PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 158, 622–625 (1978)

Changes in Androstenedione, Testosterone and Protein Metabolism as a Result of Exercise (40260)

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We recently reported that endurance training increases urea excretion, decreases nitrogen balance, increases amino acid oxidation, and decreases protein synthesis in liver, heart and the stromal fraction of muscle (1, 2). In continuing this line of research we were particularly interested in elucidating the factors that control protein metabolism during exercise and had noted that exercise alters the plasma concentrations of several hormones. Hartley *et al.* (3) found that exercise lowered plasma levels of insulin and increased both growth hormone and cortisol. The changes in the levels of insulin and cortisol during exercise would be consistent with the changes in protein metabolism that we reported and the known physiological roles of these hormones.

Androgens are known to alter nitrogen balance (4) and muscle protein metabolism (5) and a number of investigators have shown that exercise alters plasma testosterone and/or androstenedione (6–8). Galbo *et al.* (7) have reported that plasma testosterone was lowered after an exhaustive bout of exercise or 6 hr after performing an "anaerobic" type of exercise. We reasoned that lowered testosterone secretion might explain the altered protein metabolism that we observed in endurance trained rats. Thus, we undertook this study to measure and correlate the changes in plasma concentrations of androgens with changes in protein metabolism during adaptation to endurance training and exhaustive exercise.

Materials and methods. Male and female Holtzman rats weighing approximately 200 g at the start of the experiment were housed in individual cages and given water and commercial lab chow (Wayne Lab Blox, Allied Mills, Inc., Chicago, IL) *ad libitum.* In one experiment male rats weighing approximately 150 g were castrated and then training was begun after they had recovered and weighed approximately 200 g. Rats were divided into two groups: untrained, which remained sedentary in their cages; and trained, which were subjected to daily treadmill running at 35 m/min, 8% grade, 1 hr/day, 6 days/week for 6 weeks as previously described (9).

During the last week of training a 24-hr urine collection was taken and frozen for urea analysis at a later time. Urea was analyzed using urease and glutamic dehydrogenase in a coupled enzyme assay essentially as described by Talke and Shubert (10). The reagents were purchased from Sigma Chemical Co., St. Louis, MO as the single reagent BUN assay system.

Untrained rats were sacrificed in a resting condition while trained rats were sacrificed in either a rested (6 hr or 24 hr after the last bout of exercise), exercised (killed immediately after exercise), or exhausted condition. The exhausted rats were run at 35 m/min, 8% grade until they could no longer continue (1–3 hr) and then they were sacrificed. In the exhausted state they could not right themselves when put on their backs. Rats were anesthesized by injection of nembutal (50 mg/kg) intraperitoneally, the abdominal cavity was opened by a midline incision and blood was drawn from the vena cava into a heparinized syringe. Plasma was prepared and frozen for later hormone analysis.

The solvents and chemicals used in the assay of androstenedione and testosterone were nanograde quality. For preparation of standards androstenedione and testosterone (Sigma Co.) were weighed on a Cahn electrobalance to the nearest μg and then dissolved in absolue ethanol and stored at 4°. [^{3}H]Androstenedione (86.8 Ci/m mole) and [^{3}H]testosterone (85 Ci/m mole; New England Nuclear Corp., Boston, MA) were purified on microcelite columns with ethylene glycol as the stationary phase (11).

The celite chromatography separation and

0037-9727/78/1584-0622$01.00/0
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radioimmunoassay (RIA) of androstenedione and testosterone were identical to the method described previously (12) with these exceptions: firstly, the specificity and sensitivity of the androstenedione antibody have been previously described (13). Secondly, the testosterone antibody [TL-KM TMO-3 #74] was a gift of Dr. H. D. Hafs and its cross reactivity was tested against all steroids which had side chains which closely resembled testosterone. Cholesterol and the various corticoids were also tested and none of these compounds cross reacted significantly (<0.05%) except dihydrotestosterone (20%) and androstenedione (1%). We compared testosterone values of the first 10 samples (assayed as described above) to the same samples assayed with the chromatography step deleted. There was no difference (correlation coefficient of 0.98) between these two sets of values and therefore all remaining testosterone measurements were performed excluding the column chromatographic step.

The recovery of radiolabeled androstenedione and testosterone through the entire assay procedure was 70–80%. The solvent blank values for testosterone and androstenedione were less than 10 pg/ml. The sensitivity level for both assays was thus around 25 pg/ml. The recovery of added unlabeled androstenedione or testosterone from plasma was 95–103%. The intra- and inter-coefficient of variation for both steroids never exceeded 15%.

Data were statistically analyzed by a one way analysis of variance and group comparisons were made by the Neuman-Keul test (14). The $P < 0.05$ level was chosen for statistical significance.

TABLE I. EFFECT OF TRAINING AND CASTRATION ON UREA EXCRETION IN MALE AND FEMALE RATS.

	Urea excretion (mmoles/24 hr/kg body wt)	
Group	Males	Females
Normal-untrained	36.2 ± 1.4 (15)[a]	37.8 ± 1.5 (9)
Normal-trained	41.2 ± 2.0 (16)[b]	37.4 ± 1.3 (7)
Castrated-untrained	43.4 ± 1.8 (17)[b]	—
Castrated-trained	44.0 ± 1.8 (16)[b]	—

[a] All values are mean ± SEM with the number of observations in parenthesis.

[b] Significantly different ($P < 0.05$) than male, normal-untrained.

