# Androgen Secretion and Spermatogenesis in Rats Following Semistarvation[1] (35976)

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It is well established that undernutrition adversely affects the reproductive system in rats (1–4). While spermatogenesis appears relatively resistant to the influence of caloric restriction (5), the endocrine function of the testes appears to be highly susceptible, as determined by decreased accessory organ weights and secretory activity (6, 7). That the primary derangement from underfeeding centers in the pituitary gland, was indicated by studies in which regressive changes were corrected by injected gonadotropins (8, 9).

These conclusions based on accessory organ weights have not always been substantiated by the reports of androgen levels in blood and testes. This was demonstrated by Mann *et al.* (10) who restricted the feed intake of a bull calf to 33% of that eaten by its twin full-fed control. After 2 months, the testosterone in spermatic vein blood and testes was 25% that in the control. Continuation of underfeeding for 7 months resulted in a marked increase in testes testosterone but the underfed was 80% of the control. Semistarvation of rats suggested that a 33% loss of body weight was associated with a reduction in androgen production by testes *in vitro* (11). Although the reduction was statistically significant, it may not be biologically important since androgen synthesis was reduced only about 10%.

Although previous studies have described the alterations in reproductive functions occurring during prolonged periods of semistarvation, they have not included quantitative data on the hormonal changes or information on accessory sex organs. The present study was designed to determine the effects of semistarvation; total starvation with, or without, a previous period of semistarvation; and refeeding on testicular functions in rats.

*Materials and Methods.* Ninety, male weanling Sprague-Dawley rats were fed *ad libitum* a grain ration until 10 weeks of age. This was a corn–soy ration which contained 23% protein, 3% fat, 54% carbohydrate and provided 3.4 kcal/g. At the end of that period, five rats were autopsied to obtain organ weights. Rats were weighed weekly and mean daily feed intakes of unrestricted rats were calculated from weekly food consumption data. Water was available to all animals throughout. Rats were housed individually in wire-screened cages in a room maintained at 25–27° with a 12-hr period of illumination daily. At 10 weeks of age, 30 rats were assigned at random to *ad libitum*-fed control and 30 to a 50% restricted diet group. Half the quantity of diet consumed by rats fed *ad libitum* was given to restricted rats daily between 7 and 8 p.m. Both groups were fed the previously described grain ration.

Ten rats on the restricted diet were sacrificed when their weight loss plateaued which occurred after 8 weeks of semistarvation. Ten unrestricted controls were also sacrificed at this time. The remainder of the 20 rats on the unrestricted diet and 20 on the restricted diet were maintained on their assigned regimens until 33 weeks of age, when 10 animals from each group were sacrificed. Since at this age, several of the *ad libitum*-fed rats but none on the restricted diet were afflicted with chronic respiratory disease, it was decided to terminate this aspect of the study. After 20 weeks of restricted

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TABLE I. Body, Epididymidal and Testicular Weights, and Testicular and Epididymidal Sperm Concentration Following Semistarvation and Refeeding in Rats.

| Feeding treatment | Age (weeks) | No. of rats | Body wt (g) | Epididymidal | | | Testicular | |
|---|---|---|---|---|---|---|---|---|
| | | | | Wt (g) | Sperm [(×10⁶)/g] | | Wt (g) | Sperm [(×10⁶)/g] |
| | | | | | Head-body | Tail | | |
| *Ad libitum* | 18 | 10 | 474 ± 19[a] | 1.31 ± 0.13 | 248 ± 49 | 342 ± 63 | 3.77 ± 0.15 | 46 ± 12 |
| | 33 | 10 | 519 ± 27 | 1.14 ± 0.02 | 259 ± 58 | 295 ± 75 | 3.32 ± 0.35 | 39 ± 16 |
| Semistarved | 18 | 10 | 231 ± 10 | 0.85 ± 0.12[b] | 283 ± 55 | 262 ± 31 | 2.82 ± 0.19[b] | 40 ± 18 |
| | 33 | 10 | 234 ± 6 | 0.88 ± 0.07[b] | 231 ± 81 | 314 ± 68 | 2.82 ± 0.24[b] | 37 ± 9 |
| Semistarved–refed | 33 | 10 | 402 ± 18 | 1.02 ± 0.19[c] | 291 ± 85 | 298 ± 91 | 3.01 ± 0.17[c] | 41 ± 18 |
| *Ad libitum*–starved 6 days | 33 | 6 | 403 ± 35 | 1.06 ± 0.13[b] | 254 ± 48 | 259 ± 62 | 3.21 ± 0.43 | 41 ± 16 |

