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Depletion of [^{3}H]Methyltrienolone Cytosol Binding in Glucocorticoid-Induced Muscle Atrophy (42001)

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Abstract. The present study was undertaken to determine cytosol binding properties of [^{3}H]methyltrienolone, a synthetic androgen, in comparison with [^{3}H]dexamethasone, a synthetic glucocorticoid, under conditions of glucocorticoid excess in skeletal muscle. Male hypophysectomized rats received either seven daily subcutaneous injections of cortisone acetate (CA) (100 mg·kg^{-1} body wt) or the vehicle, 1% carboxymethyl cellulose. Following treatment, both [^{3}H]dexamethasone and [^{3}H]methyltrienolone-receptor concentrations were decreased from those in vehicle-treated rats by more than 90 and 80%, respectively, in CA-treated animals. Scatchard analysis of [^{3}H]methyltrienolone binding in muscles of vehicle-treated animals became nonlinear at high concentrations of labeled ligand and were reanalyzed by a two-component binding model. The lower affinity, higher capacity component, which was attributed to binding of methyltrienolone to a "dexamethasone" component, disappeared in muscles of CA-treated rats and Scatchard plots were linear. Receptor concentrations of the higher affinity lower capacity "methyltrienolone" component were similar in muscles of vehicle-treated and CA-treated groups. From competition studies, the high relative specificities of glucocorticoids for [^{3}H]methyltrienolone binding in muscles of vehicle-treated animals were markedly reduced by CA treatment. In addition, the binding specificity data also showed strong competition by progesterone and methyltrienolone for [^{3}H]dexamethasone binding and estradiol-17β for [^{3}H]methyltrienolone binding. These results demonstrate that most of the [^{3}H]methyltrienolone binding is eliminated under *in vivo* conditions of glucocorticoid excess. Furthermore, the competitiveness of various steroids for receptor binding suggests that rat muscle may not contain classic (ligand-specific) glucocorticoid and androgen receptors.

[^{3}H]Methyltrienolone (R1881),[1] a synthetic steroid, has been used as an androgen cytosol-receptor marker in a number of tissues including skeletal muscle (1–6), primarily because it does not bind to plasma proteins, which may be contaminants included in cytosol preparations; it is not susceptible to enzymatic modification; and it has high affinity for receptor binding (7). Nevertheless, the results of several of these studies also indicate that methyltrienolone may bind to glucocorticoid receptors (4–6). Specifically, the data points from Scatchard analyses become curvilinear at high concentrations of [^{3}H]R1881; whereas, this nonlinear pattern can be prevented by the addition of unlabeled glucocorticoid to cytosols during incubation (5). Furthermore, excess unlabeled R1881 has been shown to be effective in displacing the triamcinolone acetonide cytosol receptor (5). In view of these observations from *in vitro* experiments on the lack of specificity of R1881 binding to exclusively androgen receptors, the present investigation was undertaken to further evaluate [^{3}H]methyltrienolone binding under *in vivo* conditions of

[1] Trivial and systematic names: methyltrienolone (R1881): 17β-hydroxy-17-methyl-4,9,11-estratriene-3-one; testosterone: 17β-hydroxy-4-androsten-3-one; 5α-dihydrotestosterone: 17β-hydroxy-5α-androstan-3-one; Dianabol (methandrostenolone): 17βhydroxy-17α-methyl-androsta-1,4-diene-3-one; dexamethasone: 9-fluoro-11β, 17,21-trihydroxy-16α-methyl-1,4-pregnadiene-3,20-dione; corticosterone: 11β,21-dihydroxy-4-pregnene-3,20-dione; triamcinolone acetonide; 9-fluoro-11β,16α,17,21-tetrahydroxy-1,4-pregnadiene-3,20-dione cyclic 16,17-acetal; cortisone: 17α,21-dihydroxy-4-pregnene-3,11,20-trione; progesterone: 4-pregnene-3,20-dione; estradiol-17β: 1,3,5,(10)-estratriene-3, 17β-diol.

