DOES TRENBOLONE OR RESISTANCE EXERCISE REVERSE HYPOGONADISM INDUCED BONE AND MUSCLE LOSS?

By

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To my loving parents, my in-laws, my family and friends and Sandy and Liam

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DOES TRENBOLONE OR RESISTANCE EXERCISE REVERSE HYPOGONADISM INDUCED BONE AND MUSCLE LOSS?

By

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Supraphysiologic testosterone (T) administration attenuates high turnover osteopenia in orchiectomized (ORX) rats; however T may increase prostate mass. Trenbolone enanthate (TREN) is a highly anabolic synthetic T analogue, which is purported to result in less prostate enlargement than T. The purpose of this experiment was to determine if T, TREN, and weighted ladder climbing (EX) prevent muscle and bone loss, effect prostate mass, fat mass, bone hormone and hemoglobin concentrations, and Pax7 positive satellite cells. Fifty, 10 month old male F344/Brown Norway rats were randomized into SHAM, ORX, ORX+EX, ORX+T, and ORX+TREN groups. The prostate, retroperitoneal fat pad, flexor hallicus longus (FHL), semimembranosus (SEMI), soleus, plantaris and levator ani bulbocavernosus (LABC) muscles were removed and weighed. The FHL and SEMI were examined for Pax7 expression. Femurs were analyzed by pQCT, while tibiae were evaluated for bone hormone concentrations by EIA. Differences were evaluated using a One Way ANOVA with a Tukey's post hoc. No significant change was observed in the mass of the FHL, SEMI, soleus or plantaris. However, significant increases were seen in the LABC and hemoglobin concentrations of T and TREN treated animals while both animals reduced

retroperitoneal fat pad mass. ORX reduced trabecular and cortical bone density compared with SHAMs. EX did not prevent ORX-induced bone loss. Conversely, TREN and T and maintained tBMC, tBMD, and cBMD at control values. Both ORX groups, prostate masses were reduced by 71% compared to SHAMs. TREN caused non-significant 20% reductions in prostate mass compared with SHAM; while T nearly doubled prostate mass ($p<0.05$). Conclusions: T and TREN prevent some indices of hypogonadism-induced muscle and bone loss, and decreased visceral adiposity. Progressive resistance exercise offered no protection to muscle and bone. An added benefit of TREN was the maintenance of prostate mass at SHAM levels.

CHAPTER 1
INTRODUCTION

In males, aging results in a variety of physical decrements, including loss of skeletal muscle mass and function (i.e., sarcopenia), and reduced bone mineral density (BMD) (i.e., osteopenia), which combine to increase fall risk and consequently bone fracture risk (1). Recent guidelines from the Endocrine Society's Clinical Practice Guidelines (2010) suggest evaluating patients for initial treatment of hypogonadism when serum testosterone concentrations are less than10.4 nmol/L and patients present with additional clinical manifestations. Hypogonadism is one of several factors that underlie both sarcopenia and osteopenia (2, 3). The incidence of hypogonadism increases with age, such that approximately 20% of the male population meets the clinical definition of hypogonadism by 60 years of age, while nearly 50% of those older than 80 years of age experience hypogonadism (4). Hypogonadism may occur as a result of primary (i.e., testicular level of failure) or secondary testicular failure (i.e., central defects of the hypothalamus or pituitary), or a combination of primary and secondary testicular failure (i.e., involving both the testis and pituitary) (5). However, treatment of older hypogonadal men with replacement doses of testosterone produces only modest improvements in skeletal muscle mass/strength and BMD (2, 3); whereas, high-dose testosterone administration produces robust improvements in both muscle mass (6) and BMD (7) in humans and animal models (8-11). However, testosterone administration is associated with a variety of side-effects of which prostate enlargement and polycythemia are most prevalent (12). Thorough routine clinical follow-up and proactive monitoring of potential patient side effects can improve the benefit to risk ratio of testosterone replacement therapy. The combination of side effects and unknown

optimal dosing regimens caused the Endocrine Society Clinical Practice Guidelines to recommend rigorous monitoring of hematocrit, LH and FSH for primary and secondary causes of hypogonadism, prostate specific antigen (PSA) levels, and gross prostate examinations for individuals receiving testosterone replacement therapy (5). The Institute of Medicine has requested that small and medium sized trials be conducted to assess the safety and efficacy of testosterone replacement therapy for the treatment of a variety of conditions associated with hypogonadism, including sexual dysfunction, cognitive impairment, depression, muscle weakness, and osteoporosis from Institute of Medicine 2003 position statement. Additionally, examining alternative pharmacological therapies and non-pharmacological interventions which may enhance skeletal muscle mass/strength and BMD, without causing prostate enlargement, or exacerbating polycythemia may improve treatment options for hypogonadism-induced sarcopenia and osteopenia.

Osteoporosis affects nearly 200 million individuals worldwide making it the most prevalent metabolic bone disorder in the world (13), with falls contributing over $15 billion to US healthcare costs annually (14). Males represent approximately 25% of the total incidence of osteoporosis and the estimated cost of osteoporotic bone fractures in men exceeds $4 billion per year (15). The lifetime incidence of hip fracture in white males residing in the US approximates 5%, while black males residing in the US have approximately a 50% lower lifetime incidence risk (16). Women experience a higher incidence of osteoporotic fractures than men, although men experience a higher mortality, at least following hip fracture. In one UK based study, the likelihood of mortality following a hip fracture was eightfold higher in men compared to women (17).

Specifically, in the VA Healthcare System, men who experience a hip fracture have an approximate 32% mortality rate within 12 months of fracture incidence (18), with higher mortality rates remaining higher than women for two years post fracture (17) demonstrating the severity of this condition. Further, elderly individuals may also experience the physical and emotional burden of residual pain, short- or long-term disability, and possible deformities that limit quality of life, and perhaps a loss of skeletal muscle mass and strength following osteoporotic fractures (19).

Sarcopenia (i.e., age related loss of skeletal muscle mass) is also associated with low BMD and occurrence of falls, suggesting that sarcopenia increases osteoporotic fracture risk in the elderly (20). It is estimated that complications from falls are the sixth leading cause of death (21). Sarcopenia is specifically defined as height adjusted muscle mass score two or more standard deviations below the mean of young adults (22, 23). The definition of sarcopenia has recently been established as a multifactorial geriatric syndrome comprised of low muscle mass, impaired muscular strength, lifestyle, chronic disease, genetic susceptibility factors presenting as disordered mobility, disability, impaired quality of life, morbidity, and mortality (24). Hallmarks of sarcopenia include walking speeds of less than 1m/s and a mean lean body to fat mass ratio more than two standard deviations greater than normative data for young adults (25). Current population estimates place 45% of the population aged 60 years and older in the category of moderate to severe sarcopenia (26). The European Union Geriatric Medicine Society recently proposed classification of sarcopenia into primary (i.e., confined to age related changes), and secondary when loss of muscle mass and function is related to another disease or condition (27). Several factors may predispose

individuals to sarcopenia such as: loss of functional capacity due to disease or disuse, nutrient malabsorption or deficiency, and organ failure (27). Against the backdrop of the US population's advancing mean age, the economic impact of sarcopenia is estimated at 19 billion dollars annually making it a prominent public health concern (26). Thus, treatments which are designed to prevent the loss of both skeletal muscle mass and function, and BMD may reduce the morbidity and mortality associated with hypogonadism.

In addition, several common factors are implicated in the pathogenesis of both sarcopenia and osteopenia including reductions in endogenous sex-steroid concentrations (28), skeletal muscle apoptosis, and reduced physical activity (28). Recent research has attempted to determine the safety and efficacy of interventions designed to prevent both sarcopenia and osteopenia. Those interventions include treatment with testosterone (9, 29, 30) or selective androgen receptor modulators (SARMs) (31). Ideally, SARMs would selectively bind and activate the androgen receptors and produce anabolic responses in tissues of interest (e.g., muscle and bone), while avoiding androgenic activity in other tissues (e.g., prostate) (32).

Trenbolone is a synthetic analogue of testosterone. The affinity of trenbolone for the human androgen receptor is 3 fold higher than that of testosterone and approximately equal to that of DHT (33, 34). Trenbolone, is capable of preventing hypogonadism-induced muscle loss in animal models (35-39) and does not induce prostate enlargement when administered in low doses (40, 41). However, Freyberger and colleagues reported increased ventral prostate mass of castrated male rats when trenbolone was administered orally for ten days at a dosage of 40mg/kg, whereas the

