J. steroid Biochem. Vol. 23, No. 1, pp. 33–38, 1985
Printed in Great Britain.

0022-4731/85 $3.00 + 0.00

RESPONSE OF SERUM TESTOSTERONE AND ITS PRECURSOR STEROIDS, SHBG AND CBG TO ANABOLIC STEROID AND TESTOSTERONE SELF-ADMINISTRATION IN MAN

A. RUOKONEN[1], M. ALÉN[2], N. BOLTON[1] and R. VIHKO[1]
[1]Department of Clinical Chemistry, University of Oulu, SF-90220 Oulu and
[2]Department of Health Sciences, University of Jyväskylä, SF-40100 Jyväskylä, Finland

(*Received* 12 *November* 1984)

Summary—The influence of high doses of testosterone and anabolic steroids on testicular endocrine function and on circulating steroid binding proteins, sex hormone binding globulin (SHBG) and cortisol binding globulin (CBG), were investigated in power athletes for 26 weeks of steroid self-administration and for the following 16 weeks after drug withdrawal. Serum testosterone and androstenedione concentrations increased ($P < 0.05$) but pregnenolone, 17-hydroxypregnenolone, dehydroepiandrosterone, 5-androstene-3β,17β-diol, progesterone and 17-hydroxyprogesterone concentrations strongly decreased ($P < 0.001$) during steroid administration. Serum pregnenolone, 17-hydroxypregnenolone and dehydroepiandrosterone sulphate concentrations followed the changes of the corresponding unconjugated steroids but 5-androstene-3β,17β-diol and testosterone sulphate concentrations remained unchanged during the follow-up time. During drug administration SHBG concentrations decreased by about 80 to 90% and remained low even for the 16 weeks following steroid withdrawal. Steroid administration had no influence on serum CBG concentrations. In conclusion, self-administration of testosterone and anabolic steroids soon led to impairment of testicular endocrine function which was characterized by low concentrations of testosterone precursors, high ratios of testosterone to its precursor steroids and low SHBG concentrations. Decreased concentrations of SHBG and testicular steroids were still partly evident during the 16 weeks after drug withdrawal. The depressed circulating levels of dehydroepiandrosterone and its sulphate may indicate that the androgenic-anabolic steroids also suppress adrenal androgen production.

INTRODUCTION

Athletes have been reported to use high quantities of testosterone and anabolic steroids on the assumption that the drugs will improve performance in competition [1–5]. Androgenic-anabolic steroids have also been studied as agents for male contraception and their inhibitory influences on testicular testosterone secretion and spermatogenesis are well known. Anabolic steroid use usually results in decreases in serum gonadotrophin [6–9] and testosterone levels [1, 6–9], but in some studies a decrease in testosterone but not in gonadotrophin concentrations has been observed [10–14]. Administered testosterone and its derivates decrease serum gonadotrophin and may increase testosterone levels [15–19]. The various effects on gonadotrophin secretion seem to depend on the drug used, its dose and the route of administration.

Estrogen administration increases serum SHBG and CBG concentrations [20–23], whereas androgens have an opposite influence on SHBG [24] but usually induce no change or only a slight decrease in CBG concentrations [25]. As a result of these characteristics, CBG has been used as an indicator of the estrogenic activity and SHBG as an indicator of the combined estrogenic and androgenic effects of administered steroids [26, 27].

The purpose of the present work was to investigate in more detail the effects of very large doses of testosterone and anabolic steroids on testicular steroidogenesis and on the steroid binding proteins, SHBG and CBG, in power athletes who self-administered the drugs during training.

EXPERIMENTAL

Subjects and experimental approach

Four power athletes, who had previous experience in the use of androgenic steroids in their training, volunteered for this study and gave their informed consent. At the time when they again decided to start self-administration of anabolic steroids and testosterone, they were included in the study. The self-administration of androgens was followed by means of medication diaries. The power athletes had individual self-planned programmes in the administration of the drugs. Self-administration of anabolic

The following trivial names and abbreviations were used: Methandienone = 17α-methyl-17β-hydroxy-1,4-androstadien-3-one; Nandrolone = 17β-hydroxy-4-estren-3-one; Stanozolol = 17α-methyl-5α-androstano[3,2-C]-pyrazol-17β-ol; SHBG = sex hormone binding globulin; CBG = cortisol binding globulin.

steroids and testosterone continued for 26 weeks (phase I) followed by 16 weeks (phase II) without use of any steroid hormones. Because it is illegal in Finland for medical doctors to give a prescription for anabolic steroids for any other purpose than medical, the power athletes had obtained their drugs from the black-market and used them out of medical control. The doses and the drugs which the power athletes used are shown in Fig. 1.