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glucocorticoid excess. [^{3}H]Methyltrienolone cytosol binding capacities, dissociation constants, and specificities were determined in skeletal muscle following 7 days of cortisone acetate injections. For comparison with the effects of glucocorticoids on [^{3}H]methyltrienolone binding, the treatment responses of [^{3}H]dexamethasone, a synthetic glucocorticoid, were also determined in the same tissues.

The results indicate that to adequately determine methyltrienolone binding properties in skeletal muscle, it is essential to separate the contributions of the higher affinity "methyltrienolone" (androgen) component from the lower affinity "dexamethasone" (glucocorticoid) component. Furthermore, the absence of exclusive steroid specificity for the androgen and glucocorticoid receptors suggests that the mechanism of action of other steroid hormone classes (estrogens, progestins) in skeletal muscle might be mediated through binding to androgen and glucocorticoid receptors.

Methods. *Animal care and experimental treatments.* Male Sprague–Dawley rats were hypophysectomized at 72 days of age by Hormone Assay, Chicago, Illinois. Further procedures began when the animals were between 81 and 85 days old. The animals were individually housed and provided a diet of Purina Rat Chow and water *ad libitum.* They were arbitrarily divided into one of two groups. One group received daily subcutaneous injections of cortisone acetate (Sigma Chemical Co., St. Louis, Mo.) suspended in 1% (w/v) carboxymethyl cellulose (CMC) at a dose of 100 mg/kg body wt for 7 days. This hormone and dosage were selected because they are effective in producing muscle wasting and have been frequently used for studying the glucocorticoid effects on muscle protein turnover [cf. (8)]. The second group of animals were given daily volume–body weight matched injections of 1% CMC for 7 days. All rats were killed 15–20 hr after their last injections.

Steroid assays were performed on the gastrocnemius, a fast-twitch muscle, which undergoes marked atrophy in response to glucocorticoid treatment (8). Slow-twitch muscles were not studied because they undergo little or no change in mass with cortisone acetate injections (8).

Preparation of cytosol. Following exsanguination via the abdominal aorta under ether anesthesia, the gastrocnemius muscles were rapidly removed, kept on ice, trimmed of adjacent musculature, fat, and connective tissue, weighed, and placed in precooled polyethylene (50 ml) tubes. Subsequent cytosol preparation proceeded on ice. The samples were minced with scissors and homogenized in 4 vol of TE buffer (0.05 *M* Tris, 0.0015 *M* EDTA, pH 7.4) and 10 m*M* sodium molybdate with two 30-sec passes at approximately two-thirds speed with a Polytron (Brinkman Instruments). The homogenate was then poured into centrifuge tubes and centrifuged (Beckman L8-70) at 100,000*g* for 60 min at 4°C using a Beckman 50.2 rotor. After centrifugation, the cytosol was carefully decanted into clean, precooled polyethylene tubes, taking care not to disturb the small amount of lipid layer. Cytosol protein was determined with the biuret reagent (9) or by the method of Lowry *et al.* (10) using egg albumin as standard.

The current model of steroid hormone action is thought to involve steroid-receptor binding in the cytoplasm of the cell with subsequent translocation of the steroid-receptor complex into the nucleus where interaction with genetic material leads to further biochemical events and to specific physiological effects [cf. (11)]. However, the data from two recent studies (12, 13) have indicated that most of cellular estrogen receptors are found in the nucleoplasm of several types of target tissues. In view of these findings, the cytosol, which is defined in terms of how it is prepared, may represent both cytoplasmic and unactivated nuclear receptors, which are released during homogenization.

Assay of [^{3}H]methyltrienolone binding. Steroid binding reactions were carried out according to previously described procedures (14). Briefly, saturation analyses were performed for each group by incubating cytosols (0.5 ml, approx 7–10 mg protein) with increasing concentrations of the labeled steroid for 18 hr at 4°C. Upon completion of the incubation period, total and nonspecific binding were determined using charcoal separation procedures (11). Specific binding was determined by the difference between [^{3}H]methyltrienolone bound in the absence and presence of a 100-fold excess of unlabeled

methyltrienolone. For individual saturation analysis within each group, the muscles of 3–4 animals were pooled to obtain adequate tissue. Saturation analysis data were plotted and receptor dissociation constants and receptor binding capacities were obtained from the linear curves by the method of Scatchard (15) and for nonlinear curves by the method of Rosenthal (16). Binding specificities were determined from competition studies, in which aliquots of cytosol (0.50 ml) were incubated with a saturating concentration (8 n*M*) of [^{3}H]methyltrienolone alone or in the presence of a 100-fold excess concentration of radioinert competitors at 4°C for 18 hr. Bound and free steroids were separated using charcoal separation procedures as described above. These experiments required combining the gastrocnemius muscles of 2–4 animals per group for each competition series.

