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Research Article

EFFECTS OF METHYL TESTOSTERONE ON THYROID FUNCTION, THYROXINE METABOLISM, AND THYROXINE-BINDING PROTEIN

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Studies on the effect of androgenic steroids on thyroid function are difficult to evaluate because of a number of apparent discrepancies. Money, Kirschner, Kraintz, Merrill, and Rawson (1), using castrate rats on a low iodine diet, found that testosterone propionate had no influence on thyroid weight or on thyroid weight: body weight ratio, but did increase the radioiodine uptake. Kochakian and Evans (2) recently reported that this form of testosterone failed to influence the uptake or release of radioiodine by the thyroid of the castrate rat. Burris, Bogart, and Krueger (3), using heifers and young steers, found that testosterone propionate produced an increased gain in weight; furthermore, the thyroids of the treated animals weighed more, contained less colloid, and exhibited an increase in thyroid cell height. Voitkevich (4) is cited as stating that the goiters produced by propylthiouracil in guinea pigs and cocks are very considerably decreased by simultaneously administered methyl testosterone, and that propylthiouracil goiters are larger in castrate than in normal cocks.

In man, Kinsell, Hertz, and Reifenstein (5) demonstrated that tesosterone propionate reversed the negative nitrogen balance and creatinuria of two hyperthyroid patients, although without having clearcut effect on their hypermetabolism. Methyl tesosterone, on the other hand, induced a less sustained positive nitrogen balance, increased the creatinuria, and appeared to exacerbate the hyperthyroidism. Recently, Keitel and Sherer (6) observed that the administration of methyl testosterone to dwarfed children and to adults with metastatic breast carcinoma, produced decreases in the serum protein-bound iodine and in radioiodine uptake. The present study was undertaken to investigate the mechanism of these effects. Studies in the same subjects of thyroid radioiodine clearance, of thyroxine-binding protein (TBP), and of the rate of degradation of thyroxine, might serve to differentiate direct effects on the thyroid from extrathyroidal mechanisms.

METHODS

The subjects were three adult males and one prepubescent girl. Pertinent data are included in Table I. The subjects, with the exception of B. B., were hospitalized during the study and were on reasonably constant activity and diet. After a suitable control period, methyl testosterone was given in a dosage of 100 mg. daily (25 mg. four times daily *per os*) for seven weeks. The times of the studies in relation to duration of treatment are listed in the tables. *In vivo* counting was done with a collimated scintillation counter having a one-eighth inch lead window and placed at 40 cm. from the suprasternal notch. The collimation was such that the circle seen at 40 cm. which included the 50 per cent maximal counting rate was 12 cm. in diameter. *In vitro* counting of plasma and urine samples was done in a well-type scintillation counter.

Radiothyroxine disappearance rates, organic iodine pools, and iodine degradation were measured, using the slope and intercept method of calculation (7). I^{131}-labeled L-thyroxine [2] (5 to 10 micrograms, 50 to 100 microcuries per dose) was given intravenously, and samples of serum were assayed daily for two weeks thereafter. Thyroidal reaccumulation of iodide was blocked with methimazole, 80 mg. daily.

Basal metabolism was measured weekly. Protein-bound iodine (PBI) in serum was measured three times in each period of testing, using the modified method of Barker and Humphrey (8) or a modification of the Zak, Willard, Myers, and Boyle (9) procedure. Thyroxine-binding protein (TBP) of the serum was assayed by the method described earlier (10) and calculation of the free thyroxine level was made using equations evaluated

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[2] Obtained from Abbott Laboratories, assayed for purity by chromatography and repurified, if necessary, to a purity of at least 90 per cent.

TABLE I

Clinical data in subjects

Patient	Age	Sex	Diagnosis	Thyroid status
B. B.	40	M	Diabetes mellitus	Euthyroid
C. H.	12	F	Primary mental deficiency Epilepsy	Euthyroid
L. W.	16	M	Primary mental deficiency	Euthyroid
D. W.	31	M	Primary mental deficiency	Euthyroid

elsewhere (11). Iodide clearances were obtained from intravenous radioiodide tracers by measuring the radioactivity over the neck, in heparinized plasma, and in urine at numerous intervals for six hours (12). To determine the fraction of the neck counts due to extrathyroidal sources, a separate radioiodide tracer was given to each of three of the subjects at the conclusion of the study and after his gland had been "blocked" with 2 grams of sodium iodide. Two grams of sodium iodide is a dose sufficient to dilute the I^{131} so that if as much as 2 or 3 mg. of iodine is accumulated by the thyroid, only approximately 0.1 per cent of the I^{131} will be accumulated. From the curves of neck and plasma radioactivity so obtained, corrections for extrathyroidal activity corresponding to the plasma levels of the initial, unblocked tracer were found. Extrathyroidal neck radioactivity was then calculated on the basis of the level of I^{131} in plasma. Clearances were calculated in the usual manner from two to six hours after the dose of radioiodide.