Methandienone was taken orally with increasing daily doses towards the end of phase I. Self-injections of nandrolone phenylpropionate and stanozolol (both 50 mg per injection) were taken less frequently, usually once a week. Testosterone (250 mg/injection, consisting of 30 mg testosterone propionate, 60 mg testosterone phenylpropionate, 60 mg of testosterone isocaproate and 100 mg testosterone undecanoate) was self-administered initially once per month, but at the end of the study it was 4–8 times/month. Peripheral blood samples for the determination of testosterone, its metabolites and precursor steroids as well as for SHBG and CBG were collected at 8.00 a.m. on the sampling days. Serum samples were stored at −20°C until analysed. Control samples were taken before the first administration of the drugs and the subsequent samples at 8, 14, 20, 26 (phase I), 29, 32, 38 and 42 (phase II) weeks from the beginning of the study. Following the above schedule of sampling, SHBG and CBG concentrations were also determined in 5 athletes who did not use any drugs during training. Hence in the case of the steroid binding proteins two kinds of control values were used; the first were those before the administration and the others were from athletes who did not administer any drugs during training.

Chemicals

Sources of chemicals and steroids have been published previously [28].

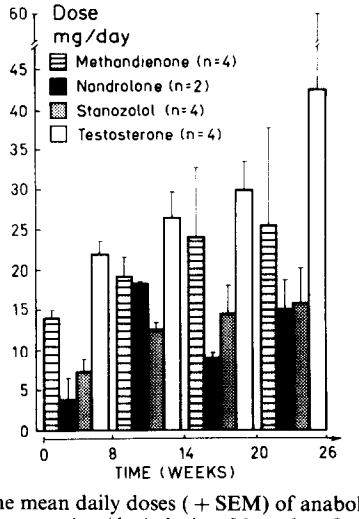

Fig. 1. The mean daily doses (+ SEM) of anabolic steroids and testosterone (mg/day) during 26 weeks of steroid self-administration.

Determination of serum steroid concentrations

For the separation of unconjugated and sulphated steroids, serum samples (1 ml) were extracted three times with ethyl ether–ethyl acetate (9:1, v/v) as previously described [28], after the addition of a known amount of tritiated testosterone in order to monitor experimental loss.

Methodology for the quantitation of unconjugated and sulphated steroids has been described previously [28, 29]. Briefly, unconjugated steroids were analysed as follows: after extraction, the combined organic phases were fractionated on Lipidex-5000™ microcolumns (Packard-Becker B.V., Chemical Operations, Groningen, The Netherlands), followed by radioimmunoassay of each steroid from the appropriate fraction, using antisera of defined specificity. The recovery of tritiated testosterone was calculated and 85% was the limit of acceptability.

Steroid sulphates were analysed from the water phases of the above mentioned extractions. They were dried under a nitrogen flow and the residue was dissolved in 3 ml of absolute ethanol. Samples were centrifuged to remove protein precipitates and the supernatant was dried and solvolysed (30) in 3 ml of ethyl acetate that had been equilibrated previously with 2 M H_2SO_4. Before overnight incubation at 37°C, an additional 50 µl of 2 M H_2SO_4 was added to the tubes to assure that the medium remained acidic. After solvolysis, the mixture was neutralized and the ethyl acetate phase was transferred to another tube, dried, and fractionated on Lipidex-5000™ microcolumns. Chromatography was followed by radioimmunoassay of steroids. Recovery and assay performance following solvolysis were monitored as described for unconjugated steroids.

SHBG and CBG determinations

Serum SHBG concentrations were measured by an immunoradiometric assay using a commercial kit donated by Farmos Diagnostica (Turku, Finland). Serum CBG concentrations were determined as described earlier [31].

Statistics

In each power athlete the values obtained during the study were compared to the control values at the beginning of the study, and in addition to this, in the case of SHBG and CBG, the values in the drug users and non-users were also compared. Comparisons were performed using either dependent or paired two-tailed Student's t-tests. A P value of < 0.05 was selected as the limit of statistical significance.