1.5mg/kg dosage tended to maintain prostate levels similar to controls (42). They reported trenbolone administration at both low and high dosages reduced prostate masses by nearly 78% (low-dosage) and 50% (high dosage) compared to animals which received subcutaneous testosterone propionate at a dosage of 0.4mg/kg (41). Similarly, Wilson and colleagues found that subcutaneous administration of trenbolone at doses below 200 micrograms/day for ten consecutive days did not significantly increase ventral prostate mass in immature male Sprague Dawley rats (34). Oral dosing with trenbolone resulted in approximately a 100 fold less induction of LABC mass compared to subcutaneous administration, similar to reduced bioavailability of oral administered testosterone proprionate (139). Wilson and colleagues state that trenbolone is not a substrate for the 5α-reductase enzyme (34), and that the levator ani bulbocavernosus complex does not contain 5α-reductase enzyme (34). Recently, Yarrow and colleagues reported significant increases of 35-40% hypertrophy in the levator ani bulbocavernosus complex compared to control animals, enhanced hemoglobin concentrations, and reduced prostate size by 34% in the low dose (1.0mg/week) trenbolone administration (40). Currently, our laboratory is assessing the most balanced dosage regimen to promote the desired anabolic/androgenic effects without inducing prostate enlargement (40, 41). Preliminary mechanistic evidence from the H295R human adrenocortical carcinoma cell line indicates that trenbolone decrease endogenous T production similar to other administered androgens. Trenbolone may also increase T metabolism through direct or indirect mechanims, and potentially upregulates the aromatase gene CYP19 *in-vitro* (43). Trenbolone concentrations greater than 0.78µg/L resulted in a four fold induction of estradiol in H295R cell lines

above values for testosterone and progresterone (43). This data may indicate a secondary effect of the trenbolone on steroidogenesis in the H295R bioassay, but may not reflect *in vivo* effects of trenbolone on other tissues. Yarrow and colleagues report that administration of trenbolone to rats increases serum, an estradiol-like or a trenbolone derived metabolite presenting as estradiol on commercially available radioimmunoassay (unpublished laboratory results). For this reason, future work with GC-MS or other advanced molecular techniques is necessary to determine the true identity of this metabolite. However, if trenbolone is truly a non-aromatizable androgenic compound as suggested by Wilson and colleagues then trenbolone may offer protection against hypogonadism-induced bone and skeletal muscle mass loss without estradiol-mediated side effect (i.e., gynecomastia) (34, 40). Specifically, androgenic and anti-glucorticoid effects may be induced by trenbolone administration, further work is necessary in determining how this compound effects both bone maintenance and development in males (29, 44-47). Estrogens are postulated to inhibit osteoclast activity (i.e., bone resorption), whereas non-aromotizable androgens (e.g., DHT) may induce bone formation and augment bone mass through enhanced anchorage of osteoblastic cells to the organic bone matrix (48). Thus, trenbolone may produce SARM-like activity, at least in skeletal muscle and prostate tissue. Recent data suggest that trenbolone may cause positive skeletal responses in orchedectomized male rats (40). Our laboratory assessed the dose dependent, anabolic/androgenic effects of trenbolone at dosages of 1.0mg/wk, 3.5mg/week or 7.0mg/week on skeletal muscle mass and bone mass prostate enlargement (40). These preliminary findings indicated that the low dose (1.0mg/wk) was most efficacious target dose of trenbolone administration for preserving

bone and muscle mass while minimizing prostate enlargement in orchiectomized rodents (40).

Progressive resistance training (PRT) (i.e., mechanical loading) is an established non-pharmacological modality that augments skeletal muscle mass and function, and BMD (49, 50), and may also reduce fracture and fall risk (51), at least in eugonadal elderly men. Few studies have specifically evaluated the skeletal muscle and bone responses to PRT in hypogonadal men or using hypogonadal animal models; however, several studies have shown that PRT combined with testosterone replacement resulted in significant improvement in leg lean muscle mass and strength compared to either PRT or testosterone alone, demonstrating that sex-hormones influence the skeletal muscle responses to PRT (52-56). Therefore, future research designed to evaluate the safety and efficacy of alternative therapies, such as trenbolone administration or PRT, using hypogonadal models may improve treatment options for both sarcopenia and osteopenia.

Specific Aim, Hypothesis, Rationale 1.

Specific Aim: To determine the effect of trenbolone-enanthate, progressive resistance exercise, and testosterone enanthate administration on skeletal muscle, bone, kidney, retroperitoneal fat pad mass, hemoglobin concentration, and prostate growth in a mature orchiectomized male rat model. **Hypothesis**: In mature orchiectomized male rats, 42 days of trenbolone-enanthate administration will attenuate the hypogonadism-induced skeletal muscle and bone loss associated with orchiectomy, maintain kidney mass, reduce retroperitoneal fat pad mass, and elevate circulating hemoglobin concentrations without inducing prostate enlargement. **Rationale:** Supraphysiological testosterone administration completely prevents the loss of skeletal

muscle mass and BMD in hypogonadal rodents, but results in significant prostate enlargement due to the 5α-reduction of testosterone to DHT. Trenbolone, a synthetic analogue of testosterone, reduces orchiectomy-induced skeletal muscle loss in rodents (34, 40), but is not thought to be a substrate for the 5α-reductase enzyme (34, 41) and thus should not induce prostate enlargement. Conversely, supraphysiological administration of testosterone-enanthate for 28 days has been shown to augment the anthropometric (i.e., length and mass) and biomechanical characteristics of the femur in both developing male and female rodents (11). However, in this study 10-month old rats are mature and present with closed epiphysis making length increases impossible. In a graded dose response study by Yarrow and colleagues, low dose trenbolone (i.e., 1.0mg/week) reduced retroperitoneal fat pad mass by 23% compared to orchiectomized animals. They reported that low dose trenbolone also augmented LABC mass by 35%, and prevented reductions in kidney mass and hemoglobin concentration without enlarging the prostate. Additionally, testosterone administration preserved cancellous bone volume and trabecular number in male rats following ORX (10, 11, 57, 58). However, a separate study examining the effects of oral administration of 50mg/day testosterone propionate administration in hypogonadal male mice reported decreases in cortical area and width (59). Unlike estradiol where reductions in cortical bone were fully compensated for by an increased trabecular structured network, testosterone administration resulted in only a partial compensation of this network (59). Thus, some controversy exists regarding the efficacy of testosterone administration in preventing hypogonadism-induced bone loss. The Fischer 344 male rat model aged 3 months shows a modest decline in the plantaris muscle (8%) (60) at eight weeks following ORX.

However, during a four week experimental period by Yarrow and colleagues observed that plantaris muscle and femoral and tibial bone mass were not significantly reduced following ORX (11). Therefore the current study was extended to six weeks to evaluate a longer period of ORX on muscle and bone mass. Unlike testosterone replacement therapy, PRT is not reported to increase prostate size, but has produced significant effects in muscle (61, 62) and bone turnover markers (63). Our proposed model of PRT in the rat (weighted ladder climbing) increases the flexor hallicus muscle mass of eugonadal rodents by 23% in eight weeks (61, 62), and increases the external load on bone. Increased external load through weight bearing, increased body weight or resistive exercise has shown positive effects on bone mass and strength, however these specific effects have not been reported. In addition, PRT is not associated with one of the most reported side effects of androgen therapy prostate enlargement. To accomplish this aim we will quantify the degree trenbolone-enanthate, PRT, testosterone-enanthate (positive control), or vehicle (negative control) alter skeletal muscle mass (wet weight), retroperitoneal fat pad mass, bone mass and morphometry (as evaluated by femoral pQCT measurements), bone biomechanical strength, hemoglobin concentration, and mass of the kidney and prostate mass (wet weight) in mature ORX male rats

Specific Aim, Hypothesis, Rationale 2.

Specific Aim: To characterize the effects of trenbolone-enanthate administration, testosterone enanthate administration or progressive resistance exercise (i.e., weighted ladder climbing) on the serum, and bone concentrations of testosterone, trenbolone, and DHT in an orchiectomized mature male rat model. **Hypothesis:** Orchiectomized male rats, rats receiving trenbolone or progressive resistance training will display lower

bone, and serum hormone concentrations of Testosterone and DHT compared to control, whereas supraphysiological testosterone administration will exhibit the highest levels of bone (T,DHT) and serum hormones (T, DHT). **Rationale:** Previous work by Yarrow and colleagues (11) has found that ORX reduces serum concentrations of both testosterone and elevates intraskeletal DHT and maintains bone concentrations of testosterone; while testosterone administration of ORX rodents significantly elevates both serum and bone testosterone and DHT concentrations. To-date, no published data exists on the effects of trenbolone or PRT on bone hormone concentrations. To accomplish this aim, we will quantify the degree to which trenbolone-enanthate, PRT, testosterone-enanthate (positive control), or vehicle (negative control) therapy alters the serum, and bone sex-hormone concentrations in mature ORX male rats.

Specific Aim, Hypothesis, Rationale 3:

Specific Aim: To characterize the effects of trenbolone enanthate administration, testosterone enanthate administration or progressive resistance exercise (i.e., weighted ladder climbing) on Pax 7 satellite cell activation, fiber type distribution, and fiber cross sectional area in the semimembranosus and flexor hallicus longus. **Hypothesis**: Administration of trenbolone enanthate and testosterone enanthate will increase Pax 7 activated satellite cell populations, increase Type II fiber cross sectional area, and maintain Type II fiber distribution in both muscles of the semimembranosus and flexor hallicus longus. Progressive resistance training will increase Pax 7 activated satellite cell numbers in the flexor hallicus longus only. **Rationale**: Administration of androgens has been shown to increase satellite cell number in humans and animals (35, 64). Progressive resistance training leads to muscular hypertrophy in exercised muscles of both humans and rodents (56, 61, 65). However, limited peer-reviewed data exists

establishing the ability of progressive resistance training to induce satellite cell activation during a superimposed hypogonadal state in either the rodent or human model.

