Regulation of Human Gonadotropins. VIII. Suppression of Serum LH and FSH in Adult Males following Exogenous Testosterone Administration

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ABSTRACT. Serum LH and FSH levels were measured by radioimmunoassay after exogenous testosterone was administered to four groups of adult males in dosages ranging from 1–25 mg per day. Serum testosterone concentrations, determined by radioimmunoassay, were elevated subsequent to the administration of the higher dosages. Dose-related depression of both LH and FSH was observed subsequent to administration of 5 or more mg of testosterone per day; the smallest dosage (1 mg) was not associated with any consistent changes in LH or FSH. The rate and extent of decline of the two gonadotropins differed. Serum LH values tended to drop and recover more rapidly than FSH following testosterone administration. These results indicate that testosterone suppresses serum levels of both LH and FSH in the human adult male. There was no evidence of stimulation of gonadotropin release at these doses. (*J Clin Endocrinol Metab* **35**: 636, 1972)

ALTHOUGH the role of testosterone in feedback inhibition of pituitary luteinizing hormone (LH, ICSH) in the adult male of various species is well established (1–8), reports on the influence of testosterone on serum FSH are varied. Thus, following testosterone administration to men, serum FSH levels were not altered significantly, although LH values fell (2–4). In contrast, pharmacologic doses of testosterone were found to suppress plasma FSH in castrate rats (8–10).

The present study, using radioimmunoassay techniques to measure LH, FSH and testosterone, was designed to determine the effects of exogenous testosterone upon gonadotropin concentrations in sera from healthy adult males. Since exogenous testosterone has a dose related suppressive effect upon endogenous testosterone production rates (11), serum testosterone levels were measured to determine the resultant serum concentrations achieved.

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Materials and Methods

Samples of venous blood were obtained from 22 healthy adult males age 19–26 before and after the im injection of testosterone in aqueous suspension (Oreton®). Blood samples were obtained 3 times a day during hormone administration and once or twice a day following administration.

Four groups of five individuals each received a different dosage of testosterone. The dosages, given for three days at 8 AM were 1.0, 5.0, 12.5 and 25.0 mg per day. One individual received 50.0 mg daily for three days.

Serum was separated from whole blood by centrifugation after clotting. Samples were stored at —20 C and assayed in duplicate. All measurements of LH, FSH and testosterone from a given subject were measured in the same assay in duplicate.

LH determinations were performed using modifications of the double antibody radioimmunoassay previously described (12,13). FSH determinations were performed as described previously (14) except that the antiserum was diluted in 0.25% normal rabbit serum. Results

were expressed as equivalents of the Second International Reference Preparation of Human Menopausal Gonadotropin (2nd IRP-HMG) in milli-International Units (mIU) per ml of serum. One mg of LER-907 is equivalent to 232 IU of 2nd IRP-HMG in the LH assay, and to 46 IU of 2nd IRP-HMG in the FSH assay.

The lower limit of sensitivity, calculated as the hormone concentration which corresponded to the lower 95% confidence limit of the non-hormone-containing control tubes run in each assay, averaged 2.1 mIU 2nd IRP-HMG/ml in the LH assays and 2.3 mIU 2nd IRP-HMG/ml in the FSH assays.

In one individual, the values of LH dropped below the limit of detectability of the assay as noted in the Results.

Serum testosterone was assayed by radioimmunoassay in control samples obtained prior to treatment, in samples obtained 24 hr after the second daily dose of testosterone, and in samples obtained 5 or 6 days after the third or final dose.

The method for quantitating serum testosterone by radioimmunoassay employed an antiserum to testosterone-3-Bovine Serum Albumin (BSA), testosterone-3-TME-^{125}I for quantitation of the degree of binding, and ^{3}H-testosterone for correcting procedural losses incurred after purification by a single thin-layer chromatographic step. 1,2-^{3}H-testosterone (4000 cpm, 37.3 Ci/mmole, New England Nuclear Corp.), purified by chromatography on silica gel impregnated glass fiber sheets (ITLC, Gelman Instrument Co., Ann Arbor, Mich.) in cyclohexane, ethyl acetate (8:1), was added to 0.5 ml plasma and incubated in 45 ml glass stoppered conical centrifuge tubes at 37 C for 30 min, cooled to room temperature and extracted with 10 volumes of glass redistilled ethyl ether (Burdick Laboratory, Muskegon, Mich.). The tubes were then immersed in a dry-ice acetone bath to freeze the aqueous phase of the serum, and the organic phase was transferred to a second tube and dried under nitrogen. The sample was then chromatographed on ITLC sheets, which had been prewashed with chloroform, using cyclohexane:ethyl acetate (8:1) as developing solvent. The area of the chromatogram with mobility similar to radioactive testosterone run in parallel on the same chromatogram was eluted with 4 ml of chloroform into disposable culture tubes. The eluates were then dried under nitrogen, and 1 ml of 0.1% gelatin (Sigma) in 0.14M NaCl buffered at pH 7.0 with 0.01M sodium phosphate (0.1% gel-PBS) was added to each tube. A 0.1-ml aliquot was transferred to a counting vial to assess procedural losses. The remainder was used for the radioimmunoassay.