RESULTS

Before the administration of testosterone and anabolic steroids (control) serum concentrations of the endogeneous steroids (ng/ml; mean ± SEM) were as follows: Pregnenolone (0.38 ± 0.15), 17-hydroxypregnenolone (0.32 ± 0.8), dehydroepiandrosterone

(8.7 ± 1.6), 5-androstene-3β,17β-diol (2.9 ± 0.1), progesterone (0.14 ± 0.02), 17-hydroxyprogesterone (1.1 ± 0.1), androstenedione (1.1 ± 0.2), testosterone (4.5 ± 0.7), 5α-dihydrotestosterone (0.50 ± 0.12), pregnenolone sulphate (43 ± 5), 17-hydroxypregnenolone sulphate (5.7 ± 0.8), dehydroepiandrosterone sulphate (2040 ± 400), 5-androstene-3β,17β-diol sulphate (420 ± 110) and testosterone sulphate (2.3 ± 0.3).

In Fig. 2, the mean concentrations ($\pm$ SEM) of unconjugated steroids at 8, 14, 20 and 26 weeks of drug administration and at 3, 6, 12 and 16 weeks after drug withdrawal, are given as percentages of the values (100%) before drug administration. Testosterone concentrations significantly increased ($P < 0.05$) during drug administration. Three weeks after drug withdrawal there was a highly significant drop ($P < 0.001$) in testosterone concentrations to the level of 20–40% of the control values. It was not until 16 weeks after steroid withdrawal that testosterone concentrations were no longer significantly different from the control values. The unconjugated testosterone metabolites, androstenedione and 5α-dihydrotestosterone, closely followed the concentration pattern of their precursor.

Of the unconjugated testosterone precursor steroids, pregnenolone, 17-hydroxypregnenolone, dehydroepiandrosterone, 5-androstene-3β,17β-diol, progesterone and 17-hydroxyprogesterone concentrations all decreased during drug administration. In 8 weeks of drug administration testosterone to 17-hydroxypregnenolone and testosterone to 17-hydroxyprogesterone ratios had increased by averages of 1100 and 660% respectively ($P < 0.001$). Slow recovery took place only in pregnenolone and 17-hydroxyprogesterone levels during the follow-up period of the study.

The concentrations of serum steroid sulphates during the study are given in Fig. 3. During steroid administration serum concentrations of pregnenolone, 17-hydroxypregnenolone and dehydroepiandrosterone sulphates were consistently very low. Of these three steroids, only dehydroepiandrosterone sulphate reached its control level at 16 weeks after drug withdrawal. Concentrations of 5-androstene-3β,17β-diol and testosterone sulphates remained essentially unchanged although the concentration pattern of the latter compound was similar to that of unconjugated testosterone (Fig. 2).

In Fig. 4, the changes of SHBG and CBG concentrations are shown in 5 athletes (○) taking no steroids and in the 4 men (●) who used testosterone