CHAPTER 2
REVIEW OF LITERATURE

Hypogonadism Induced Effects in Man

Male hypogonadism (i.e., serum T <300ng/dL) manifests as sexual dysfunction, reduced energy, depression, cognitive deficits, anemia (53) and may result in reductions in lean body mass and bone mineral density, and increased adiposity (66, 67). Together these changes limit functional independence and increase the risk of fracture (68). In males, the incidence of osteoporosis related fractures is increasing. However, osteoporosis remains under-diagnosed and under-treated area of clinical medicine in men (59) . Strikingly, hip fractures result in far greater mortality in men than in women. The one-year period post fracture mortality risk (32%) for male veterans versus 18% in females (18). Sarcopenia is an age-related decline in skeletal muscle mass and strength leading to decreased functional capacity and predisposition to chronic metabolic disease (69). The loss of skeletal muscle results in a reduced metabolic rate, a reduction in the primary storage depot for glucose, amino acids, and for lipid oxidation (70). Reductions in muscle mass also limit locomotor ability ultimately reducing an individual's functional independence and ability to maintain cardiorespiratory endurance.

Testosterone Replacement Therapy for Hypogonadism

Testosterone is the primary androgen in circulation and is closely bound to sex hormone binding globulin and albumin (95%), whereas free testosterone circulates at around 2% of total testosterone (71). The Institute of Medicine recently requested for small clinical trials to be conducted to elucidate the potential anabolic and androgenic effects of testosterone replacement therapy aimed at reducing, reversing or preventing

the catabolic effects of hypogonadism in males. The Endocrine Society's recently published Clinical Practice Guidelines, states that a male presenting with a serum total testosterone level of 300ng/dL is likely to be hypogonadal (28). A clinical test with a level between 200-400ng/dL should have the test repeated along with a measurement of free testosterone (28). Clinical measurement of luteinizing hormone (LH) and follicle stimulating hormone (FSH) are also recommended to determine whether primary testicular failure is the causative factor or a secondary disturbance of the hypothalamus-pituitary-gonadal axis (28, 72). Twenty percent of men over the age of 60 have serum testosterone levels below 11.3 nmol/L (325ng/dL) the normal range for normal eugonadal men (73).

Bhasin and colleagues first proved the clinical effectiveness of testosterone in a trial of seven hypogonadal men receiving a 100 mg*week^{-1} of testosterone enanthate by intramuscular injection for ten weeks (74). Individuals participating in this trial underwent a 12 week pre-experimental period of withdrawal from previous androgen or gonadotropin therapy, with no weight training or heavy endurance training four weeks prior to study initiation. Significant effects were seen in accrual of lean body mass (5.0 ± 0.8 kg) and increased muscle strength (22%) following ten weeks of 100 mg*week^{-1} of testosterone enanthate (74). Similarly, Wang and others administered a dosage of 5mg/ 3 times a day, sublingually in non-exercising hypogonadal men and found positive changes in fat-free mass, leg strength, and markers of bone resorption after six months of treatment (75). Administration of testosterone (100 mg/day) in a transdermal gel (which has a low rate of absorption) placed on selected skin sites raised serum testosterone concentrations from approximately 6 nmol/L, up to 29 nmol/L 24 hours

after application at one site (76). Additional evidence exists in humans that supraphysiologic doses of 600 mg*week^{-1} of testosterone for twenty weeks resulted in significant improvements in lean body mass, muscle cross sectional area, and muscle strength without exercise in eugonadal young males made hypogonadal through the administration of lupron or older hypogonadal males (6). In a recent study, by Page and colleagues (77, 78) improvements in timed functional tests, handgrip strength, bone mineral density, and lean body mass were reported for hypogonadal elderly men receiving 200 mg intramuscular testosterone bi weekly. Additionally, testosterone administration has demonstrated positive effects on body composition in older hypogonadal males. In hypogonadal men aged 65 years or older receiving 200 mg* week^{-1}, Page and colleagues (77, 78) found increases of more than 3.0kg of lean body mass and a 5% reduction in total body fat after 36 months. In another 36 month long study, Snyder and colleagues (79) investigated a testosterone replacement therapy utilizing a scrotal patch delivering 6 mg/day of testosterone. This dosage level and route of administration resulted in significant increases in lean body mass of 1.9 kg and a decreased total fat mass of 3.0 kg following the intervention. Bone mineral density improvements were found for both study populations ranging and 2-10% change in regional BMD sites of the hip and lumbar spine, respectively (7, 79, 80).

Potential Therapeutic Use of Trenbolone Administration for Hypogonadism

Trenbolone (17β-Hydroxyestra-4,9,11-trien-3-one) is a synthetic analog of testosterone that is generally administered in an acetate or enanthate form. Historically, trenbolone has been confined to experimentation in laboratory animals and livestock (81) to enhance muscle growth, and market characteristics of slaughter animals (82). Administration of trenbolone (120mg +24mg estradiol) to finishing steers (i.e., castrated

male bovines) tended to increase type IIa myosin heavy chain (MHC) mRNA concentrations after 30 days in the semimembranosus muscle (83), while sarcopenia tends to diminish type II fiber concentrations (3). In a study by Gonzalez and colleagues, crossbred cull cows had increases in Type I muscle fiber cross sectional area and diameter were larger when trenbolone acetate was co-adminstered with a beta agonist ractopamine HCL (84). Its application in humans as a method to prevent or attenuate hypogonadism induced osteopenia and sarcopenia has not been explored. Currently, human application has been hindered because the specific mechanism of growth promotion by trenbolone is not completely elucidated. However, a recent review by Yarrow and colleagues discusses the tissue selectivity and potential clinical applications of trenbolone administration in humans (41), however the dosage and safety profile of trenbolone in humans has not been established to date.

Pottier and colleagues reported that trenbolone is hydrolyzed to 17 beta trenbolone (17B-TbOH, 17 B hydroxy-estra-4,9,11,-trien-3-one) following absorption (85). In a classic study by Danhaive and Rousseau, it is stated that trenbolone exerts its anabolic action through androgenic and potent anti-glucocorticoid effects (86). Meyer and colleagues state that trenbolone binds to the androgen receptor with similar affinity to DHT (i.e., 3 times greater than testosterone) (87). Interestingly, trenbolone also binds to the progesterone receptor with the same affinity as progesterone, however the role of the progesterone receptor in tissue anabolism or catabolism warrant further study (87). The binding affinities of trenbolone for both the androgen and progesterone receptor coupled with the potent anti-gluccocorticoid effects are different than testosterone and may result in differing tissue effects in muscle and bone. However, a general consensus

regarding mechanism responsible for trenbolone's effects on tissue accretion has not been reached.

In a two week study, young female Sprague-Dawley rats (60-120g) gained 26% more weight in muscle and skeletal tissue during trenbolone administration at 80 micrograms of trenbolone /100g body weight compared to control animals (35). In addition, there was a higher efficiency of feed conversion to lean body mass by the trenbolone treated intact female rats (35). There was no significant increase in skeletal muscle (wet weight) of the gastrocnemius, peroneus, and tibialis anterior, however total DNA content was significantly increased in the peroneus and tibialis anterior (35). Interestingly, the semimembranosus a large muscle of the hindlimb saw significant increases in both skeletal muscle wet weight (24%) and total DNA/muscle (38%) following two weeks of trenbolone administration in intact, young female rats (35). Without confirmation by histological sampling, the potential growth observed cannot be attributed solely to accretion of skeletal muscle tissue and may have been through changes in lipid deposition or connective tissue.

Vernon and Buttery reported that trenbolone administration exerts a more profound effect on minimizing protein degradation compared to placebo, allowing for the accretion lean body mass (88). Similarly, total carcass nitrogen content of the trenbolone treated animals was increased while total body fat content tended to decrease (89, 90). An additional possible mechanism hypothesized by Thompson and colleagues is that trenbolone administration may increase the responsiveness of satellite cells to FGF and IGF-1 (35). However, the direct role that androgens play in

regulating muscle and plasma IGF-1 and associated binding proteins remains to be determined.

Hypogonadism Rat Model Selection

Several orchiectomized rodent models have been utilized to mimic the effects of human hypogonadism including orchiectomized Brown Norway, Wistar and Fischer 344 (F344) rats, among others. Older Brown Norway experience minor reductions in skeletal muscle mass (91), while undergoing spontaneous prostate enlargement similar to aging men. Similarly, Borst and colleagues evaluated the responsiveness of bone resorption, prostate mass, and muscle mass in a group of 13 month old Brown Norway rats receiving a 5-alpha reductase inhibitor without blocking the anabolic effects of T on muscle and bone (8). However, previous work by Borst and colleagues reported that orchiectomized Brown Norway experience only minor decrements in skeletal muscle hindlimb mass, and do not experience an increased in fat mass, or a significant alteration in serum bone resorption markers following orchiectomy (8). Therefore, Borst and colleagues evaluated three rat models (i.e., Brown Norway, Wistar and Fischer 344) to identify the most robust strain for measuring the catabolic consequences of hypogonadism and found that F344 rats were the only strain where orchiectomy induced a cascade of effects similar to that observed in hypogonadal men, including reductions in body and muscle mass, high turnover osteopenia (i.e., increased bone resorption and a reflux increase in bone formation), and increased adiposity(60). Recent data by Hershberger and colleagues, established that the levator ani bulbocavernosus muscle complex are androgen responsive skeletal muscle and tissues in the rat (40) with a greater androgen receptor density compared to human skeletal muscle (42, 92). Therefore, we evaluated F344/Brown Norway rodents to examine the robustness of this

model in mimicking the effects of human hypogonadism on skeletal muscle, bone, fat, hemoglobin, and prostate tissue.