Radioimmunoassay. Five-tenths ml of buffer (0.1% gel-PBS) containing varying amounts of standard was allowed to incubate with 0.2 ml of 1:6000 dilution anti-testosterone-3-BSA-serum (15) and 0.1 ml testosterone-3-TME^{125}I (15) at 4 C for 4 hr. Two-tenths ml of anti-rabbit gamma globulin was added, and the incubation was allowed to proceed for another 12 hr. Three ml PBS was then added, and the tubes were centrifuged at 4 C for 30 min. The supernatant was decanted, and the precipitate was counted. The radioactivity in the precipitate from assay tubes which contained radioactive hormone and antibody, but which contained no unlabeled hormone (*i.e.*, buffer control tubes) was assigned a value of 100%. A standard curve was constructed at the beginning and at the end of each assay, using unlabeled hormone at 11 dose levels, to give a range from 10–10,000 pg. The extracts of serum were assayed in duplicate at a minimum of two dose levels. The results were analyzed with a computer program which uses a logit-response, log dose transformation to obtain a linear inhibition curve (16). The amount of testosterone found in each assay tube was corrected for procedural losses and the mass of testosterone-^{3}H added initially.

Recovery. 2.5, 5.0 and 10 ng of testosterone were added to a pool of male and a pool of female plasma. The slopes of the regression lines for the male and female plasma pools were 0.97 and 0.90 with intercepts (y) of 0.075 and 0.54 ng per tube respectively. These intercepts were not significantly different from 0. The coefficient of correlation for each regression line was 1.00.

Precision. Intra-assay precision was examined by measuring the testosterone concentration of replicates from a pool of male serum. The mean ±SD testosterone concentration from 10 sam-

TABLE 1. Effect of three daily im injections of an aqueous suspension of testosterone on serum concentrations of LH, FSH and testosterone

Dosage testosterone (mg/day)	1	5	12.5	25
Number of subjects with suppressed LH and FSH	1/5	5/5	5/5	5/5
LH				
Control range (mIU/ml)	6.8–11.7	5.8–11.4	5.3–13.5	4.9–9.8
Maximal depression (mIU/ml) range as % of control	5.6 33%	2.5–8.9 30–56%	1.7–7.4 45–100%	1.8–4.5 64–72%
Hr initial/maximal depression noted	48/48	55/82	7/88	7/64
FSH				
Control range (mIU/ml)	2.3–9.5	3.3–5.1	2.5–11.3	6.3–11.3
Maximal depression (mIU/ml) range as % of control	3.5 25%	1.8–3.2 28–51%	1.5–3.4 28–75%	1.9–4.1 39–75%
Hr initial/maximal depression noted	39/65	31/63	15/108	7/120
Testosterone				
Control range (ng/ml)	7.3–12.2	5.5–9.2	3.4–9.1	6.7–9.4
Percent change 48 hr after first injection mean and (range in %)	+12.1 (−2–29)	+8.4 (−47–52)	+143 (46–290)	+99 (50–189)
Percent change 5–6 days after third injection mean and (range)	+10.7 (−26–28)	+1.0 (−23–27)	+5.3 (−28–40)	−12.8 (−34–7)

ples was 7.33 ± 0.27 ng/ml. Inter-assay precision was examined by analysis of the testosterone concentration from a pool of male serum run on separate days in each assay. In six runs, the mean testosterone concentration was 7.63 ± 0.16 (SD) ng/ml.

Specificity. The specificity of the antibody in this system has been described by Ismail *et al.* (17). Out of 19 steroids tested, only dihydrotestosterone and 5α-androstane-3α,17β-diol, with reactivities of 0.77 and 0.26 that of testosterone, have been found to cross-react with testosterone. These steroids can be separated on ITLC (18). Thus, the chromatographic step removes the major interfering steroid, dihydrotestosterone, from testosterone.

Two "blanks" were routinely included in each assay. The mean testosterone concentration found in ten 0.5-ml samples of ether-extracted 0.1% gelatin examined on separate days was 15 ± 7 pg.

Results

No discernible changes in gonadotropin levels were observed following intramuscular administration of one mg of testosterone per day for 3 days. However, im injections of 5, 12.5, 25, and 50 mg were followed by decreased levels of LH and FSH in proportion to the amount and duration of hormone administered (Fig. 1 and Table 1).

