https://ntrs.nasa.gov/search.jsp?R=19840019326 2020-03-20T23:00:17+00:00Z

# General Disclaimer

## One or more of the Following Statements may affect this Document

- This document has been reproduced from the best copy furnished by the organizational source. It is being released in the interest of making available as much information as possible.

- This document may contain data, which exceeds the sheet parameters. It was furnished in this condition by the organizational source and is the best copy available.

- This document may contain tone-on-tone or color graphs, charts and/or pictures, which have been reproduced in black and white.

- This document is paginated as submitted by the original source.

- Portions of this document are not fully legible due to the historical nature of some of the material. However, it is the best reproduction available from the original submission.

Produced by the NASA Center for Aerospace Information (CASI)

(NASA-CR-173630) SEX STEROIDS DO NOT AFFECT MUSCLE WEIGHT, OXIDATIVE METABOLISM OR CYTOSOLIC ANDROGEN RECEPTION BINDING OF FUNCTIONALLY OVERLOADED RAT PLANTARIS MUSCLES (Maryland Univ.) 23 p HC A02/MF A01 G3/51

N84-27394

Unclas
13641

# SEX STEROIDS DO NOT AFFECT MUSCLE WEIGHT, OXIDATIVE METABOLISM, OR CYTOSOLIC ANDROGEN RECEPTOR BINDING OF FUNCTIONALLY OVERLOADED RAT PLANTARIS MUSCLES

Stephen R. Max and Naomi Rance*

Department of Neurology
University of Maryland
School of Medicine
Baltimore, Maryland 21201

Running Title: Sex Steroids and Muscle Hypertrophy


RECEIVED
A.I.A.A.
1983 MAY -6 AM 9:36
T. I. S. LIBRARY

Send proofs to:

Stephen R. Max, Ph.D.
Department of Neurology
University of Maryland
School of Medicine
Baltimore, Maryland 21201
(301) 528-3436

*Present address:

Department of Pathology
Johns Hopkins University
School of Medicine
Baltimore, Maryland 21205

Synopsis:

We studied the effects of sex steroids on muscle weight and oxidative capacity of rat plantaris muscles subjected to functional overload by removal of synergistic muscles. Ten weeks after bilateral synergist removal, plantaris muscles were strikingly hypertrophic compared with unoperated controls. After this period there were selective alteratiors in the ability of the muscles to oxidize three substrates of oxidative metabolism. Thus, glucose [6-$^{14}$C] oxidation did not change on the basis of total activity (nmols $^{14}CO_2$/muscle/hour), while on a specific activity basis (nmols $^{14}CO_2$/mg non-collagen protein/hour) it was significantly reduced. Oxidation of ß-hydroxybutyrate (3-$^{14}$C) was not altered on the basis of either total or specific activity. The oxidation of pyruvate [2-$^{14}$C] was increased in overloaded muscle as total activity, but was not altered as specific activity. Succinate dehydrogenase specific activity was decreased in overloaded muscle while total activity was not altered. There was no effect of sex hormone status on any of these parameters. Finally functional overload did not influence cytosolic androgen receptor binding. These results are not consistent with the idea of synergistic effects of sex steroids and muscle usage.

## INTRODUCTION

Anabolic steroids currently enjoy widespread use as concomitants of physical training programs under the assumption that such use will enhance muscle bulk and strength. This assumption, however, is not generally supported by clinical studies in man (30). To date, few animal experiments have been conducted regarding weight-bearing. A commonly employed experimental model for weight-bearing and excessive use is compensatory overload of muscle, produced by surgical ablation of synergists. As noted by Baldwin et al. (1), the terms compensatory hypertrophy and functional overload are not specific. The terms simply imply that passive and active mechanical forces provide physical stress to the muscle (1, 29). Our initial study of oxidative metabolism in this model was made on male rats (8); most subsequent studies have employed females (1-3, 29). Ianuzzo and Chen (18) found no differences between males and females in degree of hypertrophy or in the activities of a number of enzymes at 30-day post-synergist removal. In the present report, we compare results on rats of both sexes, and on male rats subjected to bilateral orchiectomy and hormone replacement. The experiments to be described revealed no effects of hormone status; and thus they do not support the hypothesis that anabolic steroids provide beneficial effects on overloaded muscle. In addition, we found no effect of functional overload of muscle on cytosolic androgen receptor binding, further suggesting lack of synergistic effects of anabolic steroids and increased muscle usage. The data do suggest, however, that selective adaptions in muscle enzyme systems accompany muscle hypertrophy following functional overload.