Bone Effects in Orchiectomized (ORX) Rats

Previous work by Borst and colleagues determined that rapid changes in bone resorptive markers were present for both Fischer 344 and Brown Norway 3 month old rats following orchiectomy (60). Both species experienced an increase in urinary excretion of urinary DpD/creatinine 28 days and 56 days following ORX surgery. Bone resorption indices of urinary DpD/creatinine in Fisher 344 rats increased by 87% in the first month, with makers of bone resorption still increased at 60% into month two, whereas Brown Norway rats experienced a 50% increase in urinary DpD/creatinine excretion in month one and a 99% increase at month two of the study (60). Loss or withdrawal of endogenous testosterone results in catabolic effects in muscle and bone (60) that become apparent within two weeks of ORX and are steadily increased compared to control animals, over the course of 9 months (93). However administration of 1.0 mg of testosterone per day for 56 days suppressed the urinary excretion of Dpd/creatinine in ORX rats (20). Substantial osteoporotic changes occur in cancellous bone of the axial and appendicular skeleton of ORX aged 13 month old orchiectomized male Fischer 344 rats (8). In the vertebrae, the majority of cancellous bone loss consisted of reductions in trabecular number and thinning of the trabeculae, whereas tibial cancellous bone loss in the tibial consists only of the loss of trabecular number without a concommitant decrease in trabecular thickness (93). ORX also reduces periosteal appositional growth in cortical bone, an effect that is reversible by testosterone or dihydrotestosterone administration (58, 94). Within two months following orchiectomy, a rapid and large loss of cortical bone mass occurs in mature male Fischer

344 rats and is associated with increased bone remodeling and an expansion of the marrow cavity under conditions of androgen deficiency (95). However, subcutaneous administration of testosterone undecanoate 6mg/kg*week^{-1} prevented cortical bone loss in ORX animals (95). Expectedly, Erben and colleagues reported reductions in serum concentrations of estradiol following ORX in rats (93), although reductions in serum estradiol were not observed in male F344 rats following ORX in another study (11). Erben and others suggested that estradiol in serum and bone may attenuate changes in high turnover osteopenia following androgen withdrawal (93). In a human study by Riggs and colleagues, the authors stated that estradiol is a prominent hormone regulating skeletal metabolism in elderly men (average age 68 years), and in fact, is the dominant hormone governing bone resorption (96). They reported that resorption markers were reduced by 70% following estradiol administration in men deficient in T and E (96). Whereas T may have a more limited, but important role in preventing apoptosis in mature osteoblastic cells (96). In addition, Reim and colleagues suggest that circulating levels of estradiol may function in the suppression of endocortical bone resorption in aged male rats (95). These studies indicate that the loss of naturally produced estradiol, may be implicated in skeletal loss, and the presence of estradiol may maintain the balance between formation and resorption in the adult skeleton. Currently, the mechanisms governing bone resorption during androgen replacement therapy are still being refined.

At least one study has reported that subcutaneous testosterone undecanoate administration of 8 mg/kg*week^{-1} to ORX rats restores circulating levels of estradiol (93). However, a recent study by Yarrow and colleagues reported that intramuscular

administration of supraphysiologic testosterone-enanthate (7.0 mg/week) to 3 month old ORX F344 rats did not elevate the serum or bone estradiol concentrations, but did result in significant elevations in serum and bone testosterone and DHT concentrations (11). Supraphysiolgic testosterone enanthate increased tibial bone mass by 10% in the tibia, and by 23% in the femur in young rodents (144). Similarly, bone mechanical strength was improved by 12-19% in both sexes of gonadectomized rodents administered testosterone (11). Currently, the significance of bone androgen and estrogen concentrations role in bone strength and bone turnover is not understood.

Bone and Serum Hormone Concentrations in ORX rats

Recently, Yarrow and colleagues reported the first-ever measurements of the bone sex-hormone concentrations for intact, ORX, and testosterone-treated animals (11). In intact F344 rat, tibial concentrations of testosterone were reported at 2.5ng/g, whereas ORX induced a nearly 50% decline in bone testosterone concentrations. Conversely, supraphysiologic testosterone-enanthate administration to ORX male rats resulted in a 9.5-fold increase in tibial testosterone compared with control animals and a 12.5-fold greater testosterone concentration that what was found in serum (11). Tibial DHT concentrations were 91% greater in male rats receiving testosterone enanthate compared to controls, whereas serum levels of DHT were 21 fold higher than intact controls (11). However, neither ORX nor testosterone enanthate administration demonstrated altered bone estradiol concentrations in male rats (11).

Skeletal Muscle Effects in ORX rats

Orchiectomy induces rapid loss of skeletal muscle mass in some hindlimb muscles within two months following surgery (60). Coupled with reductions in muscle mass, ORX induces losses of glycogen stores and impairs protein synthesis (97). Intramuscular

testosterone-enanthate administration (7.0mg/week) has been shown to increase rat plantaris wet-weight compared to ORX rats following 28 days administration (11). Hourde and colleagues reported that while three months of androgen treatment in orchiectomized rats did not cause hypertrophy of the soleus muscle, force and endurance were increased by 69% and 35% respectively (98).

Resistance Training for Sarcopenia and Osteoporosis

Resistance training is well known to produce favorable increases in skeletal muscle mass/strength (99), functional abilities (99), and bone mineral density (BMD) (100, 101), without the deleterious side effects associated with testosterone replacement therapy. In fact, the American College of Sports Medicine's 1998 position statement recommends that resistance exercise should be performed by all healthy older adults in order to prevent the reductions in muscle function and BMD that occur primarily between 50-80 years of age (3). Further, in a recent review, Hunter and colleagues report that resistance training is the most favorable intervention for ameliorating the deleterious effects of sarcopenia (102). Despite the established benefits of resistance exercise on muscle mass and BMD, few studies have evaluated the effects of resistance training in hypogonadal older men or hypogonadal animal models.

The resistance exercise induced augmentations of muscle mass are believed to be influenced by the acute changes in the systemic and localized (i.e., within muscle) sex-hormone and growth factor concentrations that occur following exercise (103-107). Specifically, following heavy resistance exercise, the serum concentrations of growth hormone and testosterone are elevated for approximately 60 minutes in healthy young and older men (108). However, recent work by Vingren and colleagues (2008) reported

that the concentrations of testosterone within muscle were not elevated in humans following heavy resistance training (109), which is in contrast to reports in rat models undergoing swimming (110) and treadmill running (111); although, the modalities of swimming and treadmill running in the rat are not parallel stimuli to resistance training in the human.

Hornberger and colleagues have developed a weighted ladder climbing exercise protocol to model the overload stimulus typically seen in progressive resistance training programs in humans (62). An important feature of ladder climbing as a model for skeletal muscle hypertrophy is the volitional nature of the activity. Rats typically take to the ladder without induction of the noxious stimuli (i.e., forced running, over-heating, and electrical shock) that occurs with treadmill exercise. Presumably, ladder climbing initiates a lower stress response when compared to treadmill running. Indeed, Lee and colleagues have utilized the weighted ladder climbing model and observed increases of 23% in the flexor hallicus longus muscle mass following eight weeks of training (61). By the end of this eight week training period, rats were able to carry nearly 1,400g attached to their tail representing a load nearly 3 times their body weight (61). Indeed, in this study increases in skeletal muscle wet weight are not a direct reflection in accrural of muscle cross sectional area or improvements in myofibrillar content. In the study by Lee and colleagues the resistance trained group had lower levels of myofibrillar protein content when expressed as mg/g of flexor hallicus longus muscle tissue compared to control animals (61). One possible mechanism for this discrepancy suggested by Lee and colleagues is that the eccentric phase of ladder climbing induces larger degrees of muscle damage, and possibly resultant fibrosis and damage to the surrounding

sarcolemma (61). The eccentric mode was suggested by Lee and colleagues, as the animals engage the ladder rung with their toes, followed by an initial lengthening contraction of the flexor hallicus longus and flexor digitorum longus (61). The second phase consists of concentric contraction of the plantar flexors and knee extenors while loaded (61). Increased damage may lead to increased swelling as evidenced by decreased total muscle protein content in the FHL reported by Lee and colleagues (61). Another potential mechanism for the increased strength displayed with weighted ladder climbing could be attributable to learning, or growth in other muscle groups not evaluated by the researchers. Although, weighted ladder climbing is a potent stimulus for increasing skeletal muscle hypertrophy; systemic and local sex-hormone and growth factor responses to weighted ladder climbing have not been evaluated.

Osteoporosis is metabolic disease of bone tissue affecting bone microarchictecture, fracture risk, and bone mass (112). Osteoporotic fractures are estimated to occur at a rate of 1.5 million per year in individuals over the age of 50 years (100). Scane and colleagues reported that aging males experience a reduction in trabecular bone content of 15-45%, and a loss of cortical bone ranging from 5-15% (113). Resistance training is a recommended modality to combat the deleterious consequences of osteoporosis although the Centers for Disease Control and Prevention reported that only 12% of individuals aged 65-74 and 10% of individuals over 75 years of age engaged in muscular strength and endurance activities at least two days a week. Recent studies by Vincent and Braith have found increases in bone mineral density (2%) in older men and women engaged in a 24 week high intensity progressive resistance exercise study, whereas lower intensity exercises may not be as effective

(65). Little research has examined the role of resistance training in the prevention of osteoporosis in males, specifically hypogonadal males. A majority of longitudinal studies have focused on women as the critical population at risk for osteoporosis (100). However, a population at more immediate post fracture risk is elderly males as one in three elderly males dies within twelve months of a hip fracture, whereas females have more overall fracture rates (18). Therefore, non-pharmacological interventions such as resistance training and pharmacological strategies for the attenuation of hypogonadism induced sarcopenia and osteopenia in males are necessary.

