day of birth, to ensure that these effects were removed from the data prior to comparing the line means. Statistical comparison of the adjusted line means was undertaken using Student’s t-test. All analyses were performed using the computer package ‘REG’ (14).

Results
Live weights
The live weights of SOC, CRT14 and CRT42 mice of the low and high lines are shown in Fig. 1. Significant (p < 0.05) line differences became apparent at 10 days of age and persisted for the remainder of the experiment. Live weights of CRT14 mice were not significantly different to those of SOC mice at any time. However, delaying testosterone replacement in CRT42 mice resulted in a significant
(p < 0.05) retardation of growth from 35 or 39 days of age (when compared to SOC or CRT14 treatments, respectively). Testosterone replacement in the CRT42 group, beginning at day 42, resulted in accelerated growth and by day 56 between-treatment differences were no longer significant. The line x treatment interaction was non-significant at all times.

**Plasma IGF-I concentrations**

Plasma IGF-I concentrations in SOC, CRT14 and CRT42 mice and in low- and high-lines are shown in Table 1. Significant (p < 0.05) differences between lines in plasma IGF-I were apparent at days 21, 35 and 49 of age and in each case high-line mice had greater IGF-I concentrations (by 70–130 μg/l). However, by 63 days of age, these differences were small and no longer significant.

<table>
<thead>
<tr>
<th>Plasma IGF-I concentrations by line and treatment at each age.</th>
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<tr>
<td><strong>Plasma IGF-I (μg/l) at day</strong></td>
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a, b: Means with different superscripts differ at p < 0.05. Numbers in parentheses are those contributing to each mean. At day 21, blood from 3 mice was pooled to give each observation of plasma IGF-I concentration. SOC: sham-operated control; CRT14: castrated and given replacement testosterone therapy from day 14; CRT42: castrated and given replacement testosterone therapy from day 42.

There was no effect of treatment on plasma IGF-I concentrations at day 21. SOC and CRT42 mice maintained similar plasma IGF-I concentrations throughout the experiment. However, CRT14 mice had significantly (p < 0.05) higher IGF-I concentrations than SOC mice at 35, 49 and 63 days of age. CRT14 and CRT42 mice differed (p < 0.05) in IGF-I levels only at 63 days of age.

A significant interaction between the effects of line and treatment on plasma IGF-I was apparent only at day 49. At this age, CRT14 mice of the high line had plasma IGF-I levels (912 ± 45 μg/l) intermediate between those of SOC (874 ± 39 μg/l) and CRT42 mice (940 ± 35 μg/l) of the same line. However, the plasma IGF-I levels in CRT14 mice of low line (957 ± 46 μg/l) were substantially greater than those of SOC (723 ± 43 μg/l) or CRT42 mice (730 ± 39 μg/l).

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**Fig. 1.**

Body weight of sham-operated control, SOC (○, ●), castrated-replacement testosterone from day 14, CRT14 (Δ, ▲) and castrated-replacement testosterone from day 42, CRT42 (□, ■) mice of low, L (open symbols) and high, H (closed symbols) IGF-I lines. Pooled standard error (PSE) is given at each time. Number of animals in each line x treatment group was 7–12. Effects of line, treatment, and the line x treatment (L x T) interaction, were evaluated by ANCOVA as described in the text. NS = non-significant, * p < 0.05.
Discussion

Patterns of live weight gain in this study were as expected with high-line mice growing faster than low-line animals and growth of CRT12 mice being retarded until testosterone replacement therapy commenced. Growth of SOC mice was not different from that of the CRT14 group at any time. Thus the castration treatment was effective in retarding growth and testosterone was administered at a dose which successfully reversed the effects of castration on body growth.

Our results do not support the hypothesis that low-line males grow more slowly than high-line males because they fail to elevate plasma IGF-I concentrations in response to androgenic stimulation. There was no interaction between the effects of line and castration/testosterone therapy on body growth and, except at 49 days of age, no interaction in effects on plasma IGF-I. At this age, low-line mice treated with testosterone exhibited an enhanced, rather than a reduced, plasma IGF-I response to testosterone treatment compared with their high-line peers.

Nor do our results support the hypothesis that androgenic stimulation of body growth acts via circulating IGF-I. Although CRT12 mice of both lines grew more slowly than SOC mice during the period 35–56 days of age, significant differences between these groups in plasma IGF-I concentrations were not apparent at any time. Similarly, while CRT14 mice had higher plasma IGF-I levels than SOC mice at days 35–63, they did not grow more rapidly and if anything their growth was slightly reared.

It is concluded from this experiment that the greater pubertal growth in high-line males compared with low-line males is not due to a greater androgenic stimulation of endocrine IGF-I secretion (primarily by the liver). Rather, androgens appear to stimulate growth by some other mechanisms, possibly including mechanisms which do not involve IGF-I. Alternatively, the mechanisms could involve local (paracrine or autocrine) production of IGF-I to an extent which does not influence circulating concentrations of IGF-I (15–17).

References


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