## METHODS

Experimental protocol. We used 50 male and 10 female rats of the CD strain (Charles River Breeding Labs, Wilmington, MA), initially weighing about 200 g and provided with food and water ad libitum. Males were assigned to one of 5 groups: (I) intact gonads, no overload (i.e., synergist removal), no testosterone-propionate (TP) treatment; (II) intact gonads, overload, no TP; (III) orchiectomized (GDX), overload, no TP; (IV) intact gonads, overload, TP-treated; (V) GDX, overload, TP-treated. Females were assigned to one of 2 groups of 5 rats: (VI) intact gonads, no overload; and (VII) intact gonads, overload. The compositions of these groups are summarized in Table I.

Surgical procedures and hormone administration. Rats were anesthetized with chloral hydrate (400 mg/kg, i.p.). Removal of gastrocnemius and soleus muscles was performed bilaterally as described by Baldwin et al. (1). The rats were studied 10 weeks after surgery. We used the bilateral approach because it resulted in a greater hypertrophic response rather than the unilateral one used in our earlier study (8).

Testosterone propionate (Sigma) was administered by subcutaneous implantation of 62.5 mm lengths of Dow-Corning Silastic medical grade tubing (outer diameter, 0.125 inches; inner diameter, 0.062 inches) filled with crystalline hormone. This procedure results in delivery of 2.5 mg hormone/day (5). Control rats received similar Silastic implants containing cholesterol.

Bilateral orchiectomy (GDX) was carried out via the abdominal route under ether anesthesia.

### Biochemical Determinations

Substrate oxidation and succinate dehydrogenase. Rats were decapitated,

ORIGINAL PAGE IS
OF POOR QUALITY



and muscles were removed, trimmed of extraneous tissues, minced with scissors, and homogenized by hand at 0-4°C with 15 strokes of a TenBroeck homogenizer (1:20, w/v) in ice-cold Chappell-Perry medium (9), which comprises 100 mM KCl, 100 mM Tris-HCl (pH 7.4 at 20°C), 5 mM $MgSO_4$, 1 mM EDTA, and 1 mM ATP.

For measurement of substrate oxidation, incubation was carried out as described (27) in 16 x 100 mm biosilicate glass test tubes using 200 µl homogenate and 250 µl of [$^{14}$C-2] pyruvate, [6-$^{14}$C] glucose, or [3-$^{14}$C] β-hydroxybutyrate in a medium modified from that of Beatty et al. (4), consisting of 70 mM KCl, 1 mM ATP, 1 mM ADP, 4 mM CoA, 0.5 mM EDTA, mM NADP, 0.5 mM NAD, 60 mM sucrose, and 100 mM Tris-HCl (pH 7.4 at 20°C). Glucose was employed at a concentration of 5 mM β-hydroxybutyrate at 10 mM, and pyruvate at 5 mM. Incubations with pyruvate also contained 0.1 mM malate (3). About 2 x $10^5$ dpm of labeled substrate were employed. Tubes were sealed with serum caps fitted with hanging center wells containing a fluted filter paper soaked with 300 µl hyamine hydroxide and were incubated at 37°C in a Dubnoff shaking water bath for 30 min. The reaction was terminated by injecting 200 µl of 10% TCA. The tubes were then incubated for an additional 45 min at 37°C. Center wells were severed directly into scintillation vials and counted in a liquid scintillation spectrometer at approximately 84% efficiency. The production of $^{14}CO_2$ from [6-$^{14}$C] glucose, [$^{14}$C-2] pyruvate and [$^{14}$C-3] β-hydroxybutyrate was linear with respect to time and protein concentration within the ranges employed in this study (cf. 13). The release of $^{14}CO_2$ from these substrates was inhibited by 0.02 M NaCN.

Succinate dehydrogenase was assayed in a 400 x g supernatant prepared from an aliquant of the above homogenates by the method of Bonner (6).