Assay of [^{3}H]dexamethasone binding. For glucocorticoid receptor binding, aliquots (0.25 ml) of the same cytosol preparation as used for methyltrienolone binding were incubated with increasing concentrations of [^{3}H]dexamethasone for 20 hr at 4°C for exchange and assayed according to recently described procedures (17). Bound radioactivity, specific binding, determination of dissociation constants, receptor binding capacities, and binding specificities were performed as described above for methyltrienolone.

Statistical procedure. Student's *t* test was used for comparison of two means between groups and paired *t* tests were used for pre–post mean differences within the same group. Statistical significance was set at the 0.05 level.

Results. At the start of the experiments, body weights were similar between groups, but decreased 20% in the CA-treated animals after 7 days of treatment (Table I). Gastrocnemius muscle weights were 27% lighter in the CA-treated than in the vehicle-injected group.

[^{3}H]Methyltrienolone binding. Two distinct Scatchard plots for [^{3}H]methyltrienolone were observed in the vehicle-treated and cortisone acetate-treated groups (Fig. 1). In the vehicle-injected group, the data points of the Scatchard analyses became curvilinear at high concentrations of labeled ligand. In the muscles of glucocorticoid-treated rats, the Scatchard plots were consistent with a single class of receptors with high affinity and low capacity for the [^{3}H]methyltrienolone. The existence of a single class of receptor sites in the CA-treated group, which are saturable, was confirmed by observation of a plateauing of

TABLE I. BODY WEIGHT AND GASTROCNEMIUS MUSCLE WEIGHT RESPONSES TO 7 DAYS OF CORTISONE ACETATE INJECTIONS[a]

Treatment group	*n*	Body wt (g) Before treatment	Body wt (g) After treatment	Gastrocnemius weight (mg)
Vehicle treated	39	266 ± 3	272 ± 3	1316 ± 17
Cortisone acetate treated	56	271 ± 2	228 ± 2+,*	960 ± 8*

Note. Values are means ± SE. *n*, number of rats used for all experiments. +Before vs after treatment, $P \leq 0.05$). *Vehicle treated vs CA treated, $P \leq 0.05$.

[a] 100 mg · kg^{-1} body wt · day^{-1}.

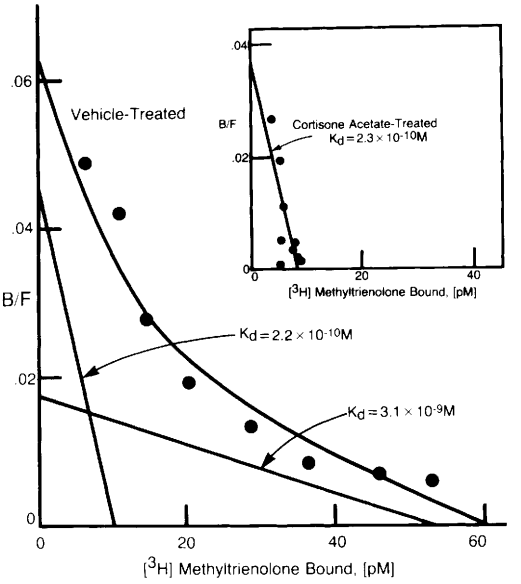

FIG. 1. Scatchard analysis of [^{3}H]methyltrienolone binding in gastrocnemius muscle cytosols from vehicle-treated (control) and cortisone acetate-treated (shown in inset) rats. The nonlinear curve of the controls was reanalyzed by the method of Rosenthal (12). Cytosols were incubated at 4°C for 18 hr with various concentrations of [^{3}H]methyltrienolone. Bound radioactivity was measured by the DCC adsorption technique. Each point is the mean of three separate saturation analyses.

data when bound ligand was plotted against the logarithm of free ligand (data not shown) (18). This was not the case for the binding in the vehicle-treated group. Receptor capacity averaged 5.33 ± 0.48 fmole/mg protein in the vehicle-treated group and was reduced by more than 80% to 0.95 ± 0.14 fmole/mg protein in cytosols of CA-treated rats.