RESULTS

Clinical changes

None of the patients showed any symptoms or signs of hypo- or hyperthyroidism, and none had any toxic symptoms from the methyl testoterone or Tapazole®. The three patients weighed repeatedly gained 1 to 2 Kg. each during the treatment period, and lost the weight within two weeks after discontinuing the drug. Basal metabolism rates proved to be uninterpretably variable. Serum cholesterol values rose in all patients during treatment and returned toward control levels after the medication was discontinued (Figure 1). In the case of D. W., values had not yet returned to pretreatment levels four weeks after treatment had been stopped.

Iodide metabolism

Thyroidal uptake of radioiodide, thyroidal iodide clearance, and renal iodide clearance were measured before methyl testosterone and after 47 to 51 days on the drug (Table II). All values were within the normal range. Although the data for each of the subjects (except L. W. on testosterone) were internally compatible in that the 24 hour uptake could be reasonably predicted from the corresponding thyroidal and renal clearances, the differences observed between control and treatment values revealed no consistent effect of the drug.

Thyroxine metabolism

The changes in hormonal iodine levels in serum are shown in Table III. There was a small but consistent reduction in protein-bound iodine after 28 to 29 days of treatment with testosterone. Although one value was in the hypothyroid range, in general the changes were not so marked as those found by Keitel and Sherer (6). The mean pretreatment value was 4.9 micrograms per cent; dur-

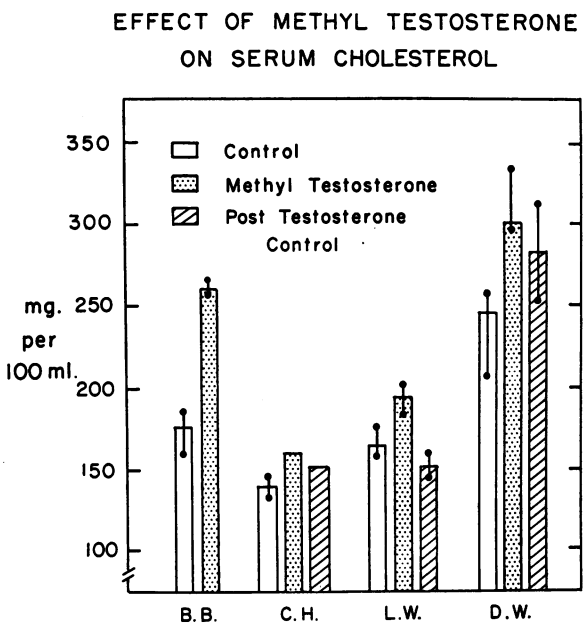

FIG. 1. EFFECT OF METHYL TESTOSTERONE IN THE SERUM CHOLESTEROL

The height of the bars represents the mean level of cholesterol for the period; the vertical lines represent the range of values for the period. No post-treatment values were obtained on Patient B. B.

TABLE II

The influence of methyl testosterone on thyroidal uptake and thyroidal and renal clearance of iodide

	Patient B. B.		Patient C. H.		Patient L. W.		Patient D. W.	
	Control	Methyl testosterone (51)*	Control	Methyl testosterone (47)	Control	Methyl testosterone (48)	Control	Methyl testosterone (48)
Radioiodide uptake, %								
3 hours	29	16	20	10	12	12	15	16
6 hours	36	21	18	12	14	14	17	22
24 hours			24	13	19	18	27	33
Thyroidal iodide clearance, ml./min./1.73 M.²	39	20†	20	20	9	13	8	18
Renal iodide clearance, ml./min./1.73 M.²	43	35	56	100	43	36	27	34

* The numbers in parentheses indicate days on testosterone at time of study.
† Since the neck: plasma radioiodine ratio could not be determined in B. B. after iodide "block" (see text), the average of the values obtained in the other subjects was employed.

ing treatment it was 3.7 micrograms per cent. For consistency in presentation of the data in Table III, protein-bound iodine has been converted to molar concentration of thyroxine.