Satellite Cells

Definition

Satellite cells are globally defined as a heterogenous population of mononuclear myogenic precursors that contribute to the regulation, maintenance and function of essential for muscle cell hypertrophy, and repair; satellite cells are histologically defined by their location beneath the basal lamina of the muscle fiber. Satellite cells have the potential to proliferate to fuse with the myofiber or form new myofibers giving rise to multi-nucleated myotubes. Dhawan and colleague state that satellite cells can be termed muscle stem cells of postnatal skeletal muscle (140). In a similar light, other authors refer to adult skeletal muscle stem cells as satellite cells (141). By the definition of Hawke and colleagues, once satellite cells begin expressing myogenic markers they are termed myoblasts (142). Collins and colleagues (2005) report that 3,000 myonuclei can originate from a single satellite cell, highlighting the enormous potential for satellite cells to address skeletal muscle myopathies (143). As the field of studying satellite cell activation, quiescence, and signaling pathways evolve, so will the current definitions.

Quiescence

Satellite cells exist in a quiescent state until recruited for repair, hypertrophy, renewal or differentiation. The homeostatic balance of quiescence and activation is necessary to maintain satellite cell function (144). In response to disease or injury, satellite cells of the Pax 7+ lineage may enter the cell cycle to initiate myofiber repair while a sub-population returns to quiescence. During the proliferation phase, myoblasts expand their cytoplasmic-nuclei ratio and initiate fusion with existing fibers or initiate de novo myofiber synthesis (142). Recent research suggests that Sprouty1, a receptor tyrosine kinase signaling inhibitor, is highly expressed by satellite cells in the quiescent state (144) and is down regulated during the proliferative phase of muscle regeneration (145). Shea and colleagues suggest that the expression of Sprouty1 occurs in a sub-population of satellite cells and is essential for their return to the quiescent state (145). Subsequently, Sprouty1 appears critical for regulating quiescence but is not an obligatory component of muscle differentiation. In contrast, Abou-Khalil and colleagues, suggest that quiescence may be regulated by the interaction of satellite cells with the vascular bed, and the simultaneous promotion of endothelial growth, muscle regeneration and angiogenic factors (14). Angiopoieitin-1/Tie-2 (e.g., an angiogenesis promoting factor/tyrosine kinase endothelial receptor) is described as maintaining vascular integrity; where Angiopoietin-1/Tie-2 increases the number of satellite cells in the quiescent state. Whereas, blocking Ang-1/Tie-2 initiates satellite cells to re-enter the cell cycle (146). The role Ang-1/Tie-2 is theorized to regulate angiogenesis in response the satellite cell pool, ensuring that muscle growth does not exceed angiogenesis. The Ang-1/Tie-2 regulation of satellite cell pools entry and emergence from the quiescent state requires further elucidation to advance the field of muscle regeneration.

Self-Renewal, Differentiation, and Activation

In a classic study by Reznik, satellite cells were characterized as having the potential to be mobilized from their quiescent state, expand, generate myoblasts and terminally differentiate to fuse with surrounding fibers for growth or repair (114). In the rodent, satellite cells represent approximately four percent of the total myonuclei, and decrease to two percent of the myonuclei in senescence (146). Occurrence of myotrauma through exercise or injury results in the activation, proliferation, and migration of satellite cells to the injured fiber. Upon arriving at the area of trauma satellite cells which will then fuse with the damaged myofiber (hypertrophic response) or fuse together to produce additional myofibers (hyperplastic response). The regenerated fiber will then appear with central nuclei, these newly added nuclei will then migrate to the periphery. Satellite cells not directly committed to muscle repair or growth are involved in satellite cell self-renewal (142). Moss and colleagues confirmed that a sub-population of satellite cells exists that do not terminally differentiate but which function to renew the satellite cell pool (115).

Recently, Notch signaling and the canonical Wnt (ligand for Frizzled family of receptors) pathway signaling are implicated in the myogenic properties of muscle regeneration, growth, and activation of satellite cells. In response to muscle injury, Notch signaling components (Delta-1, Notch-1, and active Notch) are upregulated and are necessary to activate satellite cells into a highly proliferative state (147). Wnt functions in recovery from muscle injury and has a prominent role in myoblast differentiation and myotube fusion (140). Under conditions of injury or exercise, satellite cells initiate proliferation and exhibit a myoblast cell fate under the regulation and control of Myf-5, MyoD, α integrin, and desmin (149). The specific mechanisms by

which Wnt and Notch regulate or co-regulate cell fates during postnatal myogenesis remain to be determined.

During the postnatal period, myonuclei remain in a mitotically inactive state—quiescence in the absence of appropriate stimuli (116). Pax-7 is expressed universally in satellite cells in rats, other mammals, salamanders, and zebrafish (117-120). Deletion of the Pax-7 gene in knockout models causes depletion of satellite cells by causing embryonic progenitors to apoptose or adopt alternative non-myogenic cell fates (121-123). Expresssion of Pax-7+ in satellite cells allows for their self-renewal and survivability, while removal of Pax-7+ leads to decline in their number as determined in Pax-7 null mice (122-124). Therefore, measuring and monitoring the expression of Pax-7 will allow for the detection of early and late phase therapeutic interventions aimed at increasing skeletal muscle in at risk populations.

Pax 7+ Responsiveness to Growth Factors

Transforming growth factor–β isoforms have dual roles in the muscle satellite cells depending upon the phase of the cell cycle. Transforming growth factor–β isoforms are implicated in reducing migration in C2C12 skeletal muscle satellite cells, while reducing the ability of IGF-1 to increase migration. However, during the regenerative process, the expression of transforming growth factor- β receptor and transforming growth factor ligand are required for proliferation and differentiation (150). The insulin like growth factor family is a known inducer of satellite cell proliferation and differentiation (151). IGF-1 may support the satellite cell pool by down-regulating caspace/apoptotic pathways and activating the serine threonine protein kinase Akt (152). Following exercise, the induction of IGF-1 signaling also promotes the fusion of satellite cells to the myofiber, and committment of the satellite cell to participate in hypertrophy (142).

Androgen Effects on Satellite Cells

In a recent study by Sinha-Hikim, it was suggested that testosterone may partially regulate the cycle of cell proliferation, and commitment of daughter cells through the activation of Notch (153). Sinha-Hikim and colleagues reported that supraphysiological doses of testosterone enanthate (300 vs.600 mg/week) resulted in a dose dependent increase in satellite cell number, size, and motility in healthy men (125). However, a lower dose of testosterone (125mg/week), which remains anabolic, did not increase satellite cell number (125).

Androgens may directly affect the proliferation of satellite cells through binding with androgen receptors present on muscle satellite cells (126). Alternatively, androgens may indirectly stimulate satellite cell activation through the presence of muscle IGF-1, although the relative contributions of these two mechanisms are unknown. Similarly, in cultured satellite cells nuclei and myotubes from an in vitro porcine model administered testosterone; Doumitt and colleagues reported increased androgen receptor numbers were present on satellite cells after incubation with testosterone (127). Lastly, Sinha-Hikim suggest that testosterone may function through regulating satellite cell replication, inhibition of satellite cell apoptosis or dedication of stem cells into a myogenic lineage(125).

The combination of trenbolone and estradiol, when implanted in the semimembranosus muscle of yearling steers, causes a significant increase in satellite cell number (39). In addition, trenbolone and estradiol increased daily carcass protein accretion by 82% over the course of 40 days of administration (39). Kamanga-Sollo and others have reported that trenbolone also increases IGF-1 mRNA in bovine satellite cell cultures as a possible mechanism for muscle cell hypertrophy (128, 129). Thompson

and colleagues reported that trenbolone administration increases muscle size and DNA content when administered to mice and that trenbolone increases satellite cell sensitivity to IGF-I and to fibroblast growth factor (FGF) in a culture system (35). However, the mechanisms by which trenbolone or testosterone regulate directly or indirectly induce satellite cell proliferation and differentiation remain to be elucidated.

CHAPTER 3
METHODS

Experimental Design

All experimental procedures conformed to the ILAR Guide to the Care and Use of Experimental Animals and were approved by the Institutional Animal Care and Use Committees at the Malcolm Randall VA Medical Center. Fifty Fisher 344/Brown Norway F1 (N=50) intact male rats, were obtained from Charles River Laboratories (Wilmington, MA), and were housed in separate cages at 20º C under a 12 hour light cycle. All rats underwent a minimum one week acclimatization phase in the laboratory prior to any procedural intervention. Rats were allowed to move freely and were fed standard rat chow and water. Food intake was measured on a weekly basis.

Borst and Conover have established F344 and Brown Norway male rats as models for studying the anabolic/androgenic pathways and catabolic state associated with hypogonadism(60). For this study, male F344/Brown Norway F1 rats, aged 10 months, were matched for initial body weight and randomized into the following groups (n = 10/group; Total=50) and treated for 42 days as follows:

1) Sham + vehicle (Sham)
2) ORX + vehicle (ORX),
3) ORX + testosterone (ORX+T),
4) ORX + trenbolone (ORX+TREN),
5) ORX + progressive resistance exercise (ORX+EX).