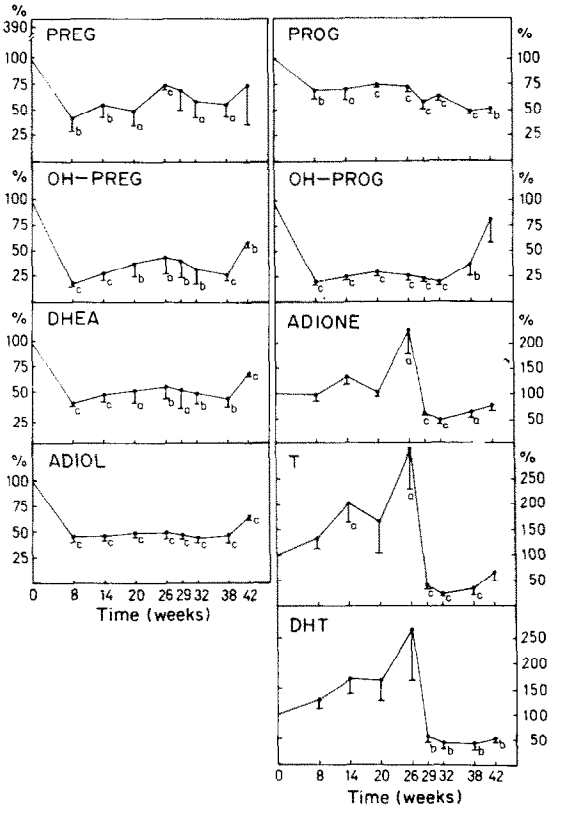

Fig. 2. Relative serum concentrations (mean + SEM) of testosterone, its unconjugated precursor steroids and 5α-dihydrotestosterone before (100%) administration of testosterone and anabolic steroids, during 26 weeks of steroid administration (weeks 0–26) and for the following 16 weeks after drug withdrawal (weeks 27–42). PREG = pregnenolone, PROG = progesterone, OH-PREG = 17-hydroxypregnenolone, OH-PROG = 17-hydroxyprogesterone, DHEA = dehydroepiandrosterone, ADIONE = androstenedione, ADIOL = 5-androstene-3β,17β-diol, T = testosterone, DHT = 5α-dihydrotestosterone. Significance of difference, compared to the 0-point level: a = $P < 0.05$, b = $P < 0.01$, c = $P < 0.001$.

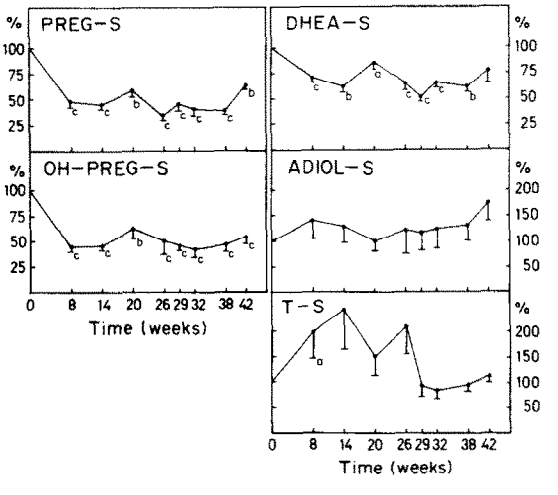

Fig. 3. Relative serum concentrations (mean + SEM) of the sulphate conjugates of testosterone and its precursor steroids before (100%) administration of testosterone and anabolic steroids, during 26 weeks of steroid administration (weeks 0–26) and for the following 16 weeks after drug withdrawal (weeks 27–42). Significance of difference, compared to the 0-point level: a = $P < 0.05$, b = $P < 0.01$, c = $P < 0.001$. S = sulphate, the abbreviations of steroids are given in Fig. 2.

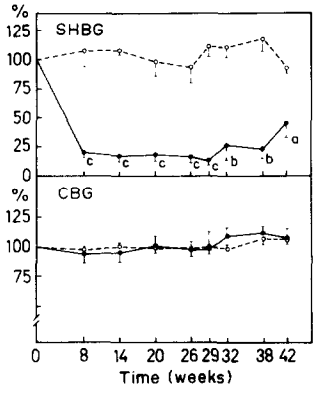

Fig. 4. Relative serum concentrations (mean + SEM) of SHBG and CBG before (100%) administration of testosterone and anabolic steroids, during 26 weeks of steroid administration (weeks 0–26) and for the following 16 weeks after drug withdrawal (weeks 27–42). Symbols: ● = steroid users ($n = 4$), ○ = non-users ($n = 5$). Significance of difference, compared to the 0-point level: a = $P < 0.05$, b = $P < 0.01$, c = $P < 0.001$.

and anabolic steroids during training. At the beginning of the study, serum concentrations (mean ± SEM) of SHBG were 28 ± 4 nmol/l in the non-treatment group and 30 ± 3 nmol/l in the treatment group. CBG concentrations were 431 ± 35 nmol/l and 438 ± 22 nmol/l, respectively.

During drug administration SHBG concentrations decreased by about 80–90% of the pretreatment values and they had not reached the control level even 16 weeks after drug withdrawal. Apart from the samples before steroid administration, SHBG concentrations were constantly lower (P-values from less than 0.001 to less than 0.05) in the drug users than in the non-users. Steroid administration had no influence on serum CBG concentrations.

DISCUSSION

In this study we investigated the influences of large doses of testosterone and anabolic steroids on testicular steroidogenesis in power athletes who self-administered high doses of the drugs on the assumption that they would improve performance in competition. The endogenous precursor steroids of testosterone have not been studied earlier in this context.