The thyroxine-binding capacity of the thyroxine-binding protein (TBP) was normal in all patients before treatment (mean equals 2.4×10^{-7} M).[8] During treatment, the concentration of TBP sites fell to abnormally low levels (mean equals 0.98×10^{-7} M). Because of the relatively greater decrease in thyroxine-binding protein than in PBI the calculated level of free thyroxine rose, although the small change in B. B. and C. H. is of questionable significance (*cf.* Discussion). The mean control free thyroxine was 7.0×10^{-11} M; the mean during treatment was 10.9×10^{-11} M. In all but C. H., the value during treatment was above the range seen in a series of normal adults (11, 13). No qualitative alteration in the distribution of thyroxine among the serum proteins, as determined

[8] This value, expressed as molar concentration, is presumably equivalent to the concentration of TBP sites for thyroxine. If the number and availability of sites per molecule of TBP does not vary, then this value would be proportional to the concentration of TBP itself. Although there is no evidence on this point, these terms will be used interchangeably in this discussion.

TABLE III

The influence of methyl testosterone on iodine metabolism: Protein-bound iodine, thyroxine-binding protein, and free thyroxine levels in serum

	Patient B. B.		Patient C. H.		Patient L. W.		Patient D. W.	
	Control	Methyl testosterone (29)*	Control	Methyl testosterone (28)	Control	Methyl testosterone (29)	Control	Methyl testosterone (29)
Endogenous thyroxine,† $\times 10^{-7}$ M‡	1.4	0.90	0.77	0.44	0.89	0.71	0.78	0.71
TBP sites for thyroxine, $\times 10^{-7}$ M	3.0	1.5	1.7	0.66	2.1	0.67	2.6	1.1
Free thyroxine, $\times 10^{-11}$ M	8.9	10	7.3	8.2	7.1	16	4.6	9.9

* In parentheses is the number of days on testosterone at time of study.
† Calculated from the protein-bound iodine on the assumption that it consists entirely of thyroxine.
‡ Conversion factor: Protein-bound iodine (micrograms per cent) equals $5.03 \times 10^{7} \times$ molar concentration thyroxine.

by electrophoresis at pH 8.6, was induced by the testosterone.

The fractional rate of thyroxine disappearance was increased in all patients during treatment, from a mean of 13 per cent per day to 19 per cent per day after 23 to 27 days of treatment (Table IV). The extrathyroidal organic iodine pool was unchanged in three patients, reduced in one (B. B.). The higher fractional degradation resulted in an increased absolute degradation of hormonal iodine in all patients, although the differences were small and questionably significant in two cases. The mean pretreatment was 53 micrograms iodine per day per 1.73 M.^{2}; the mean during treatment was 68. Following cessation of testosterone treatment, the rate of thyroxine disappearance fell and the amount of iodine degraded per day decreased despite an apparent rise in the extrathyroidal organic iodine pool. The significance of the pool change is uncertain. Although not given in the table, the volume of distribution of thyroxine increased during treatment in all cases and fell after cessation of testosterone. These changes are consistent with known effects of testosterone on fluid retention.

Data for two patients who were studied at intervals after cessation of methyl tesosterone are presented in Figure 2. The minimal concentrations of TBP sites for thyroxine were found in the blood obtained seven days after treatment was stopped. Subsequently there was a slow return toward normal levels, although the concentration of TBP sites was still subnormal at 35 days. The levels of free thyroxine rose during treatment and fell after discontinuance of the methyl testosterone. There appeared to be a second rise some 35 days after the drug was stopped.