[a] Mean ± standard error.
[b] Differs from rats fed *ad libitum* at same age ($p < .05$).
[c] Differs from semistarved rats at same age ($p < .05$).

feeding, 10 rats were permitted free access to the diet and sacrificed following a 3-week refeeding period (33 weeks of age). At the same time, six previously unrestricted rats were deprived of all food for 6 days at the end of which they were killed (33 weeks of age). All rats were killed with an overdose of ether; blood was obtained from the abdominal aorta. The serum was stored at —15°. For testosterone analysis, the serum was first extracted with diethyl ether. Purification was done by methanolic precipitation of lipids and thin-layer chromatography to isolate the androgens. The diheptafluorobutyrate derivative of testosterone was used for quantification by electron capture using gas–liquid chromatography (12).

Seminal vesicles were weighed and homogenized for fructose analysis (14). Weights of adrenals, anterior pituitary, and liver were secured at autopsy. Epididymides and testes were homogenized in 0.85% sodium chloride (with 0.01% Triton-X) to determine sperm contents (13).

*Results.* Semistarvation for 8 weeks produced a significant decrease ($p < .05$) in the weights of the testes and epididymides (Table I). However, since body weight loss was proportionately greater than that of the testes or epididymides, the relative weights of the testes and epididymides increased when expressed as a fraction of the body weight. Semistarvation for 8 weeks also caused reduced ($p < .05$) seminal vesicular citric acid and coagulating gland fructose (Table II). Relative to values at 18 weeks of age, caloric restriction for another 15 weeks proportionately increased weights of seminal vesicles and coagulating glands. During this time, seminal vesicular citric acid increased while coagulating gland fructose decreased (Table II). Refeeding for 21 days caused increases in relative weights and secretory activity of seminal vesicles, as measured by citric acid content, and coagulating glands, as measured by fructose content (Table II) and in absolute testicular and epididymal weights (Table I). When completely deprived of food for 6 days, serum testosterone declined to undetectable levels (Table II), but proportional weight of coagulating glands

and absolute testicular weight were not affected ($p>.05$). However, in conjunction with reduced testosterone, citric acid in seminal vesicles and fructose in coagulating glands were markedly reduced ($p<.05$). Concentration of sperm in testes and epididymides was not affected significantly after any period of restricted feeding or refeeding (Table I).

Correlation analyses (Table III) indicated that proportional weights of seminal vesicles paralleled serum testosterone levels in control at 18 and 33 weeks and in restricted rats at 18 weeks of age. During semistarvation the weight of testes varied inversely with serum testosterone ($r = -1.0$); however, level of testosterone was positively correlated with body weight, fructose in coagulating glands, and with weights of seminal vesicles, coagulating gland, and epididymides ($p<.01$). In refed rats, serum testosterone was negatively correlated with total fructose and adrenal gland weights ($p<.01$).

*Discussion.* The relative concentration of sperm in the testes and epididymides was unimpaired by the food restrictions applied in this experiment. This concentration of sperm per gram of tissue was maintained even after 23 weeks when food was restricted to half that of the *ad libitum* controls and when body weights were proportionately reduced.

Previous work suggests that caloric restriction initiated early in life impaired spermatogenesis (2, 5). However, once sexual maturity was achieved, gametogenic function of the testes was maintained in spite of undernutrition. The present results agree with previous data, which showed that spermatogenesis continued in vitamin B-deficient rats (15) and in semistarved adult bulls (16).

Classically, relative weights of seminal vesicles, coagulating glands, the prostate and their secretory activities have been used as indicators of androgen secretion (13). Methods sufficiently sensitive to detect serum testosterone allow definitive quantification of gonadal endocrine function. High variation among animals within each age group typified our testosterone data, as it did in bulls (17). The levels of testosterone listed in Table II

TABLE II. Criteria of Androgen Secretion in Rats Following Semistarvation and Refeeding.