ORIGINAL PAGE IS
OF POOR QUALITY



The $^{14}CO_2$ production and succinate dehydrogenase data are expressed using 2 reference bases. The first is total muscle activity. This mode of expression is commonly employed in situations in which a tissue is undergoing weight changes; the results are not influenced by hypertrophy. The second means of expressing the data is as specific activity, i.e., with respect to non-collagen protein (20), determined as described (26), using the method of Lowry (21).

Cytosolic androgen receptor binding. Plantares from separate groups of rats were employed. These muscles were removed from decapitated rats, dissected free of extraneous tissues, minced, and frozen in liquid $N_2$. The frozen pieces of muscle were pulverized using a mortar and pestle cooled with liquid nitrogen. The powder was then homogenized (1:2, w/v) in buffer comprising 0.05 mM Tris, 0.1 mM EDTA, and 0.25 mM dithiothreitol, pH 7.4 at 20°C, using 3 5-sec. bursts with a Polytron homogenizer at setting #5. The homgenate was centrifuged at 110,000 x g in a Sorvall OTD-50 preparative ultracentrifuge. The supernatant fraction (cytosol) was used for determination of specific binding of [$^3$H] methyltrienolone (New England Nuclear) with inclusion of triamcinolone acetonide (16, 33). The pellet was used for DNA assay (7). Specific methyltrienolone binding was determined as described in detail in (23) and (24), except that bound and unbound ligand were separated on columns of Sephadex G-75. Scatchard analysis (not shown) revealed binding parameters identical to those previously published from this laboratory (23).

For the studies described herein we used a "one-point" assay at a saturating concentration of [$^3$H] methyltrienolone (8 nM). This was necessary because paucity of muscle material precluded determination of a full binding isotherm. Specific binding of this synthetic androgen is expressed as fmoles/mg DNA, determined according to Burton (7).

ORIGINAL PAGE IS
OF POOR QUALITY



Statistical determinations. These were made using a t-test or a one-way analysis of variance.

RESULTS

Muscle wet weight and non-collagen protein (NCP). Muscle wet weight was normalized to per cent body weight to avoid subtle influences of small body weight variations. As seen in Table II, all hypertrophy groups, irrespective of hormone status (i.e., intact gonads, GDX or TP-treated) showed striking increases in muscle wet weight compared with intact gonads, no overload, no hormone-treatment controls. It can be seen that TP treatment had no significant effect on muscle wet weight in any group, and males and females did not respond differently (Table III). However, total NCP content (per muscle) was influenced by the presence of TP (Table III). There was no effect in these same animals on NCP concentration (i.e., mg NCP/gram wet weight). Again there was no significant difference between males and females (Table III).

The experimental treatments did not result in a uniform change in the pattern of oxidation of the 3 substrates. Thus, [6-$^{14}$C] glucose oxidation, when expressed as total activity, either did not change or was somewhat diminished among the various treatment groups (Table IV). [3-$^{14}$C] β-hydroxybutyrate oxidation behaved differently; there were no significant differences among any of the groups, either in terms of total activity or specific activity (Table V). [2-$^{14}$C] pyruvate oxidation (total activity) was increased as a function of overload compared with unoperated controls, although there was no difference in this increase between males and females (+33% in males and +33% in females). On a specific activity basis there were no significant differences in [2-$^{14}$C] pyruvate oxidation (Table VI).

ORIGINAL PAGE IS
OF POOR QUALITY

Succinate dehydrogenase specific activity (Table VII) was significantly decreased in the 2 TP-treatment groups compared with intact or GDX animals without hormone.

Finally, the overload paradigm had no effect on the specific cytosolic androgen receptor binding in plantaris muscles (Table VIII). As reported by us (25) and by others (10), the level of receptors increased following gonadectomy and returned to control levels following androgen repletion. These changes were uninfluenced by synergist removal.

DISCUSSION

The data described above confirm and extend the findings on oxidative metabolism of hypertrophic muscle from this laboratory (8) and of others (1-3; 18). In the present work we employed the bilateral surgical procedure of Baldwin et al. (1) rather than the unilateral approach we previously employed (8). Indeed, the bilateral approach and the longer time-course resulted in doubling of muscle wet weight. Values reported from other studies range from +40% (1) to double (29). Most studies were done in female rats. We employed males and females in the present study; we found no differences in response to synergist removal.