DISCUSSION

It is clear from this study that methyl testosterone causes a profound decrease in the thyroxine-binding capacity of TBP in serum, presumably as a result of a decrease in the concentration of this protein. It seems likely that this is a primary effect of the hormone unrelated to any direct effects which testosterone might have on thyroid function and thyroxine metabolism. It is attractive, therefore, to consider the possibility that changes in thyroid function may be the result of this primary effect. According to this theory, one would expect the fall in TBP to result in an increased level of free thyroxine. The elevated free thyroxine might then result in an increased availability of thyroxine for metabolic degradation. Either as a direct effect of the ele-

TABLE IV

The influence of methyl testosterone on iodine metabolism: Radiothyroxine disappearance rate, organic iodine pool, and organic iodine degradation rate

Patient	Status	PBI* (μg./100 ml. serum)	Thyroxine disappearance rate (% per day)	Extra thyroidal organic iodine pool (μg. iodine/1.73 M.^{2})	Organic iodine degradation (μg. iodine degraded/day/1.73 M.^{2})
B. B.	Control	7.2	11	590	65
	Methyl testosterone (26)†	4.6	16	450	70
C. H.	Control	3.8	14	390	55
	Methyl testosterone (27)	2.2	22	390	85
	Control (159)	4.0	13	490	64
L. W.	Control	4.5	15	340	50
	Methyl testosterone (23)	3.6	18	300	54
	Control (30)	4.6	12	380	45
D. W.	Control	4.0	13	330	43
	Methyl testosterone (23)	3.6	19	330	61
	Control (130)	5.6	9.9	500	50

* Protein-bound iodine.

† The number in parentheses indicates days on testosterone, or off testosterone, at time of study.

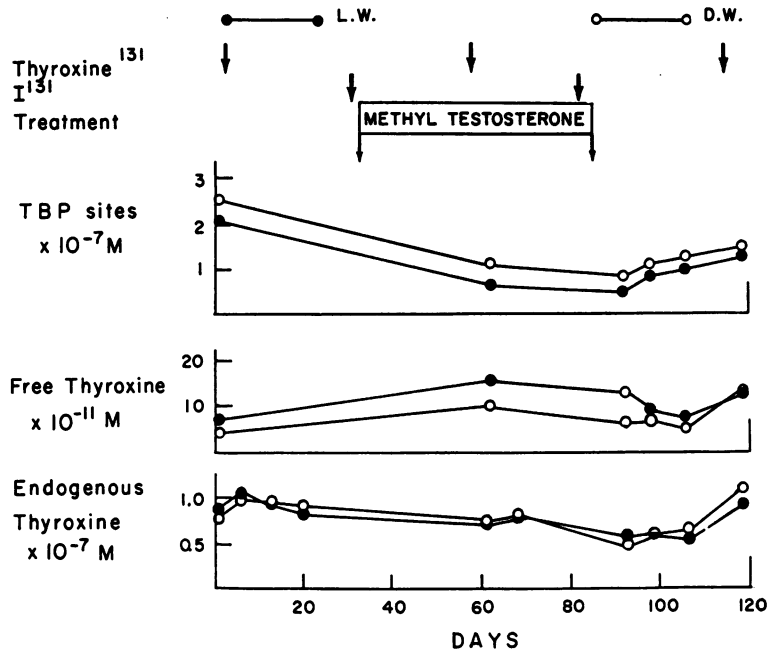

FIG. 2. EFFECT OF METHYL TESTOSTERONE ON THYROXINE-BINDING PROTEIN, FREE THYROXINE, AND PROTEIN-BOUND IODINE

The arrows after thyroxine^{131} and I^{131} represent, respectively, the times at which the labeled thyroxine was injected for the turnover studies and the time the iodide tracer studies were done.

vated free thyroxine or as a result of the increased thyroxine metabolism, pituitary secretion of thyrotropic hormone might then be suppressed, resulting in a decrease in the turnover and uptake of iodine by the thyroid gland. The combined effect of increased thyroxine degradation and decreased thyroxine secretion would lead to a fall in serum thyroxine and, ultimately, a new steady state in which the serum PBI is depressed, the free thyroxine level is normal, and thyroid iodide clearance, thyroid hormone secretion, and thyroxine degradation are normal.

In keeping with such a theory, this study revealed an increase in the free thyroxine level in serum, an increase in thyroxine degradation, and a fall in serum PBI in some or all of the subjects. It would appear that a new steady state had not yet been reached after four weeks of testosterone treatment, when TBP and thyroxine disappearance were determined, although after seven weeks it might have been (*vide infra*).

Several objections to the above interpretation should be considered.

1) The method of calculation of extrathyroidal organic iodine (by intercept), of degradation (by final slope), and of organic iodine degradation rate is at best approximate. Only if there are no changes in diffusion rates is the use of these values valid for comparison, even in the same patient.