Following randomization animals underwent Sham vs. ORX surgery and while receiving the following treatments for 42 days: testosterone-enanthate (7.0mg/week), trenbolone-enanthate (1.0mg/week), vehicle (sesame oil) or exercise (i.e., weighted ladder climbing performed 3 days/week+vehicle) treatments for 42 days. Blood was sampled every two weeks via the tail tip ambulation method (130) prior to and during

drug administration or progressive resistance training exercise and by a single intracardiac puncture at sacrifice (131), in order to evaluate serum hormone concentrations and to verify drug delivery. During all surgical procedures, animals were kept on a circulating heat pad to maintain body temperature, remained under isoflurane anesthesia (5% induction, 1.5-2.5% maintenance), and received buprenophine analgesia to reduce pain. Following surgery, a nutritional supplement (Jell-O cube with added protein and fat) (NIH protocol diet) was provided for one week in order to minimize weight loss resulting from surgery. The rats were sacrificed at day 42, via an intraperitoneal injection of 120mg/kg pentobarbital blood, bone, muscle, and prostate tissues were removed for group comparisons. There were a total of ten animals per group, as determined by a power analysis, for a total of 50 rats.

Drug Administration

The slow-releasing enanthate esters of both testosterone(11) and trenbolone (unpublished laboratory results) result in a sustained supraphysiological drug serum concentrations for at least seven days following intramuscular injection. Testosterone-enanthate (Savient Pharmaceutical, East Brunswick, NJ), trenbolone-enanthate (Steraloids, Newport, RI), and vehicle were dissolved in sesame oil and administered (0.1mL) once every seven days, under isoflurane anesthesia, into the quadriceps musculature. Injections were alternated between legs in order to reduce possible discomfort of repeated injections. Additionally, previous studies from our laboratory have reported that once-weekly supraphysiological (7.0 mg/week) testosterone-enanthate administration successfully prevents skeletal muscle mass/strength and BMD loss in growing ORX rodents (aged 3 months) (8, 11, 30).

Weighted Ladder Climbing

Weighted ladder climbing is an established form of progressive resistance training (PRT) in rodents that results in overload of the flexor hallicus longus musculature(62). During training, rats repeatedly climbed a 1 meter ladder inclined 85º while carrying progressively heavier (in 25 g increments) lead weights. The weights were placed in a nylon bag and hung from a Velcro strap that was secured by a protective foam pad on the rat's tail. Prior to weighted training, rats underwent a 3-day acclimatization period, carrying their body weight up the ladder. If the rat paused during the climb, it was induced to complete the climb with a brief pulse of compressed air. On the fourth training day, rats carried a series of progressively increasing loads in order to establish the baseline maximum. Over the 42 day experimental period, exercising rats performed the PRT protocol consisting of five completed climbs per session (3 sessions/week), during which time the weight were progressively increased following successful completion of the training protocol. Each week the load was increased between 10-30% over the load carried the previous week. If the rat was unable to complete greater than 3 sessions at the new workload, the load was reduced by 5-10% on the subsequent session. Next, weights were reduced to most recently utilized load to ensure 5 weighted climbs were completed. Each climb was separated by two-minutes. Testing was completed when the required number of repetitions were completed or when a brief pulse of compressed air didn't elicit further climbing.

Hemoglobin and Serum Sex-Hormone Measurements

Whole blood samples were acquired from the tail at baseline and week 2, week 4, and week 6 under isoflurane anesthesia and assayed in duplicate using the Hgb Pro (ITC, Edison, NJ) photometer, which has an intra-assay CV of less than 2.41%. Briefly,

approximately 20µl of whole blood was placed on Hgb Pro test strips membrane. Once dispersed in the membrane the red blood cells contact a lysing agent and release their hemoglobin. The photometer analyzed the hemoglobin concentration at a wavelength of 522nm.

The remaining blood samples were centrifuged at 3000g for 12 minutes and serum aliquots were separated and stored at -80ºC for later analysis. All serum hormone measurements were determined in duplicate within the same plate. Testosterone was determined by EIA which requires 50 µl serum and has a reported sensitivity of 0.04ng/ml with an intra-assay CV of 5.3% (Alpco Diagnostics, Windham, NH). DHT was determined by EIA which requires 20 µl serum and has a reported sensitivity of 6.0 pg/ml with and intra-assay CV <11% (Alpco Diagnostics, Windham, NH). Trenbolone was determined by modifying a qualitative EIA that requires 20 µl serum (Neogen Corporation, Lexington, KY) with a sensitivity of 0.1ng/ml and an intraassay CV of 3.76%, according to methods previously devised in our laboratory (unpublished laboratory results). Specifically, trenbolone (Sigma-Aldrich) as dissolved in 100% ethanol (1:1) and subsequently serially diluted in hormone-free EIA buffer (Neogen Corporation, Lexington, KY) to produce a quantitative standard curve.

In-Vivo Muscle Strength Testing (Grip Strength)

The Ring Grip Performance Test (Columbus Instruments, Columbus, OH,USA) is an established *in-vivo* muscle strength test, which measures the maximal gripping strength of the forelimb (i.e., digital flexors) muscles (132). The test measures the peak tension generated from the forelimbs of the rodent during testing. For this test, animals were placed in a horizontal position and allowed to grasp the wire mesh. Subsequently, the researcher grasps the animal by the tail and force is applied in an opposing direction

to the ring until the animal's grasp on the ring is broken. The average of three successful trials is recorded and utilized to calculate specific force similar to procedures reported by Borst and colleagues (30).

Fiber Type and Fiber Cross Sectional Area Measurements

The right flexor hallicus longus, and semimembranosus muscles were excised, pinned at resting length, coated with OTC, and frozen on a slurry of isopentane, cooled with liquid nitrogen and stored at -80ºC prior to analysis. Serial cross sections (10μm) in width were taken from the mid-belly of each muscle. Initially, plated muscle cross section slides were first permeabilized with 0.5% Triton-X100 in phosphate-buffered saline (PBS) and subsequently rinsed with a series of PBS washes. The samples were incubated with primary antibodies for laminin (Lab Vision, Fremont, CA), type I myosin heavy chain (A4.840), and type IIa myosin heavy chain (SC-71). Secondary antibody treatments were completed with rhodamine, Alexa Fluor 350 and Alexa Fluor 488 (Invitrogen, Carlsbad, CA). Cover slips were mounted with Vectashield fluorescent mounting medium (Vector Labs, Burlingame, CA).

Samples were visualized using fluorescence microscopy (10x magnification) with N21, GFP, and A4 cube filters (Leica DM LB, Solms, Germany), and then imaged with a digital camera. The CSA, fiber type, and the area fraction (A_A) occupied by each fiber type were recorded from a minimum of 250 fibers. Encoded images were calculated for fiber CSA using Scion Image (NIH) software. Fibers that fluoresced blue were assigned as Type I (slow fibers), those fluorescing green were assigned to Type IIa fibers, whereas the remainder of non-fluourescing fibers were assigned to Type IIb/x for each muscle. The A4.840 and SC-71 antibodies were obtained from the Developmental

Studies Hybridoma Bank developed under the auspices of the NICHD and maintained by The University of Iowa, Department of Biology, Iowa City, IA 52242.

Hemotoxylin and Eosin Staining

Serial cross-sections of the semimbranosus and flexor hallicus longus (10 μm) in width were taken from the mid-belly of each muscle. Muscle sections were placed on a glass slide and subjected to a progressive hydration protocol beginning with an initial exposure to 100% ethanol (ETOH) solution for one minute, followed by immersion in a 70% ETOH bath for one minute. A final rinse in deionized water for two minutes completed the first phase of the protocol. Muscle sections were immersed in hematoxylin for one minute, followed by a rinsing in deionized and tap water for three minutes to reduce non-specific tissue staining. Briefly, differentiation of hematoxylin staining was accomplished by immersing muscle sections in Scott's solution for 15 seconds followed by a 15 second rinse in deionized water. Next, muscle samples were partially dehydrated in a 70% ETOH solution for one minute, followed by placement in Eosin stain for two minutes. Muscle sections were progressively dehydrated by submersion in a 95% and 100% ETOH bath for two minutes. Last, samples were clarified in xylene for four minutes. Samples were allowed to air dry and were mounted with a glass cover slip using Crystal Mounting Medium (Genetex, Irvine, CA, USA). Muscle cross sections were visualized with brightfield microscopy (Leica DM LB, Solms Germany) at 20x magnification. Ten randomly-selected images were analyzed from each right flexor hallicus longus and semimembranosus for a minimum of 200 fibers analyzed. An investigator blinded to the treatment group analyzed and recorded the presence on centralized or internalized nuclei present in the corresponding number of fibers per field of view. Individual images were randomly selected from each group and

analyzed in the following directional pattern: top-left, top-right, bottom-right, bottom-left until the required numbers of muscle fibers were analyzed. Centralized nuclei were defined as nuclei residing in a position equidistant from two or more sides of the myofiber. Internalized nuclei were classified as nuclei residing greater than 20 pixels from the inner membrane of the fiber. Data are MEAN ± SE, expressed per 100 fibers.