The quantity of hormone administered was reflected by proportionate increases of serum testosterone. The mean control serum level of testosterone among the 13 individuals (18 determinations) measured was 8.3 ng/ml. The control levels of serum testosterone as well as levels 24 hr after the second daily dose of exogenous testosterone and 5 or 6 days after the final or third dose are presented in Table 1. Although serum testosterone concentrations were significantly higher 48 hr following the lowest dose (1 mg), significant elevation was not observed following a five-fold higher dosage. Determinations of testosterone at 8-hr intervals following the third daily injection of 5 mg showed no consistent pattern with serum concentrations greater than control levels occurring only at 8 or 16 hr after the injection, at 8, 16 and 24 hr, but never only at 24 hr. Twenty-four hr after 2 daily doses

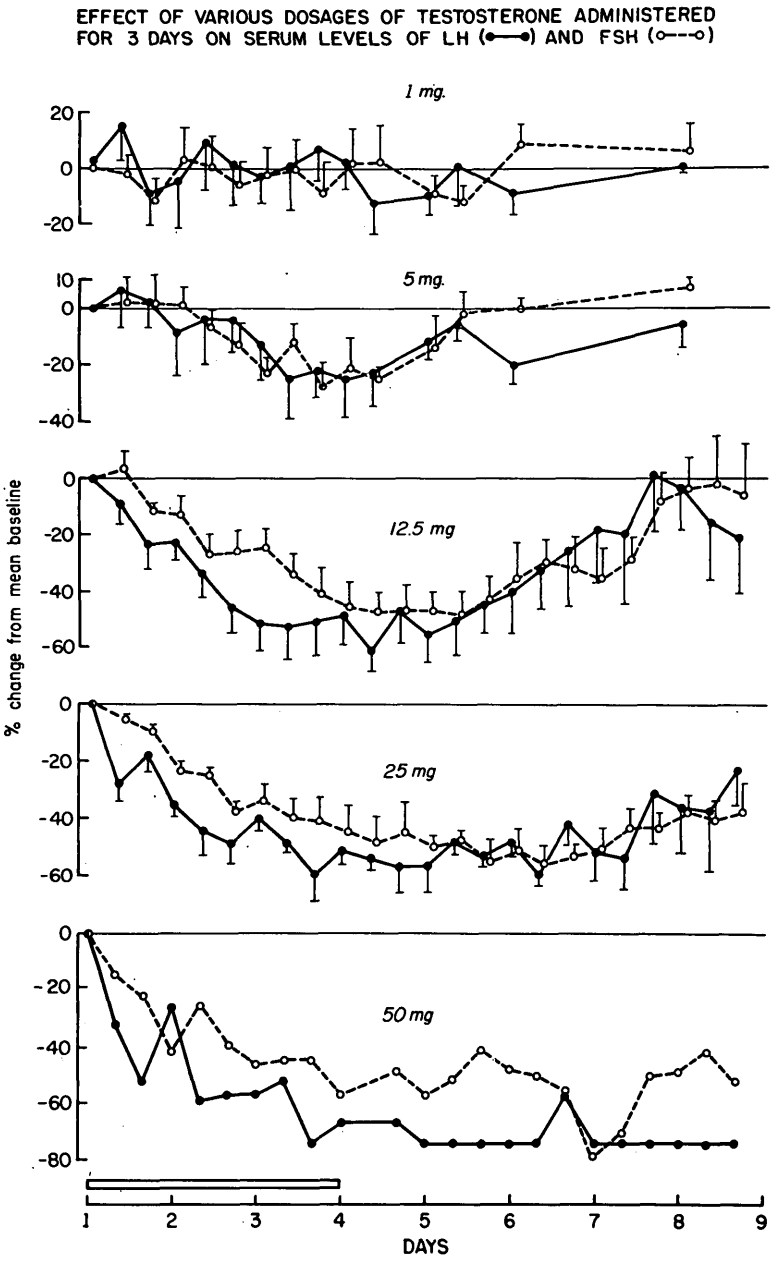

FIG. 1. Percentage change of serum concentrations of LH and FSH in groups of adult males treated for 3 days. Each of 5 groups received a different dosage of testosterone in aqueous suspension. Five individuals received each of 1.0-, 5.0-, 12.5- and 25.0-mg doses. One individual received 50 mg. Bars indicate SEM calculated from the percent change measured for each individual.

of 12.5 or 25 mg im testosterone, serum levels were considerably higher than before drug administration and higher than following administration of either the one or the 5 mg daily doses. These levels were consistently elevated above the control means at 8, 16 and 24 hr after the third injection. A subject who received 25 mg daily with a control value of 9.4 ng/ml showed a typical response with 27.0, 20.0 and 20.4 ng/ml at the respective times.