The nonlinearity of the Scatchard analyses in the vehicle-treated group led us to attempt to fit the data to a two-component binding model (16). The resultant curves for [^{3}H]methyltrienolone binding in the gastrocnemius muscle cytosols of the vehicle-treated group represent a higher affinity "methyltrienolone" component and a lower affinity "dexamethasone" component (Fig. 1). Binding capacity of the methyltrienolone component, plotted from the individual [^{3}H]-methyltrienolone saturation analyses, averaged 1.07 ± 0.04 fmole/mg protein. This is almost identical to that found in gastrocnemius muscles after glucocorticoid treatment.

In competition experiments on muscles of the vehicle-treated group, progesterone was

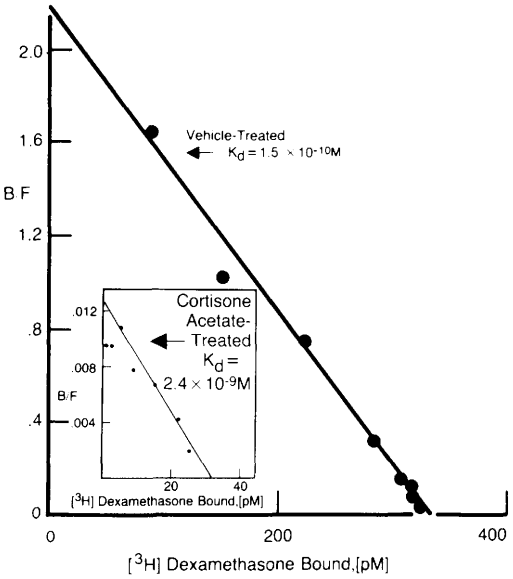

FIG. 2. Scatchard analyses of specific [^{3}H]dexamethasone binding in gastrocnemius muscle cytosols from vehicle-treated (control) and cortisone acetate-treated (shown in inset) rats. Cytosols were incubated at 4°C for 20 hr with various concentrations of [^{3}H]dexamethasone. Bound radioactivity was measured by the DCC adsorption technique. Each point is the mean of four separate saturation analyses.

TABLE II. RELATIVE STEROID SPECIFICITY OF RAT GASTROCNEMIUS MUSCLE CYTOSOL METHYLTRIENOLONE BINDING COMPONENT

Competing steroid	% Inhibition	
	Vehicle treated	Cortisone acetate treated
Methyltrienolone	100	100
Progesterone	102 ± 7	42 ± 15
Triamcinolone acetonide	93 ± 6	9 ± 7
Dexamethasone	82 ± 9	8 ± 6
Dianabol	75 ± 6	125 ± 41
Corticosterone	72 ± 6	39 ± 13
Testosterone	64 ± 5	85 ± 26
Estradiol-17β	59 ± 7	92 ± 44
Cortisone	50 ± 8	27 ± 26
DHT	33 ± 12	125 ± 23

Note. The data indicate the percentage inhibition of binding of 8 nM [^{3}H]methyltrienolone by 100-fold molar excess unlabeled steroid. Assay procedures and incubation conditions were performed as described under Methods. The difference between 8 nM [^{3}H]methyltrienolone binding in the absence of competing steroid and binding in the presence of 8×10^{-7} M unlabeled methyltrienolone was defined as 100% inhibition. There are between 4 and 11 determinations per point. Values are means ± SE.

equally as effective as methyltrienolone in displacing [^{3}H]methyltrienolone binding and the glucocorticoids—triamcinolone acetonide, dexamethasone, and corticosterone—were beter competitors than testosterone or 5α-dihydrotestosterone (DHT) (Table II). However, in the cortisone acetate-treated group, competition of the glucocorticoids and progesterone was markedly reduced; whereas, Dianabol (a synthetic anabolic steroid, CIBA Pharmaceutical Co., Summit, N.J.), DHT, testosterone, and estradiol-17β were essentially equal competitors for [^{3}H]methyltrienolone. The increased variability in preparations from CA-treated animals was a result of the reduction in total binding relative to nonspecific binding.