Our results on substrate oxidation are different from those we originally reported, probably owing to the bilateral operation and the longer time-course studied. Thus, on a total activity basis oxidation of [6-$^{14}$C] glucose was decreased, that of [3-$^{14}$C] ß-hydroxybutyrate was unchanged, while oxidation of [2-$^{14}$C] pyruvate was increased. The conclusion of our early work on short-term overload (8) was that the mitochondrial function was diluted in relation to other fractions during compensatory growth. This conclusion is supported, to a certain extent by Baldwin et al. (1, 3) who further suggested that selective changes might occur independent of muscle enlargement (1, 3). Our new data argue for selective changes

ORIGINAL PAGE IS
OF POOR QUALITY



after long-term hypertrophy.

It has been asserted (3) that oxidation of [6-$^{14}$C] glucose may be independent of mitochondrial function and that the decrease noted earlier (8), and hence in the present case, may reflect reported decreases in glycolytic enzyme activities (3). However, the maximum capacity of the glycolytic enzymes exceeds by 10-100 fold the levels noted for basal glycolysis (31). Therefore, small changes in glycolytic enzymes (1) may make little impact on the flux of glucose through glycolysis; oxidation of [6-$^{14}$C] glucose therefore reflects mitochondrial activity.

Succinate dehydrogenase was not different from control in overloaded plantaris muscles (with or without alterations of hormone status) (Table VII). This is of interest in view of reports that mitochondrial density is increased in hypertrophic muscles (22, 23).

The major result of this study, however, concerns the lack of beneficial effect of sex-hormone status on the process of hypertrophy and on the biochemical changes in overloaded muscle. Testosterone propionate had no effect on muscle weight gain or substrate metabolism 10 weeks after synergist removal in male or female rats. The lack of sex difference is in agreement with the result of Ianuzzo and Chen (18), whose experiments were performed 30 days after synergist removal. TP did, however, increase total NCP per muscle (Table III), but not protein concentration. Excepting a few small changes, neither GDX nor TP therapy altered the response of oxidative activity to functional overload.

The lack of effect of TP on muscle weight is curious in view of the well known anabolic effects of androgens (15), and in view of the report by Evans and Ivy (14) that TP had a significant effect in increasing the size of muscles undergoing atrophy secondary to limb immobilization. The result of Evans and Ivy (14) may be attributable to inhibitory effects

of TP on muscle protease activities (11, 12), as well as to stimulation of protein synthesis. Such effects were apparently not manifested in the present study.

Finally, we measured cytosolic androgen receptor binding in muscles undergoing compensatory hypertrophy. We saw no effect of synergist removal on receptor binding. This lack of an enhancement of receptors may be related to the poor responses noted above. Rogozkin (28) reported an increase in the number of cytosolic androgen receptors in muscle from rats subjected to endurance training. Our discrepant result may be related to differences between these models of muscle usage.

ACKNOWLEDGEMENTS

We thank Drs. Barbara H. Sohmer and J. Tyson Tillon for helpful comments, Ms. C. Matthias for expert technical assistance, and Ms. B. Pasko for preparation of the typescript. Supported by grants from NASA (NAG 2-100), NIH (NS 15766), and by the Short-term Research Program of the University of Maryland School of Medicine.

ORIGINAL PAGE IS
OF POOR QUALITY



REFERENCES

1. Baldwin, K. M., W. G. Cheadle, D. M. Martinez, and D. A Cooke. Effect of functional overload on enzyme levels in different types of skeletal muscle. J. Appl. Physiol: Respirat. Environ. Exercise Physiol. 42: 312-317, 1977.

2. Baldwin, K. M., V. Valdez, R. E. Herrick, A. M. MacIntosh, and R. R. Roy. Biochemical properties of overloaded fast-twitch skeletal muscle. J. Appl. Physiol.: Respirat. Environ. Exercise Physiol. 52: 467-472, 1982.

3. Baldwin, K. M., V. Valdez, L. F. Schrader, and R. E. Herrick. Effect of functional overload on substrate oxidation capacity of skeletal muscle. J. Appl. Physiol.: Respirat. Environ. Exercise Physiol. 50: 1272-1276, 1981.

4. Beatty, C. H., M. K. Young, and R. M. Bocek. Respiration and metabolism by homogenates of various types of muscle. Am. J. Physiol. 223:1232-1236, 1972.