The mean concentration of LH in control samples from the 21 subjects was 8.1 mIU/ml (range 4.9–11.7) and the mean concentration of FSH in control samples was 5.6 mIU/ml (range 2.3–11.3). The mean values of LH and FSH after administration of one mg of testosterone for three days to five

normal adult males did not show a significant departure from mean control values (Fig. 1). However, one of these individuals had levels consistently below the range of his 3 control levels beginning 16 hr after the first hormone injection was given and continuing until the second day after hormone administration had been completed. The mean values of LH for the five males who received 5 mg daily for three days dropped below control means for 2 days. Similar changes in serum FSH were observed in four of these same five individuals (Table 1, Fig. 1).

The mean and individual concentrations of LH and FSH for males receiving 12.5 and 25.0 mg of testosterone (Fig. 1) were below control values by 7 to 15 hr after the first im injection of hormone and continued to decline until the third day of hormone injection (Table 1). The LH levels in one individual receiving 12.5 mg were depressed below the confidence limits of the assay from 40 hr after the first hormone injection until the fourth day after the final dose was administered. No differences in the amount of depression of LH and FSH could be observed for subjects receiving these two dosages until two days after the last day of administration. At this time, the mean levels of the individuals receiving 12.5 mg per day began rising toward normal. Six days after the final hormone injection, at the completion of the serum collection schedule for the individuals receiving 25 mg of testosterone, mean serum LH values remained below control levels although a rising trend was noted. In the five males receiving 12.5 mg daily for three days (Fig. 1), mean FSH levels dropped until 24 hr after the final dose of testosterone was given, with a rise beginning during the second day after receiving hormone. All five of the individuals, when evaluated separately, responded in a similar manner. Mean levels for those receiving 25 mg daily for three days (Fig. 1) declined until one day after administration was complete and remained well below control means at the end of scheduled serum collections five days after receiving the last injection of testosterone.

Discussion

The administration of the 12.5 and 25.0 mg of testosterone daily was reflected by markedly higher levels of serum testosterone. After the third dose of 25.0 mg, this level reached a peak within 16 hr after im administration and remained above control levels for at least 24 hr after the higher doses were administered. The rate of decline and the magnitude and duration of depression below mean control levels in serum concentrations of both LH and FSH were related to the amount and duration of drug administration. This indicates that testosterone suppresses pituitary release of LH and FSH in a dose related fashion. The possibility that suppression of LH and FSH might so inhibit endogenous production of testosterone (11) that net serum concentration of testosterone would fall appears to have been eliminated. The three larger dosage levels are greater than the daily endogenous production rate in normal adult males (11).

When administered in doses of 12.5 mg per day and above, testosterone suppressed adult male LH levels to prepubertal levels (2.80 ± 0.15) (13) in 11 out of 13 individuals. FSH levels in 17 of 18 individuals receiving at least 5 mg testosterone per day were at or below prepubertal levels (4.59 ± 0.17) (13).

At no time were levels of LH and FSH higher after administration of testosterone than levels in the same individual before treatment. Thus, no evidence was obtained to indicate that testosterone in this dose range enhances release of LH or FSH. Moreover, except after 1 mg for 3 days, a suppressive influence of testosterone was observed regardless of dosage or duration of treatment.

A similar trend for serum concentrations of LH and FSH was observed, although at

none of the testosterone levels was there an identical response of LH and FSH. LH tended to drop more rapidly and return toward control mean levels before FSH. The percentage decline of LH after a given dosage of testosterone tended to be greater than the decline of FSH.

The present report shows a consistent depression of LH after 4 dose levels of testosterone in aqueous suspension. This confirms previous reports of LH suppression after administration of androgens (1–4). Serum concentrations determined by radioimmunoassay were consistently depressed in human males receiving testosterone propionate (1,2,4) as well as fluoxymesterone (3).

In our study, FSH levels were depressed below controls at all dosages except 1 mg/day. This consistent influence of testosterone in aqueous suspension differs with previous reports of effects of testosterone propionate (1,2,4) and fluoxymesterone (3). The latter showed either no influence or no consistent effect upon FSH levels. The present study indicates that FSH, as well as LH, decreases as testosterone levels increase. Conversion of androstenedione and testosterone to estradiol has recently been reported to occur in the anterior hypothalamus, limbic system and pituitary gland (19,20). Aromatization of testosterone may have occurred in individuals in the current study and if so, may have partially mediated the suppression of LH and FSH. This may explain the discrepancy between the present study and the study using fluoxymesterone (3) where conversion to estradiol is unlikely. Gay and Dever (8) also found similar responses of FSH and LH in orchidectomized male rats receiving testosterone benzoate and estradiol benzoate.

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