[^{3}H]Dexamethasone binding. Specific binding of [^{3}H]dexamethasone in gastrocnemius muscles of vehicle-treated rats showed high affinity (K_d 1.5×10^{-10} M) with binding capacity extrapolated to 321 ± 24 pM (45.2 ± 4.4 fmole/mg protein) (Fig. 2). Cortisone acetate treatment reduced cytosol-specific binding from the vehicle-treated groups by

approximately 90% to 31.9 ± 3.7 p*M* (4.3 ± 0.6 fmole/mg protein). These remaining measurable binding sites had a somewhat decreased affinity (K_d 2.4 × 10^{-9}) for dexamethasone as compared to that in the vehicle-treated rats.

In the binding specificity studies of vehicle-treated rats triamcinolone acetonide, corticosterone, and progesterone competed equally effectively as unlabeled dexamethasone for the dexamethasone receptor (Table III). Methyltrienolone also was a strong competitor; however, Dianabol, testosterone, DHT, estradiol-17β, and cortisone showed little cross-reactivity. This pattern of steroid competition remained unchanged in muscles of CA-treated rats despite the reduced specific binding.

Discussion. One new observation from these experiments was the marked depletion of [^{3}H]methyltrienolone cytosol binding component in gastrocnemius muscles after 7 days of glucocorticoid treatment. This finding supports the results of previous work, in which [^{3}H]methyltrienolone high-affinity binding was reduced and the nonlinear Scatchard plots were eliminated, when glucocorticoid (triamcinolone acetonide) was added to cytosol preparations (5). In addition, the present results show the relative magnitude of decrease in [^{3}H]R1881 receptor binding (80%) was similar to the loss (90%) of [^{3}H]dexamethasone cytosol binding in muscles of CA-treated rats.

TABLE III. RELATIVE STEROID SPECIFICITY OF RAT GASTROCNEMIUS MUSCLE CYTOSOL DEXAMETHASONE BINDING COMPONENT

Competing steroid	% Inhibition	
	Vehicle treated	Cortisone acetate treated
Dexamethasone	100	100
Triamcinolone acetonide	102 ± 1	97 ± 7
Corticosterone	99 ± 1	105 ± 13
Progesterone	96 ± 1	110 ± 10
Methyltrienolone	86 ± 1	78 ± 7
Dianabol	22 ± 1	12 ± 5
Estradiol-17β	16 ± 5	17 ± 5
Testosterone	15 ± 1	18 ± 5
Cortisone	13 ± 1	7 ± 3
DHT	9 ± 1	2 ± 1

Note. The data indicate the percentage inhibition of binding of 18 n*M* [^{3}H]dexamethasone by 100-fold molar excess unlabeled steroid. Assay procedures and incubation conditions were performed as described under Methods. The difference between 18 n*M* [^{3}H]dexamethasone binding in the absence of competing steroid and binding in the presence of 1.8 × 10^{-6} *M* unlabeled dexamethasone was defined as 100% inhibition. There are between 4 and 13 determinations per point. Values are means ± SE.

A logical conclusion to be drawn from these data is that [^{3}H]R1881 displays binding more like a glucocorticoid than an androgen as found in the more commonly studied target organs such as the prostate and thymus. However, unlike [^{3}H]dexamethasone, the relative binding affinity of [^{3}H]methyltrienolone was markedly changed by glucocorticoid treatment. In addition, the competition by glucocorticoids for [^{3}H]R1881 binding was reduced in muscles of CA-treated animals. At the same time the apparent affinities of androgens for [^{3}H]methyltrienolone binding increased. With [^{3}H]dexamethasone binding, the relative steroid specificities remained essentially the same in muscles of either vehicle- or CA-treated groups. An additional complexity of [^{3}H]R1881 is in its ability to be displaced by progesterone. This observation has been well established particularly in the prostate with [^{3}H]methyltrienolone binding to progesterone as well as to androgen receptors (19, 20). However, progesterone receptors have not been detected in skeletal muscle (4, 5, 14). Thus, the progesterone affinity for [^{3}H]R1881 binding can be directly related to binding of methyltrienolone to the "dexamethasone" or lower affinity component. This is further supported by the fact that progesterone competition for [^{3}H]methyltrienolone was reduced in muscles of CA-treated rats while progesterone competition for [^{3}H]-dexamethasone binding remained unchanged.