5. Berndtson, W. E., C. Desjardins, and L. L. Ewing. Inhibition and maintenance of spermatogenesis in rats implanted with polydimethylsiloxane capsules containing various androgens. J. Endocrinol. 62: 1765-1767, 1982.

6. Bonner, W. D. Succinic dehydrogenase. Adv. Enzymol. 1:722-729, 1955.

7. Burton, K. A study of the conditions and mechanism of the diphenylamine reaction for the colorimetric estimation of deoxyribonucleic acid. Biochem. J. 62:315-323, 1956.

8. Carlo, J. W., S. R. Max, and D. H. Rifenberick. Oxidative metabolism of hypertrophic skeletal muscle in the rat. Exp. Neurol. 48:222-230, 1975.

ORIGINAL PAGE IS
OF POOR QUALITY



9. Chappell, J. B. and S. V. Perry. Biochemical and osmotic properties of skeletal muscle mitochondria. Nature (London) 173:1091-1095, 1953.

10. Dahlberg, E., M. Snochowski, and J.-A. Gustaffson. Regulation of the androgen and glucocorticoid receptors in rat and mouse skeletal muscle cytosol. Endocrinology 108:1431-1440, 1981.

11. Dahlmann, B., B. Mai, and H. Reinauer. The influence of testosterone on the alkaline proteolytic activity in rat skeletal muscle. Biochim. Biophys. Acta 641:479-486, 1980.

12. Dahlmann, B., A. Widjaja, and H. Reinauer. Antagonistic effects of endurance training and testosterone on alkaline proteolytic activity in rat skeletal muscles. Eur. J. Appl. Physiol. 46:229-235, 1981.

13. DuBois, D. C. and S. R. Hoy. Effect of denervation and reinnervation on oxidation of [6-$^{14}$C] glucose by rat skeletal muscle homogenates. J. Neurochem. 40:727-733, 1983.

14. Evans, W. J., and J. L. Ivy. Effects of testosterone propionate on hindlimb-immobilized rats. J. Appl. Physiol. Respirat. Environ. Exercise Physiol. 52:1643-1647, 1982.

15. Grigsby, J. S., W. G. Berger, and R. A. Merkel. The effect of testosterone on skeletal muscle development and protein synthesis in rabbits. Growth 40:303-316, 1976.

16. Ho-Kim, M. A., R. R. Tremblay, and J. Y. Dubé. Binding of methyltrienolone to glucocorticoid receptors in rat muscle cytosol. Endocrinology 109:1418-1423, 1981.

17. Hubbard, R. W., C. D. Ianuzzo, W. T. Matthew, and J. D. Linduska. Compensatory adaptations of skeletal muscle composition to a long-term functional overload. Growth 39:85-93, 1975.

18. Ianuzzo, C. D. and V. Chen. Metabolic character of hypertrophied rat muscle. J. Appl. Physiol.: Respirat. Environ. Exercise Physiol. 46:738-742, 1979.

ORIGINAL PAGE IS
OF POOR QUALITY

19. James, N. T. Studies on the responses of different types of muscle fiber during surgically induced compensatory hypertrophy. J. Anat. 129:769-776, 1979.

20. Lilienthal, J. L., K. L. Zierler, B. P. Folk, R. J. Boka, and M. J. Riley. A reference base and system for analysis of muscle constituents. J. Biol. Chem. 182:501-508, 1949.

21. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193:265-275, 1951.

22. MacDougall, J. D., D. G. Sale, J. R. Moroz, G. C. B. Elder, J. R. Sutton, and H. Howard. Mitochondrial volume density in human skeletal muscle following heavy resistance training. Med. Sci. Sport. 11:164-166, 1979.

23. Max, S.R.: Cytosolic androgen receptor in muscle from normal and testicular feminization mutant (Tfm) rats. Biochem. Biophys. Res. Commun. 101:792-799, 1981.

24. Max, S. R., S. Mufti, and B. M. Carlson. Cytosolic androgen receptor in regenerating rat levator ani muscle. Biochem. J. 200:77-82, 1981.

25. Rance, N. and S. R. Max. Modulation of cytosolic androgen receptor level in striated muscle by sex steroids. Endocrine Society Abstracts, In press, 1983.

26. Rifenberick, D. H., C. L. Koski, and S. R. Max. Metabolic studies of skeletal muscle regeneration. Exp. Neurol. 45:527-540, 1974.