Satellite-Cell (Pax-7) Staining

The right flexor hallicus longus and semimembranosus muscle were pinned at resting length covered with OTC medium and frozen in isopentane chilled by liquid nitrogen. Satellite cell staining for the presence of Pax-7 was conducted on 10 µm muscle sections previously frozen at -80 ºC. Muscle sections were rinsed with 100 mL of 1x PBS. Muscle sections were fixed with a methanol:acetone (1:1) for 3-5 min at room temperature. Fixed muscle sections were rinsed in 1x PBS (3x) with a rinse duration of 5 minutes. Muscle sections were blocked with a Superblock (Pierce Biotechnology, Rockford, IL) in 1x PBS for 60 min at room temperature. Again, the fixed sections were rinsed with PBS a total of three times with a 5 minute rinse duration. Next, the primary antibody (Pax7(1:50)) and rabbit laminin(1:200) were incubated with the muscle section for 1-2 hours at room temperature. After the incubation time, rinse the section with 1x PBS 4-5 times with a five minute rinse duration. Then, add the secondary antibody (Alexa fluor 488(1:300)); Rhodamin (Gand R(1:500)) and incubate for one hour in diminished light. Rinse the muscle section with 1x PBS 5 times with a five minute rinse duration under diminished light. Cover slips were mounted over fixed muscle sections with Vectashield fluorescent mounting medium with DAPI (Vector Labs, Burlingame, CA) and sealed with nail polish.

Representative muscle samples from the flexor hallicus longus and semimembranosus for Pax 7 positive nuclei were visualized using fluorescence microscopy (Leica DM LB, Solms Germany) at 20x. A miminum of ten fields of view were recorded for each muscle sample. The images were loaded into Scion Image and separated into their respective layers of N21, GFP, and A4. Representative populations of Pax7 nuclei per expressed per 100 fibers, and as the percentage of Pax7 positive per myonuclei population from a sample of 200 fibers. Pax7-positive nuclei were identified by yellow-green fluorescent nuclei located between the basal lamina and the extra-cellular membrane, overlayed by DAPI-positive tissue. The investigator was blinded to the group assignments during analysis. Individual images were randomly selected from each group and analyzed in the following directional pattern: top-left, top-right, bottom-right, bottom-left until the required numbers of muscle fibers were analyzed

Bone Sex Steroid Hormone Measurements

The right tibiae were harvested and the sex- hormones (i.e., testosterone, DHT) and trenbolone were extracted according to the methods of Yarrow and others(11). First, the tibia was cut into small pieces, pulverized with a liquid nitrogen cooled Spex Certiprep freezer mill (Edison, NJ, USA) for two minutes and stored at -80ºC until homogenization. Bone powder was homogenized in 20 volumes of 4ºC Krebs-Ringer phosphate buffer that consisted of 116mM NaCl, 10 mM of phosphate, 4.5mM KCL, 2.5mM $MgCl_2$, 1.2mM $CaCl_2$, 5% glycerol (pH 6.9), 2mM EDTA and 4mM DTT (Sigma-Aldrich, St. Louis, MO). The bone homogenate was disrupted via a high speed polytron (15s) and probe sonication (30s). The homogenate was then diluted with 1:2 chloroform-methanol (2:1 v:v), vortexed for 45 seconds and centrifuged at 1500 rpm for 10 minutes in order to separate the organic aqueous layers. The upper aqueous layer is

re-diluted in 2 ml chloroform, vortexed for 45 seconds and re-centrifuged at 1500rpm for 10min. Next, the bottom organic layers from both extraction steps were combined, dried under a gentle stream of nitrogen, and equilibrated for 48 hours at room tempeature.

Bone extracts were reconstituted in manufacturer's hormone free assay buffer solutions prior to analyses. All reconstituted samples were assayed in duplicate on a single plate, with standard dilutions performed according to manufacturer's instructions. Previous work by Yarrow and colleagues demonstrated greater than 90% recovery for all hormones and minimal interference(11).

Prostate, Levator Ani Bulbocaveronsus Complex, Kidney, Adipose and Muscle Tissue Mass

Prostates were cleaned of adipose tissue, sectioned and weighed in order to compare the effects of the sex-hormone testosterone, trenbolone, and resistance training on prostate mass. The LABC was excised, weighed and cleaned of adipose tissue to examine the effects of the sex-hormone testosterone, dehydrotestosterone, trenbolone, and resistance training on tissue mass. The left kidney and retroperitoneal fat pad were removed to determine the effects of the sex-hormone testosterone, trenbolone, and resistance training on mass. The soleus, plantaris, flexor hallicus longus, and semimembranosus muscles were removed and weighed to evaluate changes in mass associated with administration of testosterone, trenbolone or weighted ladder climbing on mass.

Bone Mechanical Strength and Peripheral Quantitative Computed Tomography (pQCT)

After excision, the right and left femora were weighed and femoral length was measured using a digital caliper (Mitutoyo, Aurora, IL). Femora was immediately wrapped in saline-soaked gauze to prevent dehydration, and stored at -20ºC in order to

maintain the mechanical properties of the bone (133). Prior to mechanical testing, femora were thawed to room temperature and were kept wrapped in saline-soaked gauze except during measurements. The midshaft of the left femur was subjected to a medial/lateral three-point bending test, using an MTS material testing machine (MTS Systems Co., Erden Prairie, MN) as described by Leppanen and others (134), while the neck of the right femur was subjected to an axial load biomechanical test. Before mechanical testing, a preload (10 N/0.1 mm/s) was applied at 1.0 mm/s until failure. From the load-deformation curve, the following parameters were determined for the femoral shaft: breaking load, yield load. Bone mechanical strength is expressed both as force (measured in Newtons) as previously described by Yarrow and colleagues (11).

Prior to pQCT assessment, the saline soaked gauze was removed from the femora and it was placed into the polycarbonate holding device. The femora was placed in position within the holding device and aligned with the specific landmarks for the condyles. A test scan was performed to verify the correct positioning of the femur within the holder device, prior to the final scan being performed. Cross sections of the femoral diaphysis and metaphysis were scanned by peripheral quantitative computerized tomography (pQCT). The left femoral diaphysis and metaphysis were scanned with a Stratec XCT Research M Instrument (Norland Medical Systems,Fort Atkinson, WI). Scans were performed at a distance of 5mm (metaphysis) and 18mm (diaphysis) proximal to the distal end of the femur for total, trabecular, and cortical bone area (mm^2), content (mg/mm), and density (mg/cm^3).

Statistical Analysis

A One Way ANOVA using SPSS (version 18) were utilized to examine differences between treatments on normally distributed data for the variables of muscle mass and

strength, bone mass, strength and cross sectional area, serum sex hormones, bone hormone concentrations and prostate mass. All results were reported as Mean ± SE. Prior to anlaysis, outcome variables were tested for normality. If the recorded data for the primary outcome variables were unable to be transformed and meet the conditions of a normal distribution a non-parametric test were performed. Non-parametric data was assessed by either the Kruskal Wallis or Mann-Whitney test for final analyses. A power analysis based on previous work from our laboratory (11) comparing group means between testosterone administration, and ORX+Vehicle animals during a 28 day intervention utilizing pooled standard deviations, powered at 80%, with a type I error of 0.05% was conducted for the following primary outcome variables: femoral load, femoral mass, tibial mass, plantaris muscle mass, and prostate mass.

Similarly, a power analysis was constructed for progressive resistance training (i.e., weighted ladder climbing) based on the work Lee and colleagues (61) evaluating hypertrophy in the flexor hallicus longus. The muscle hypertrophic response to trenbolone was based on the findings of Thompson and colleagues based on a lower dose of trenbolone resulting in an 8% increase in gastrocnemius mass and a 24% increase in the mass of the semimembranosus (35). The estimated minimal group sizes necessary for to achieve statistical and clinically relevant data from the intervention groups (ORX+T, ORX+Tren, ORX+PRT) are reported as follows (Group size/% Expected change: femoral load (n=13/10%), femoral mass (n=6/10%), tibial mass (n=6/10%), plantaris mass (with T) (n=9/15%), gastrocnemius mass (with TREN) (n=11/8%), semimembranosus (with TREN) (n =10/15%), prostate growth n=10/10%). In addition, the extension of the ORX treatment period by two weeks from the

preliminary data utilized for the power analysis is expected to induce a greater osteopeanic and sarcopenic effect. Therefore, group sizes were set at N=10/group to ensure adequate power >80%, for the expected effect size of treatment and an alpha of $p<0.05$ for the primary outcome variables.

CHAPTER 4
RESULTS

Body Weights at Sacrifice

Administration of testosterone enanthate significantly lowered overall body mass compared to control animals. At sacrifice, the body weights were 443.5 ± 17.1 g (SHAM), 419.9 ± 12.5 g (ORX), 404.0 ± 5.17 g (ORX+EX), 404.8 ± 10.9 g (ORX+T), and 412.7 ± 7.64 g (ORX+TR) (see Figure 4-1). Orchiectomy resulted in a non-significant reduction in body weight of 5.3% in the ORX ($p>0.05$) group and significantly decreased bodyweight by 8.9% ORX+EX compared with SHAM at sacrifice ($p<0.05$). ORX+TR treated animals body weights were 6.9% below SHAM animals, however this reduction was not statistically significant ($p>0.05$). Whereas, ORX+T resulted in a significant reduction 8.7% in body weight compared to SHAM animals ($p<0.05$). Removed testes and epididymal fat weights were similar between surgical groups 15.5 ± 0.3 g (ORX), 14.8 ± 0.3 g (ORX+EX), 15.1 ± 0.1 g (ORX+T), 14.9 ± 0.2 g (ORX+TR), ($p>0.05$), removed weights represented approximately 3% of pre-surgical body mass. All time course comparisons are based on post surgical bodyweight. Following six weeks of treatment, ORX+TR animals were not significantly different than ORX+V, ORX+EX, or ORX+T animals ($p>0.05$). Similarly, ORX+T animals were not statistically different from ORX+V, ORX+EX, or ORX+TR ($p>0.05$).