Results. Training increased the excretion of urea in male rats (Table I) as we have previously reported (1). In female rats there was no alteration in urea excretion as a result of endurance training. This suggested the possibility that the male sex hormones might somehow be involved in the changes observed in trained male rats. This hypothesis seemed even more attractive with the finding that castration of male rats increased urea excretion to levels like those observed in trained rats and training did not further increase urea excretion.

The plasma concentrations of androstenedione and testosterone in male rats are shown in Table II. The plasma values reported for testosterone agree with previously reported values for this hormone (15). Assays for these hormones were also performed on the plasma of castrated male rats but concentrations were below the sensitivity limit of the assay. In general, these hormones were lower in the trained animals (combined groups) than in the untrained animals; however, the variability for androstenedione precluded statistical significance being attained ($P < 0.1$). Blood taken from trained rats after an exhaustive bout of exercise had the lowest testosterone concentration and the hormone level was different ($P < 0.05$) than that from animals that had exercised for only 1 hr. Testosterone concentration also declined in the period 6 hr after the end of the last regular exercise bout but returned to the postexercise level within 24 hr.

The concentration of androstenedione in female rats (Table III) was similar to that for males while testosterone levels were much lower, as was expected. There did not appear to be an alteration in the level of these hormones as a result of training. The effects of acute exercise and exhaustion were not investigated in female rats.

Discussion. Since plasma testosterone concentrations undergo diurnal variations (16), we sacrificed all animals at the same time (from 8 to 10 AM). Plasma testosterone has been shown to be rather constant during this time of the day (16). The trained-rested 6-hr group of rats were run on the treadmill from 2 to 3 AM and then sacrificed at 9 AM. There was a slight rise in testosterone after an hour of exercise (trained-rested 24 hr vs. trained-

TABLE II. EFFECT OF EXERCISE AND ENDURANCE TRAINING ON PLASMA LEVELS OF ANDROSTENEDIONE AND TESTOSTERONE IN MALE RATS.

Group	Androstenedione (pg/ml)	Testosterone (ng/ml)
Untrained-rested	484 ± 118 (9)[a]	2.3 ± 0.6 (8)
Trained-combined groups	311 ± 42 (29)	1.3 ± 0.2 (31)[b]
Trained-exercised	442 ± 62 (7)	1.9 ± 0.3 (9)
Trained-rested 6 hr	234 ± 87 (6)	0.9 ± 0.3 (6)[b]
Trained-rested 24 h	321 ± 97 (9)	1.6 ± 0.4 (9)
Trained-exhausted	224 ± 58 (7)	0.4 ± 0.1 (7)[c]

[a] All values are mean ± SEM with the number of observations in parenthesis.

[b] Significantly different ($P < 0.05$) than untrained-rested.

[c] Significantly different ($P < 0.05$) than untrained-rested and trained-exercised.

exercised, Table II) which is consistent with reports of others (6). This rise may be due to increased release from the testis or it may be due to a drop in hepatic blood flow during exercise (17). Testosterone declined to less than 50% of the preexercise value ($P < 0.05$) 6 hr after exercise in the trained rats. This decrease could possibly be explained by the existence of a mechanism for storage of intratesticular androgens (18) which may be modified by hemodynamic conditions brought on by exercise. Indeed, this possibility has also been suggested by others (8). Alternatively, this decrease could be a result of enhanced intrahepatic metabolism of androgens or a decrease in the rate of synthesis of the steroid.

The most pronounced change in testosterone was found in rats immediately after an exhaustive exercise bout (75% decrease). This change in plasma testosterone may be related to other hormonal changes that occur in these animals. Doerr and Pirke (19) reported that cortisol causes a suppression of plasma testosterone in normal adult males. Thus, the decrease in testosterone in the exhausted rats may be the result of elevated glucorticoids since we found exhaustive exercise to increase corticosterone two to threefold in rats (20).

The anabolic effect of androgens on muscle tissue is well established (4, 5) and the increased excretion of urea that we observed in the castrated rats would seem to be consistent with the absence of a protein anabolic factor in the castrated animals. Because of the previous reports of lowered testosterone after exercise (6–8) we had hypothesized that decreased androgen concentrations might be responsible for the increased urea excretion that we had observed in trained rats. Urea excretion was not changed by training in castrated male rats or in female rats, which seems to support the hypothesis that an alteration in plasma androgens secreted by the testis may be responsible for the increased urea excretion that we observed in trained rats.

TABLE III. EFFECT OF ENDURANCE TRAINING ON ANDROSTENEDIONE AND TESTOSTERONE LEVELS IN FEMALE RATS.

	Hormone level (pg/ml plasma)	
Group	Androstenedione	Testosterone
Untrained-rested	490 ± 118 (5)[a]	63.6 ± 13.7 (5)
Trained-rested	401 ± 35 (6)	60.2 ± 4.2 (8)

[a] All values are mean ± SEM with the number of observations in parenthesis.

Summary. The effects of endurance training on urea excretion and plasma androgen concentrations were investigated in male, female, and castrated male rats. Training increased urea excretion in male rats but not in female rats. Castration of male rats increased urea excretion to levels like those observed in trained rats and training did not further increase urea excretion. The level of testosterone in trained rats was lower than that of untrained rats. In trained rats there was a slight ($P > 0.05$) increase in testosterone immediately after exercise but the concentration of testosterone was significantly ($P < 0.05$) lowered 6 hr after the exercise bout. Plasma testosterone was also significantly lowered after an exhaustive exercise run. These results suggest that an alteration in plasma androgen levels may be responsible for the increased urea excretion that we observed in trained rats.

This work was supported by National Institutes of Health Grant No. AM 19116.

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Received March 6, 1978. P.S.E.B.M. 1978, Vol. 158.