Cumulatively, the foregoing results of competition by glucocorticoids and androgens for [^{3}H]methyltrienolone in muscles of vehicle-treated rats, the subsequent reduction in glucocorticoid and increase in androgen affinities for [^{3}H]methyltrienolone after CA treatment, as well as the linear Scatchard plots in muscles of CA-treated rats provided sufficient background for us to attempt to resolve the nonlinear Scatchard plots in muscles of vehicle-treated rats using a two-component binding model. Consequently, the

calculated K_d and maximum specific binding of the higher affinity component were then found to be comparable to that of the linear plots obtained from muscles of cortisone acetate-treated rats.

The rather high competition of progesterone (110%) and methyltrienolone (78%) for [^{3}H]dexamethasone binding was not anticipated. The relative binding affinity of progesterone and methyltrienolone for the [^{3}H]dexamethasone rat thymus receptor appears to be much lower than that presently observed in skeletal muscle (21). Furthermore, estradiol-17β does not appreciably cross-react with testosterone or methyltrienolone in the classical androgen receptor preparations from a number of other tissues (19, 22–24). Yet, estradiol-17β was found to be equal to testosterone in inhibiting [^{3}H]R1881 binding in the skeletal muscle. Several reports (2, 25, 26) have observed steroid specificity for methyltrienolone in rat skeletal muscle to resemble that of classical androgen target tissues. However, the competition experiments of Snochowski *et al.* (2) were performed at a subsaturating concentration (2 n*M*) of methyltrienolone, where the results would reflect binding almost exclusively to the higher affinity "methyltrienolone" component. In the second instance (25), competition experiments were performed on the "androgen sensitive" levator ani muscles at a near saturating concentration (10 n*M*) of methyltrienolone with the addition of excess triamcinolone acetonide to block binding to the lower affinity "dexamethasone" component. As in the present study, they also observed competition for methyltrienolone binding by estradiol-17β (25). In a third investigation (26), estradiol-17β was a good competitor, almost equal to the natural androgens, for methyltrienolone binding over a range of steroid concentrations, whereas, displacement of methyltrienolone binding by corticosterone occurred only at very high concentrations of competitor.

The overall lack of specificity by the [^{3}H]methyltrienolone and [^{3}H]dexamethasone receptors suggests the possibility that rat muscle does not contain clasic (ligand-specific) glucocorticoid and androgen receptors. In support of this possibility, Michel and Baulieu also (27) observed that the binding of 5α-dihydrotestosterone can be displaced by estradiol-17β in rat skeletal muscle. Additionally, Mayer and Rosen (28) have found that androgen administration can deplete glucocorticoid cytosol-receptor binding in skeletal muscle of rats. These findings support the assumption that the mechanism of action in skeletal muscle by other classes of steroid hormones (estrogens, progestins) may also be regulated through androgen and glucocorticoid cytosol receptors.

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1. Tremblay RR, Dubé JY, Ho-Kim MA. Determination of rat muscles androgen-receptor complexes with methyltrienolone. Steroids **29**:185–195, 1977.
2. Snochowski M, Dahlberg E, Gustafsson J-A. Characterization and quantification of the androgen and glucocorticoid receptors in rat skeletal muscle cytosol. Eur J Biochem **111**:603–616, 1980.
3. Michel G, Baulieu E-E. Androgen receptor in rat skeletal muscle: characterization and physiological variations. Endocrinology **107**:2088–2098, 1980.
4. Dahlberg E, Snochowski M, Gustafsson J-A. Regulation of the androgen and glucocorticoid receptors in rat and mouse skeletal muscle cytosol. Endocrinology **108**:1431–1440, 1981.
5. Ho-Kim MA, Tremblay RR, Dubé JY. Binding of methyltrienolone to glucocorticoid receptors in rat muscle cytosol. Endocrinology **109**:1418–1423, 1981.
6. Max SR. Cytosolic androgen receptor in skeletal muscle from normal and dystrophic mice. J Steroid Biochem **18**:281–283, 1983.
7. Bonne C, Raynaud J-P. Methyltrienolone, a specific ligand for cellular androgen receptors. Steroids **26**:277–232, 1975.
8. Hickson RC, Davis JR. Partial prevention of glucocorticoid-induced muscle atrophy by endurance training. Amer J Physiol **241**:E226–E232, 1981.
9. Gornall AJ, Bardawill CJ, David MM. Determination of serum proteins by means of the biuret reaction. J Biol Chem **177**:751–766, 1949.
10. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem **193**:265–275, 1951.
11. King WJ. Cytoplasmic receptors for steroid hormones—Characteristics and assay. In: Zanefeld LJD, Chatterton RT Jr, eds. Biochemistry of Mammalian Reproduction. New York, Wiley-Interscience, pp455–481, 1982.