27. Rifenberick, D. H., and S. R. Max. Substrate utilization by disused rat skeletal muscles. Amer. J. Physiol. 226:295-297, 1974.

28. Rogozkin, V. A. Anabolic steroid metabolism in skeletal muscle. J. Steroid. Biochem. 11:923-926, 1979.

ORIGINAL PAGE IS
OF POOR QUALITY



29. Roy, R. R., I. D. Meadows, K. M. Baldwin, and V. R. Edgerton. Functional significance of compensatory overloaded rat fast muscle. J. Appl. Physiol. Respirat. Environ. Exercise Physiol. 52:475-478, 1982.

30. Ryan, A. J. Anabolic steroids are fool's gold. Federation Proc. 40:2682-2688, 1981.

31. Scrutton, M. C. and M. F. Utter. The regulation of glycolysis and gluconeogenesis in small animal tissues. Ann. Rev. Biochem. 37: 249-302, 1968.

32. Seiden, D. A quantitative analysis of muscle cell change in compensatory hypertrophy and work-induced hypertrophy. Am. J. Anat. 145:459-468, 1976.

33. Zava, D. T., B. Landrum, K. B. Horwitz, and W. L. McGuire. Androgen receptor assay with [$^3$H] methyltrienolone (R1881) in the presence of progesterone receptors. Endocrinology 104:1007-1012, 1979.

**TABLE I**

**Summary of Treatments**

| Group | Sex | GDX | Overload | TP |
|---|---|---|---|---|
| I | Male | (-) | (-) | (-) |
| II | Male | (-) | (+) | (-) |
| III | Male | (+) | (+) | (-) |
| IV | Male | (-) | (+) | (+) |
| V | Male | (+) | (+) | (+) |
| VI | Female | (-) | (-) | (-) |
| VII | Female | (-) | (+) | (-) |

ORIGINAL PAGE IS
OF POOR QUALITY



TABLE II

Wet Weight and Non-Collagen Protein Content

of Hypertrophic Plantaris Muscles

| | Group | n | Muscle Weight (% body wt.) | Non-Collagen Protein (mg/muscle) | (mg/gram wet wt.) |
|---|---|---|---|---|---|
| (I) | intact gonads, no overload | 5 | 0.12 ± 0.03 | 54.30 ± 5.25 | 0.13 ± 0.01 |
| (III) | orchiectomy, overload | 4 | 0.24 ± 0.02* | 73.30 ± 3.56 | 0.096 ± 0.01 |
| (IV) | intact gonads, overload, TP | 6 | 0.26 ± 0.02* | 98.20 ± 12.88$^{\delta}$ | 0.13 ± 0.01 |
| (V) | orchiectomy, overload, TP | 5 | 0.22 ± 0.01* | 93.00 ± 5.21$^{\delta}$ | 0.15 ± 0.01 |

*Significantly different from control, $p < 0.05$. $^{\delta}$Significantly different from groups I and III, $p < 0.05$. Data are means ± SEM. Experimental procedures are described in the text.

ORIGINAL PAGE IS
OF POOR QUALITY

**TABLE III**

**Wet Weight and Non-Collagen Protein Content of Hypertrophic Plantaris Muscles from Male and Female Rats**

| | Group | Muscle Weight (% body wt.) | Non-Collagen Protein (mg/muscle) | Non-Collagen Protein (mg/gram wet wt.) |
|---|---|---|---|---|
| (I) | males, no overload | 0.10 ± 0.002 | 36.34 ± 3.75 | 0.15 ± 0.01 |
| (II) | males, overload | 0.25 ± 0.005* | 75.96 ± 2.37* | 0.13 ± 0.01 |
| (VI) | females, no overload | 0.10 ± 0.002 | 40.96 ± 1.44 | 0.15 ± 0.01 |
| (VII) | females, overload | 0.26 ± 0.002* | 82.04 ± 5.31* | 0.13 ± 0.01 |

Data are means ± SEM of 5 determinations. *Significantly different from controls of the same sex, $p < 0.05$. Experimental procedures are described in the text.