| Feeding treatment | Age (weeks) | No. of rats | Serum testosterone (ng/ml) | Seminal vesicles | | Coagulating gland | |
|---|---|---|---|---|---|---|---|
| | | | | % Body wt $\times 10^{-3}$ | Citric acid (mg) | % Body wt $\times 10^{-3}$ | Fructose (mg) |
| *Ad libitum* | 18 | 10 | 3.90 ± 3.45[a] | 261.0 ± 28.9 | 314.8 ± 72.0 | 30.2 ± 5.1 | 119.6 ± 62.3 |
| | 33 | 10 | 1.48 ± 1.08 | 223.3 ± 35.6 | 331.5 ± 51.5 | 23.2 ± 2.3 | 119.9 ± 69.9 |
| Semistarved | 18 | 10 | 1.22 ± 2.01 | 77.9 ± 7.3[b] | 17.2 ± 8.1[b] | 14.9 ± 4.0[b] | 56.8 ± 33.5[b] |
| | 33 | 10 | 1.22 ± 0.74 | 109.2 ± 20.6[bc] | 57.7 ± 21.5[bc] | 17.1 ± 0.7[c] | 20.6 ± 9.4[bc] |
| Semistarved–refed | 33 | 10 | 3.01 ± 3.05 | 258.2 ± 38.4[d] | 299.3 ±110.6[d] | 37.2 ±16.6[d] | 81.8 ± 28.6[d] |
| *Ad libitum*–starved 6 days | 33 | 6 | nd[e] | 154.0 ± 66.5[b] | 16.5 ± 3.4 | 25.4 ± 9.2[b] | 19.5 ± 1.2[b] |

[a] Mean ± standard error.
[b] Differs from rats fed *ad libitum* at same age ($p < .05$).
[c] Differs from rats fed *ad libitum* at 18 weeks ($p < .05$).
[d] Differs from semistarved rats at same age ($p < .05$).
[e] Nondetectable.

TABLE III. Correlation Coefficients Between Serum Testosterone and Some Endocrine or Reproductive Criteria.

| Experimental condition: | Control | Semistarved | Control | Semistarved | Refed 3 weeks |
|---|---|---|---|---|---|
| Age of rats (weeks): | 18 | 18 | 33 | 33 | 33 |
| Body wt | .40 | .98[a] | —.39 | .63 | .59 |
| Testicular wt | .35 | —1.00[a] | —.32 | —.41 | —.69 |
| Epididymidal wt | .35 | .96[a] | .73 | —.12 | —.58 |
| Seminal vesicles (% body wt) | .73[a] | .97[a] | .62 | —.39 | —.24 |
| Coagulating glands (% body wt) | .42 | .91[a] | .63 | .18 | —.14 |
| Seminal vesicular citric acid | —.42 | —.64 | .52 | .54 | .59 |
| Coagulating gland fructose | —.38 | .97[a] | .65 | —.52 | —.82[a] |
| Adrenals (% body wt) | —.48 | —.66 | .29 | —.09 | —.82[a] |
| Anterior pituitary (% body wt) | .84[a] | —.21 | .63 | .52 | —.14 |
| Liver (% body wt) | —.02 | —.94[a] | —.12 | —.74 | —.08 |

[a] Values significantly different from zero ($p < .01$).

are similar to those reported by Resko *et al.* (18) in plasma from adult rats (1.10 to 2.04 ng/ml) as determined by gas–liquid chromatography, but lower than others reported by Bardin (19) (41.0 to 50.4 ng/ml), and Frick *et al.* (20), using another technique. In the present study, serum testosterone values for adult rats varied from 3.90 at 18 weeks to 1.48 ng/ml at 33 weeks of age. Others (17, 21) found decreasing levels of testosterone in blood serum of bulls as they advanced from puberty to 12 months of age.

Based on accessory organ data and on the efficacy of gonadotropic hormones in reversing starvation-atrophy of the reproductive tract, Mulinos and Pomerantz (8) and Lutwak-Mann and Mann (22) suggested that undernutrition impairs hormone secretion. Although large variation (see standard errors Table II) within groups precluded significance, group means for serum testosterone indicated that semistarvation (1.22 ng/ml) and total starvation for 6 days (undetectable) reduced levels of circulating testosterone.

Reproductive performance appears to be enhanced by a period of food restriction followed by free access to food. Rats appear to adapt to a period of prolonged caloric restriction and after arriving at a new equilibrium, secrete an increased amount of hormone when presented with a surplus of food. This is suggested by the elevated testosterone blood levels in the rats that had been semistarved for 20 weeks and then fed *ad libitum* for 3 weeks. The blood testosterone levels (3.0 ng/ml) in those rats were twice those of the controls that had never been starved (Table II). Additional evidence for this hypothesis comes from the work of Jackson (23). He reported that reproductive performance of restricted-refed rats, as measured by mating tests actually surpassed that of full-fed controls. Adaptation to semistarvation also appears in the accessory organ weights which increased between 8 and 23 weeks of semistarvation and significantly increased further after refeeding.