12. King WJ, Green GL. Monoclonal antibodies localize oestrogen receptor in the nuclei of target cells. Nature (London) **307**:745–747, 1984.
13. Welshons WV, Lieberman ME, Gorski J. Nuclear localization of unoccupied oestrogen receptors. Nature (London) **307**:747–749, 1984.
14. Hickson RC, Galassi TM, Kurowski TT, Daniels DG, Chatterton RT Jr. Skeletal muscle cytosol [^{3}H]methyltrienolone receptor binding and serum androgens: Effects of hypertrophy and hormonal state. J Steroid Biochem **19**:1705–1712, 1983.
15. Scatchard G. The attractions of proteins for small molecules and ions. Ann NY Acad Sci **51**:660–672, 1949.
16. Rosenthal HE. A graphic method for the determination and presentation of binding parameters in a complex system. Anal Biochem **20**:525–532, 1967.
17. Kurowski TT, Chatterton RT Jr, Hickson RC. Countereffects of compensatory overload and glucocorticoids in skeletal muscle: Androgen and glucocorticoid cytosol receptor binding. J Steroid Biochem **21**:137–145, 1984.
18. Klotz IM. Numbers of receptor sites from Scatchard graphs: Facts and fantasies. Science (Washington, DC) **217**:1247–1249, 1982.
19. Dubé JY, Chapdelaine P, Tremblay RR, Bonne C, Raynaud J-P. Comparative binding specificity of methyltrienolone in human and rat prostate. Horm Res **7**:341–347, 1976.
20. Asselin J, Labrie F, Gourdeau Y, Bonne C, Raynaud J-P. Binding of [^{3}H]methyltrienolone (R1881) in rat prostate and human benign prostatic hypertrophy (BPH). Steroids **28**:449–459, 1976.
21. Raynaud JP, Bouton MM, Moguilewsky M, Ojasoo T, Philibert D, Beck G, Labrie F, Mornon JP. Steroid hormone receptors and pharmacology. J Steroid Biochem **12**:143–157, 1980.
22. Naess O. Characterization of the androgen receptors in the hypothalmus, preoptic area and brain cortex of the rat. Steroids **27**:167–185, 1976.
23. Wilson EM, French FS. Binding properties of androgen receptors. Evidence for identical receptors in rat testis, epididymis, and prostate. J Biol Chem **251**:5620–5629, 1976.
24. Asselin J, Melancon R. Characteristics of androgen binding in rat adrenal tissue using [^{3}H]methyltrienolone as tracer. Steroids **30**:591–604, 1977.
25. Max SR, Mufti S, Carlson BM. Cytosolic androgen receptor in regenerating rat levator ani muscle. Biochem J **200**:77–82, 1981.
26. Max SR. Cytosolic androgen receptor in skeletal muscle from normal and testicular feminization mutant (Tfm) rats. Biochem Biophys Res Commun **101**:792–799, 1981.
27. Michel G, Baulieu E-E. Recepteur cytosoluble des androgenes dans un muscle strie squelettique. CR Acad Sci (Paris) **279**:421–424, 1974.
28. Mayer M, Rosen F. Interaction of anabolic steroids with glucocorticoid receptor sites in rat muscle cytosol. Amer J Physiol **229**:1381–1386, 1975.

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