ORIGINAL PAGE IS
OF POOR QUALITY

TABLE IV

**Oxidation of [6-$^{14}$C] Glucose by Hypertrophic Plantaris Muscles from Male Rats**

| | Group | n | Specific Activity (nmols/mg NCP/ 30 min.) | Total Activity (nmols/muscle/ 30 min.) |
|---|---|---|---|---|
| (I) | intact gonads, no overload | 5 | 0.79 ± 0.08 | 42.39 ± 4.84 |
| (III) | orchiectomy, overload | 4 | 0.49 ± 0.08* | 33.37 ± 4.98 |
| (IV) | intact gonads, overload, TP | 6 | 0.27 ± 0.07** | 24.02 ± 5.68 |
| (V) | orchiectomy, overload, TP | 5 | 0.35 ± 0.04** | 32.36 ± 2.55 |

*Significantly different from group I, $p < 0.025$, **$p < 0.0025$. Data are means ± SEM.

Experimental procedures are described in the text.

ORIGINAL PAGE IS
OF POOR QUALITY

TABLE V

Oxidation of [3-$^{14}$C] β-hydroxybutyrate Hypertrophic Plantaris Muscles from Male Rats

| | Group | n | Specific Activity (nmols/mg NCP/ 30 min.) | Total Activity (nmols/muscle/ 30 min.) |
|---|---|---|---|---|
| (I) | intact gonads, no overload | 5 | 0.59 ± 0.12 | 30.43 ± 7.18 |
| (III) | orchiectomy, overload | 4 | 0.54 ± 0.18 | 38.73 ± 10.20 |
| (IV) | intact gonads, overload, TP | 6 | 0.56 ± 0.16 | 43.36 ± 3.36 |
| (V) | orchiectomy, overload, TP | 5 | 0.40 ± 0.08 | 36.83 ± 5.23 |

Data are means ± SEM. Experimental procedures are described in the text.

TABLE VI

Oxidation of [2-$^{14}$C] Pyruvate by Hypertrophic Plantaris Muscles of Male and Female Rats

| | Group | n | Specific Activity (nmols/mg NCP/ 30 min.) | Total Activity (nmols/muscle/ 30 min.) |
|---|---|---|---|---|
| (I) | males, no overload | 5 | 38.96 ± 3.26 | 1484 ± 178 |
| (II) | males, overload | 5 | 27.70 ± 2.15 | 2231 ± 156* |
| (VI) | females, no overload | 5 | 44.75 ± 3.16 | 1813 ± 105 |
| (VII) | females, overload | 5 | 32.10 ± 3.28 | 2614 ± 285* |

*Significantly different from control, $p < 0.05$. Data are means ± SEM. Experimental procedures are described in the text.

TABLE VII

Succinate Dehydrogenase Activity of Hypertrophic Plantaris Muscles from Male Rats

| | Group | n | Specific Activity ( O.D./min/ mg NCP) | Total Activity ( O.D./min/muscle) |
|---|---|---|---|---|
| (I) | intact gonads, overload | 5 | 0.02 ± 0.04 | 0.58 ± 0.08 |
| (III) | orchiectomy, overload | 6 | 0.007 ± 0.0013 | 0.54 ± 0.09 |
| (IV) | intact gonads, overload, TP | 4 | 0.005 ± 0.0003* | 0.43 ± 0.06 |
| (V) | orchiectomy, overload, TP | 5 | 0.005 ± 0.0007* | 0.45 ± 0.05 |

Data are means ± SEM. Experimental procedures are described in the text. *Significantly different from group I, $p < 0.025$.

## TABLE VIII

### Cytosolic Androgen Receptor Binding of Hypertrophic Plantaris Muscles from Male Rats

| | Group | [$^3$H] Methyltrienolone Specific Binding | |
|---|---|---|---|
| | | fmols/mg prot. | fmols/mg DNA |
| (I) | intact gonads, no overload | 1.14 ± 0.10 | 68.1 ± 14.4 |
| (II) | intact gonads, overload | 0.93 ± 0.06 | 53.5 ± 3.3[6] |
| (III) | orchiectomy, overload | 1.27 ± 0.07* | 79.7 ± 4.4* |
| (V) | orchiectomy, overload, TP | 0.95 ± 0.08 | 59.9 ± 4.7 |

Data are means ± SEM of 5 determinations. *Different from groups II and V, $p < .01$, [6]different from group I, $p < .05$. Experimental procedures are described in the text.