Administration of large doses of testosterone significantly increased its concentration in peripheral blood (Fig. 2). Its metabolites, androstenedione, 5α-dihydrotestosterone and testosterone sulphate closely followed the concentration pattern of testosterone, although the increases in 5α-dihydrotestosterone and testosterone sulphate were not statistically significant. In a previous study testosterone proprionate also resulted in increased plasma testosterone levels but at the same time testicular testosterone (in biopsy samples) decreased by about 95% [3]. In this study, during drug administration, all the unconjugated precursor steroids of testosterone decreased in peripheral blood by about 30–80%. The greatest relative decrease was observed in 17-hydroxypregnenolone and 17-hydroxyprogesterone concentrations and the ratios of testosterone to these precursor steroids increased by 1100 and 660% respectively. The sulphate conjugates of pregnenolone, 17-hydroxypregnenolone and dehydroepiandrosterone decreased during drug administration by about 40–60%. The decreases of steroid concentrations provide indirect evidence that the testis is an important source of these peripheral blood steroids in man. Although in these subjects peripheral cortisol concentrations remained unchanged [37], we cannot exclude the possibility that these anabolic androgens also influenced adrenal androgen production, reflected in the strongly depressed circulating levels of dehydroepiandrosterone and its sulphate.

In peripheral blood, 5-androstene-3β,17β-diol is present both as a mono- and a disulphate [33] and in this study we determined these steroid conjugates together. Why no change was observed in 5-androstene-3β,17β-diol sulphate concentrations during the study is probably due to the fact that in peripheral blood $\frac{2}{3}$ of the steroid is disulphated [33]. The testes significantly secrete only 5-androstene-3β, 17β-diol 3-sulphate [34, 35] and during the study peripheral changes in this minor steroid conjugate may have been hidden by the main components from other sources such as the liver [36]. Some cross-reactivities in the radioimmunoassays between the endogenous steroids and the drugs administered, or between their metabolites, cannot be completely excluded. However, no such cross-reactivity seems to take place that would essentially change the results. Specific antibodies and Lipidex chromatography of steroids before the radioimmunoassays [28] were used to minimize the possibility of cross-reaction. With the exception of 5-androstene-3β,17β-diol sulphate all the other unconjugated and sulphated precursor steroids of testosterone were significantly decreased in peripheral blood during drug administration and their concentrations remained low for a long time after drug withdrawal. Irrespective of drug administration, concentrations of 5-androstene-3β,17β-diol sulphate remained unchanged, indicating that anabolic steroids did not significantly cross-react in the assay. These results practically exclude the possibility of significant cross-reactions of the anabolics in the radioimmunoassays of endogenous steroids.

The duration of altered hormone levels in peripheral blood depends on the drug used, its dose and the route of administration [6–14]. The main mechanism by which testicular steroidogenesis was suppressed seems to be mediated by reduced pituitary secretion of LH and by associated inhibition of cholesterol side chain cleavage in the testis. In a previous study, where LH and FSH concentrations were determined in the same athletes, administration of high doses of testos-

terone and anabolics significantly decreased the peripheral blood concentrations of these gonadotrophins. After drug withdrawal, serum FSH and LH returned in 6–12 weeks to concentrations not significantly different from those seen at the beginning of the study [37]. On the other hand, in the present study, the concentrations of testosterone and its precursors recovered more slowly after drug withdrawal and some of them had not reached control levels during the 16 week follow-up period. The finding that testosterone concentrations returned to normal later than those of LH and FSH, after treatment with the anabolic steroid 19-nortestosterone, has recently been described by Schürmeyer *et al.*[9].

Levels of SHBG increase in the blood during estrogen treatment [20, 21] and decrease during androgen treatment [24]. Due to these characteristics it has been used in several studies as an indicator of both estrogenic and androgenic effects of administered steroids [27]. In the present study peripheral SHBG concentrations were strongly reduced during the administration of testosterone and anabolic steroids and they had not reached control levels even after 16 weeks following drug withdrawal, indicating long lasting androgenicity of the combination of the drugs administered. The androgenic effects of the steroids administered were therefore strengthened by the reduction of their binding protein and the consequent increase in the biologically active free fraction of the steroids bound by SHBG. Peripheral CBG concentrations increase during treatment with estrogenic steroids but are mainly unchanged during androgen administration [22, 23, 25]. In the present study CBG concentrations remained unchanged, supporting the earlier results which have indicated that androgens have no major influence on the synthesis of this protein. Peripheral estradiol concentrations also significantly increased in the same athletes [37], but in these conditions it was not enough to stimulate the synthesis of CBG.