Total inanition for 6 days markedly reduced seminal vesicular citric acid, coagulating gland fructose, and serum testosterone, illustrating that adaptation did not occur after 6 days of complete food deprivation.

Some, but by no means all, accessory organ data were positively correlated with serum testosterone values. As measured in this experiment, no single accessory organ weight or secretory activity appears to be sufficiently dependent upon testosterone to serve as a reliable index of blood testosterone levels. Under our conditions, level of testosterone at any given moment may reflect accessory organ criteria after a suitable interval of time. Testicular weights generally were negatively correlated with serum testosterone, suggesting that maintenance of spermatogenesis is independent of circulating testosterone. Relative liver weights were negatively correlated with

serum testosterone in all groups. This may be associated with the role of the liver in regulating blood steroid levels through their removal and deactivation.

*Summary.* Male Sprague-Dawley rats were allowed to feed freely until 10 weeks of age and then were restricted to 50% of the *ad libitum* food intake of controls for 8 or 23 weeks. Serum testosterone, determined by gas–liquid chromatography, decreased during the first 8 weeks of food restriction to 31% of that in full-fed controls, but then rebounded to control levels following another 15 weeks of semistarvation. Similarly, weights and secretory activities of accessory reproductive organs declined during the first 8 weeks and increased during the last 15 weeks of food restriction. When allowed to refeed for 3 weeks following 20 weeks of semistarvation, serum testosterone rose to 3.0 ng/ml, twice the 1.5 ng/ml in fully fed controls and three times the 1.2 ng/ml for chronically semistarved rats at the same age.

The complete absence of food from previously full-fed rats for 6 days lowered testosterone levels to undetectable quantities.

Absolute weights of testes and epididymides and their concentration of sperm (per gram of tissue) were affected less than androgens during a 50% reduction in feed intake as well as during the subsequent *ad libitum* refeeding period.

1. Keys, A., Brozek, J., Henschel, A., Mickelsen, O., and Taylor, H. L., "The Biology of Human Starvation," Vol. 2. Univ. of Minnesota Press, Minneapolis (1950).

2. Berg, B. N., J. Nutr. **87**, 344 (1965).

3. Hahn, P., and Koldovsky, O., "Utilization of Nutrients During Postnatal Growth." Pergamon, New York (1966).

4. Moore, C. R., and Samuels, L. T., Amer. J. Physiol. **96**, 278 (1931).

5. Widdowson, E. M., Mavor, W. O., and McCance, R. A., J. Endocrinol. **29**, 119 (1964).

6. Pazos, R., and Higgins, C., Endocrinology **36**, 416 (1945).

7. Scott, P. P., and Scott, M. G., J. Reprod. Fert. **8**, 270 (1964).

8. Mulinos, H. G., and Pomerantz, L., Endocrinology **29**, 267 (1941).

9. Mann, T., and Rowson, L. E. A., Proc. Nutr. Soc. **16**, xviii (1957).

10. Mann, T., Rowson, L. E. A., Short, R. V., and Skinner, J. D., J. Endocrinol. **38**, 455 (1967).

11. Berliner, D. L., and Ellis, L. C., Radiat. Res. **24**, 572 (1965).

12. Clark, S. J., and Wotiz, H. H., Steroids **2**, 535 (1963).

13. Mann, T., "The Biochemistry of Semen and of the Male Reproductive Tract." Methuen, London (1964).

14. Roe, J. H., J. Biol. Chem. **107**, 15 (1934).

15. Kirton, K., Desjardins, C., and Hafs, H. D., Anat. Rec. **158**, 287 (1967).

16. Mann, T., and Walton, A., J. Agr. Sci. **43**, 343 (1953).

17. Rawlings, N. C., and Hafs, H. D., J. Anim. Sci. In Press.

18. Resko, J. A., Federer, H. H., and Goy, R. W., J. Endocrinol. **43**, 485 (1968).

19. Bardin, C. W., Endocrinology **84**, 435 (1969).

20. Frick, J., Steroids **13**, 21 (1969).

21. Swanson, L., Wetteman, R., Rawlings, N. C., Hafs, H. D., and Magee, W. T., J. Anim. Sci. **33**, (1971). In Press.

22. Lutwak-Mann, C., and Mann, T., Nature (London) **165**, 556 (1950).

23. Jackson, C. M., Amer. J. Anat. **18**, 75 (1915).

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