2) The errors and assumptions involved in calculation of free thyroxine have been detailed previously and still obtain (11, 14). A further problem in the present study is the fact that with such low values for thyroxine-binding capacity of TBP, the accuracy of the free thyroxine value is strongly dependent on the precision with which the PBI has been measured. Thus, at low PBI concentrations, variation within the limits of error of the PBI method will produce large changes in the calculated free thyroxine. This may account for the erratic results of this calculation (Figure 2).

3) All the calculations assume a steady state situation. As indicated above, this probably did not obtain during the study. The use of methimazole during the thyroxine turnover studies introduces a further complicating factor since it

places the thyroid in an unsteady state. It secretes iodine but cannot bind it. In normal subjects there is a large thyroid iodine pool, so it may be presumed that a few weeks exposure to methimazole will not produce major changes in the thyroid.

4) The changes in thyroidal iodide clearance seen were not consistent and hence are difficult to interpret. It should be noted that all of these clearances and the uptake values were within the normal range (12, 15). Biological variation, alterations in iodine in diet, changes in secretion of adrenal steroids, goiterogens in food, and so forth, all contribute toward the difficulty of obtaining exactly reproducible data over a period of months for thyroid iodide clearance. A new steady state of thyroid activity may have been reached after 47 to 51 days of treatment with methyl testosterone. It was, unfortunately, not feasible to perform iodide tracers and thyroxine disappearance studies simultaneously. The serial determination of thyroidal iodide clearance in a large number of subjects before and during the administration of methyl testosterone would be desirable and probably necessary to determine precisely changes in this function during the unsteady state.

Other mechanisms for the effects noted during methyl testosterone administration could be mentioned. A direct effect on the pituitary to suppress its output of thyroid stimulating hormone is possible. The PBI would fall (as observed) but a marked fall in thyroid iodide clearance would also be expected (this was not observed). Furthermore, another mechanism to account for the increase in rate of degradation of thyroxine and fall in TBP would still be necessary. A direct effect on the thyroid to decrease secretion of thyroxine could explain the fall in PBI. If this mechanism were similar to the effect of iodine, there might be little change in thyroid iodide clearance. Nevertheless, the increased degradation of thyroxine and fall in TBP would still be unexplained. An effect similar to that produced by dinitrophenol, which increases the rate of metabolism of thyroxine and also suppresses the pituitary, is possible (16). Usually a marked fall in thyroid iodide clearance is seen, however, and again the fall in TBP is not explained.

Therefore, although the theory presented, which proposes that the effects of methyl testosterone result from a primary alteration in TBP, is not proven, it seems the most likely working hypothesis at present.

It is of considerable interest that estrogenic hormones and pregnancy, which have been shown to produce a rise in PBI in serum, have an effect on thyroxine-binding of TBP which is opposite to that found with methyl testosterone (11, 14, 17, 18). Detailed studies of other aspects of thyroid function during estrogen treatment in humans have not been reported.

The change in serum cholesterol cannot be directly inferred from this hypothesis, since, if anything, one would expect a fall in the level to correspond with the elevated free thyroxine level and utilization. An increase in serum cholesterol in male subjects with coronary artery disease and hypercholesterolemia when they were given methyl testosterone has been noted previously (19). No data are available to suggest whether this effect is upon cholesterol synthesis or excretion, or whether it is direct or indirect.

It is possible that the exacerbation of hyperthyroidism observed by Kinsell and co-workers in patients receiving methyl testosterone might be explained by the fall in TBP and the consequent elevation of the level of free thyroxine. Since testosterone propionate did not produce such an exacerbation, it should prove valuable to determine whether testosterone propionate will affect thyroxine-binding by TBP.

SUMMARY

A study of the influence of methyl testosterone on human thyroid function is presented. The drug induced a striking fall in thyroxine-binding capacity of the thyroxine-binding alpha globulin of serum in all patients, and a less striking but consistent increase in the fractional rate of thyroxine disappearance from blood. Other findings were a slight fall in the serum protein-bound iodine, an increase in circulating free thyroxine, and an increase in the amount of thyroxine degraded per day. There were inconstant effects on the thyroidal and renal clearance of iodide. The hypothesis is offered that a fall in concentration of thyroxine-binding protein in serum is responsible for the changes observed. A consistent rise in serum cholesterol was also noted during the administration of methyl testosterone.

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