It seems very likely that novel ways of detecting anabolic-androgenic steroid use could be developed on the basis of the present results. Development of such means would be associated with the use of blood specimens.

Acknowledgements—We wish to thank Mrs Liisa Ollanketo for her skilful technical assistance.

REFERENCES

1. Kilshaw B. H., Harkness R. A., Hobson B. M. and Smith A. W. M.: The effects of large doses of the anabolic steroid, methandrostenolone, on an athlete. *Clin. Endocr.* **4** (1975) 537–541.
2. Freed D. L. J., Banks A. J., Longson D. and Burley D. M.: Anabolic steroids in athletics: crossover double-blind trial on weightlifters. *Br. med. J.* **ii** (1975) 471–473.
3. Johnson L. C. and O'Shea J. P.: Anabolic steroid: effect on strength development. *Science* **164** (1969) 957–959.
4. Hill J. A., Suker J. R., Sachs K. and Brigham C.; The athletic polydrug abuse phenomenon. A case report. *Am. J. Sports Med.* **11** (1983) 269–271.
5. Strømme S. G., Meen H. D. and Aakvaag A.: Effect of an androgenic-anabolic steroid on strength development and plasma testosterone levels in normal males. *Med. Sci. Sports* **6** (1974) 203–208.
6. Holma P. and Aldercreutz H: Effect of an anabolic steroid (methandienon) on plasma LH, FSH, and testosterone and on the response to intravenous administration of LRH. *Acta endocr., Copenh.* **83** (1976) 856–864.
7. Sherins R. J. and Loriaux D. L.: Studies on the role of sex steroids in the feedback control of FSH concentration in man *J. clin. Endocr. Metab.* **36** (1973) 886–893.
8. Bijlsma J. W. J., Duursma S. A., Thijssen J. H. H. and Huber O.: Influence of nandrolondecanoate on the pituitary–gonadal axis in males. *Acta endocr., Copenh.* **101** (1982) 108–112.
9. Schürmeyer T., Belkien L., Knuth U. A. and Nieschlag E.: Reversible azoospermia induced by the anabolic steroid 19-nortestosterone. *Lancet* **i** (1984) 417–420.
10. Aakvaag A. and Strømme S. B.: The effect of mesterolone administration to normal men on the pituitary-testicular function. *Acta endocr., Copenh.* **77** (1974) 380–386.
11. Fahey T. D. and Brown C. H.: The effects of an anabolic steroid on the strength, body composition and endurance of college males when accompanied by a weight training program. *Med. Sci. Sports* **5** (1973) 272–276.
12. Hervey G. R., Hutchinson J., Burkinshaw L., Jones B. R. M., Norgan N. G. and Levell M. J.: "Anabolic" effects of metandienone in men undergoing athletic training. *Lancet* **ii** (1976) 699–702.
13. Remes K., Vuopio P., Järvinen M., Härkönen M. and Adlercreutz H.: Effect of short-term treatment with an anabolic steroid (methandienone) and dehydroepiandrosterone sulphate on plasma hormones, red cell volume and 2,3-diphosphoglycerate in athletes. *Scand. J. clin. Lab. Invest.* **37** (1977) 577–586.
14. Jones T. M., Fang V. S., Landau R. L. and Rosenfield R. L.: The effects of fluoxymesterone administration on testicular function. *J. clin. Endocr. Metab.* **44** (1977) 121–129.
15. Mauss J., Börsch G., Bormacher K., Richter E., Leyendecker G. and Nocke W.: Effect of long-term testosterone oenanthate administration on male reproductive function: clinical evaluation, serum FSH, LH, testosterone and seminal fluid analyses in normal men. *Acta endocr., Copenh.* **78** (1975) 373–384.
16. Snyder P. J. and Lawrence D. A.: Treatment of male hypogonadism with testosterone enanthate. *J. clin. Endocr. Metab.* **51** (1980) 1335–1339.
17. Caminos-Torres R., Ma L. and Snyder P. J.: Testosterone induced inhibition of the LH and FSH responses to gonadotropin-releasing hormone occurs slowly. *J. clin. Endocr. Metab.* **44** (1977) 1142–1153.
18. Swerdloff R. S., Campfield L. A., Palacios A. and McClure R. D.: Suppression of human spermatogenesis by depot androgen: potential for male contraception. *J. steroid Biochem.* **11** (1979) 663–670.
19. Cunningham G. R., Silverman V. E., Thornby J. and Kohler P. O.: The potential for an androgen male contraceptive. *J. clin. Endocr. Metab.* **49** (1979) 520–526.
20. Briggs M. H.: Hormonal contraceptives and plasma sex-hormone binding globulin. *Contraception* **12** (1975) 149–153.
21. van Kammen E., Thyssen J. H. H., Rademaker B. and Schwarz F.: The influence of hormonal contraceptives on sex hormone binding globulin capacity. *Contraception* **11** (1975) 53–59.

22. Schwartz U. and Hammerstein J.: The oestrogenic potency of various contraceptive steroids as determined by their effects on transcortin-binding capacity. *Acta endocr., Copenh.* **76** (1974) 159–171.
23. Moore D. E., Kawagoe S., Davajan V., Nakamura R. M. and Mishell D. R.: An *in vivo* system in man for quantitation of estrogenicity. II. Pharmacologic changes in binding capacity of serum corticosteroid-binding globulin induced by conjugated estrogens, mesteranol and ethinyl estradiol. *Am. J. Obstet. Gynec.* **130** (1978) 482–486.
24. Anderson B. C.: Sex-hormone-binding globulin. *Clin. Endocr.* **3** (1974) 68–96.
25. Barbosa J., Seal U. S. and Doe R. P.: Effects of anabolic steroids on hormone-binding proteins, serum cortisol and serum non-protein-bound cortisol. *J. clin. Endocr. Metab.* **32** (1971) 232–240.
26. Schwartz U. and Hammerstein J.: The oestrogenic potency of various contraceptive steroids as determined by their effects on transcortin-binding capacity. *Acta endocr., Copenh.* **76** (1974) 159–171.
27. El Makhzangy M. N., Wynn V. and Lawrence D. M.: Sex hormone binding globulin capacity as an index of oestrogenicity or androgenicity in women on oral contraceptive steroids. *Clin. Endocr.* **10** (1979) 39–45.
28. Hammond G. L., Ruokonen A., Kontturi M., Koskela E. and Vihko R.: The simultaneous radioimmunoassay of seven steroids in human spermatic and peripheral venous blood. *J. clin. Endocr. Metab.* **45** (1977) 16–24.
29. Ruokonen A., Lukkarinen O. and Vihko R.: Secretion of steroid sulfates from human testis and their response to a single intramuscular injection of 5000 IU hCG. *J. steroid Biochem.* **14** (1981) 1357–1360.
30. Vihko R.: Gas chromatographic–mass spectrometric studies on solvolyzable steroids in human peripheral plasma. *Acta endocr., Copenh. Suppl.* **109** (1966) 1–67.
31. Hammond G. L. and Lähteenmäki P. L. A.: A versatile method for the determination of serum cortisol binding globulin and sex hormone binding globulin binding capacities. *Clin. chim. Acta* **132** (1983) 101–110.
32. Morse H. C., Horike N., Rowley M. J. and Heller C. G.: Testosterone concentrations in testes of normal men: effects of testosterone propionate administration. *J. clin. Endocr. Metab.* **37** (1973) 882–886.
33. Jänne O., Vihko R., Sjövall J. and Sjövall K.: Determination of steroid mono- and disulfates in human plasma. *Clin. chim. Acta* **23** (1969) 405–412.
34. Laatikainen T., Laitinen E. A. and Vihko R.: Secretion of free and sulfate-conjugated neutral steroids by human testis. Effect of administration of human chorionic gonadotropin. *J. clin. Endocr. Metab.* **32** (1971) 59–64.
35. Ruokonen A. and Vihko R.: Steroid metabolism in human and boar testis tissue. Steroid concentrations and the position of the sulfate group in steroid sulfates. *Steroids* **23** (1974) 1–16.
36. Baulieu E. E. and Robel P.: Catabolism of testosterone and androstenedione. In *The Androgens of the Testis* (Edited by K. B. Eik-Nes). Marcel Dekker, New York (1970) p. 49, pp. 55–58.
37. Alén M., Reinilä M. and Vihko R.: Response of serum hormones to androgen administration in power athletes. *Med. Sci. Sports* (1985) In press.