Treatment Effects on Serum T

Intramuscular injections of testosterone enanthate at 7.0 $mg \cdot week^{-1}$, resulted in supraphysiologic concentrations (6 fold) above SHAM. Notably, intramuscular injections of trenbolone enanthate at 1.0 $mg \cdot week^{-1}$ maintained testosterone levels similar to the other castrated groups. Following six weeks of treatment, serum testosterone levels

were 2.46 ± 0.51 ng/mL (SHAM), 0.12 ± 0.05 ng/mL (ORX+V), 0.06 ± 0.03 ng/mL (ORX+EX), 14.8 ± 2.02 ng/mL (ORX+T), and 0.20 ± 0.15 ng/mL (ORX+TR) (see Figure 4-2). ORX+V animals serum testosterone levels were significantly lower 95% than SHAM six weeks following surgery ($p<0.05$). Similarly, ORX+EX animals were significantly lower 98% than SHAM ($p<0.05$). Also, ORX+TR animals were significantly lower 92% than SHAM ($p<0.05$). In contrast, the superphysiologic administration of testosterone enanthate in the ORX+T resulted in (6 fold) increase of serum testosterone levels compared to SHAM ($p<0.01$). ORX+TR treated animals were significantly lower 99% than ORX+T treated animals ($p<0.05$). Serum levels of T were not significantly different between ORX+TR, ORX+V, and ORX+EX animals ($p>0.05$). However, serum levels of T in the ORX+T treated group were significantly greater than OVX+V (10-fold) and OVX+EX (20-fold) treated animals ($P<0.01$).

Treatment Effects on Serum DHT

Intramuscular injections of testosterone enanthate at 7.0 mg·week^{-1}, resulted in supraphysiologic concentrations (~9 times control) of serum dihydrotestosterone in orchiectomized male rats. Following six weeks of treatment, serum DHT levels were 461.1 ± 70.6 pg/mL (SHAM), 112.7 ± 14.0 pg/mL (ORX+V), 99.0 ± 13.2 pg/mL (ORX+EX), 4427.0 ± 383.4 pg/mL (ORX+T), and 62.7 ± 11.6 pg/mL (ORX+TR) (see Figure 4-3). At sacrifice, OVX+V serum DHT levels were reduced by 75.6% compared to SHAM ($p<0.01$). OVX+EX animals had a similar reduction in serum DHT levels of 78.5% ($p<0.01$). ORX+TR treated animals had significantly lower 86.4% serum DHT levels compared to SHAM ($p<0.01$). Indeed, treatment with T significantly increased serum DHT levels by (9-fold) compared to SHAM conditions ($p<0.01$). OVX+TR animals had significantly lower serum DHT levels than OVX+V 44.4% and OVX+EX 36.7%

($p<0.05$). Also, OVX+TR had lower 99% serum DHT levels compared to ORX+T treated animals ($p<0.01$). Whereas, serum DHT in the group undergoing testosterone supplementation (ORX+T) were (4-fold) greater than ORX+V and were similarly increased compared to ORX+EX (4-fold) ($p<0.01$). Serum DHT levels were not significantly different in ORX+EX animals compared to ORX+V ($p>0.05$).

Treatment Effects on Serum TREN

Intramuscular injections of trenbolone enanthate at 1.0 mg·week^{-1}, resulted in elevated serum trenbolone concentrations in orchiectomized male rats. Following six weeks of treatment, serum TREN levels were 0.72 ± 0.21 ng/mL (SHAM), 0.64 ± 0.16 ng/mL (ORX+V), 0.51 ± 0.08 ng/mL (ORX+EX), 1.11 ± 0.27 ng/mL (ORX+T), and 4.77 ± 0.42 ng/mL (ORX+TR) (see Figure 4-4). Administration of synthetic TREN resulted in significantly elevated levels of serum trenbolone compared to SHAM (6-fold), ORX+V (6-fold), ORX+EX (8-fold), and ORX+T (3-fold) treated animals ($p<0.05$). Serum TREN levels were not significantly different in ORX+EX animals compared to ORX+V ($p>0.05$). Since, trenbolone is an administered synthetic analog of testosterone, no trenbolone should be present in the SHAM, ORX+V, ORX+EX, or ORX+T groups. A small percentage (3%) of cross reactivity is present between trenbolone and testosterone at a physiologic concentration of 3.2 ng/mL as reported by the manufacturer (Neogen Corporation, Lexington, KY). The SHAM group had serum T values with a mean of 2.5 ng/mL. However, superphysiologic administration of testosterone in these animals resulted in mean serum T values nearly 4.6 times the stated concentration used by the manufacturer to determine cross-reactivity which may account for the apparent presence of trenbolone in these groups. In contrast, the cross-reactivity of trenbolone is negligible when assayed on the testosterone assay.

Treatment Effects on Retroperitoneal Fat Mass

Androgen administration significantly reduced retroperitoneal fat pad mass, whereas progressive resistance in the hypogonadal male rat model tended to increase fat pad mass. At sacrifice, retroperitoneal fat pad masses were 3.26 ± 0.33 g (SHAM), 4.06 ± 0.20 g (ORX+V), 3.77 ± 0.27g (ORX+EX), 2.25 ± 0.08g (ORX+T), and 2.84 ± 0.12 g (ORX+TR) (see Figure 4-5). ORX+V animals experienced a 24.7% increase in fat pad mass six weeks following surgery compared to SHAM animals, however this was not statistically significant ($p=0.052$). Similarly, ORX+EX animals increased retroperitoneal fat pad mass by 15.9% compared to SHAM, however this was not statistically significant($p>0.05$). Indeed, significant decreases in retroperitoneal fat pad mass were evident for ORX+TREN 12.7% and ORX+T 31% compared to SHAM animals ($p<0.05$). In a similar fashion, androgen treatment with TREN significantly decreased fat pad mass 30% compared to the ORX+V treatment group ($p<0.01$). TREN treatment decreased fat pad mass 25% compared to ORX+EX ($p<0.01$). The ORX+T treatment significantly reduced retroperitoneal fat pad mass by 44.7% compared to the ORX+V group ($p<0.01$). A similar reduction in retroperitoneal fat pad mass by the ORX+T group 40.5% was observed when compared to the ORX+EX group ($p<0.05$). ORX+T animals experienced significant retroperitoneal fat loss compared ORX+TR animals ($p<0.01$). Weighted ladder climbing had no effect in altering retroperitoneal fat pad mass compared to ORX+V animals ($p>0.05$).

Treatment Effects on Hemoglobin at Sacrifice

Androgen administration of testosterone and trenbolone enanthate significantly elevated circulating hemoglobin levels by the conclusion of the study. In contrast, progressive resistance exercise in the hypogonadal male rat model tended to maintain

circulating hemoglobin at control levels. At sacrifice, hemoglobin values were 15.28 ± 0.44 g/dL (SHAM), 15.18 ± 0.25 g/dL (ORX+V), 15.16 ± 0.26 g/dL (ORX+EX), 16.79 ± 0.16 g/dL (ORX+T), and 16.98 ± 0.33 g/dL (ORX+TR) (see Figure 4-6). Following six weeks of orchiectomy, no significant changes in hemoglobin concentrations were present for either vehicle injections ORX+V or ORX+EX compared to SHAM ($p>0.05$). Whereas, ORX+TR treated animals had a significant elevation of 11.1% in circulating hemoglobin levels compared to SHAM ($p<0.01$). Similarly, ORX+T resulted in a significant elevation 9.9% in circulating hemoglobin levels compared to SHAM ($p<0.01$). Androgen administration of ORX+TR and ORX+T had similar responses for hemoglobin concentration ($p>0.05$). ORX+T administration resulted in significant elevations of circulating hemoglobin compared to ORX+V 10.6% and ORX+EX 10.8%, ($p<0.01$) respectively. Weighted ladder climbing (ORX+EX) had no significant effect on circulating hemoglobin compared to vehicle injections (ORX+V) ($p>0.05$).

Time Course of Treatment Effects on Hemoglobin

Androgen administration of testosterone and trenbolone enanthate significantly elevated circulating hemoglobin levels by week four of the study, with non-significant elevations from week four to six. Hemoglobin (Hb) levels were not significantly different for any treatment groups when assessed at week 0 and week 2 of the experimental period ($p>0.05$) (see Figure 4-7). At week 4, significant elevations were evident for ORX+TREN 9% and ORX+ T 8% treatment groups compared to SHAM and both ORX groups 11% vs 10%, respectively ($p<0.01$). However, there were no significant differences between the androgen treatment (ORX+TR and ORX+T) groups at week 4 ($p>0.05$). In addition, elevations in hemoglobin levels were present in both androgen administration groups compared to SHAM and both ORX groups at week 6 ($p<0.01$).

Weighted ladder climbing exerted no measurable effect on Hb levels over SHAM or ORX+V treatments ($p>0.05$).

Treatment Effects on the Levator Ani Bulbocavernosus Complex (LABC)

Androgen administration of testosterone and trenbolone enanthate to the highly androgen sensitive levator ani bulbocavernosus muscle resulted in significant hypertrophy. In contrast, orchiectomy and orchiectomy coupled with exercise resulted in significant reductions in the LABC. At the conclusion of the experiment, LABC masses were 1.07 ± 0.04 g (SHAM), 0.68 ± 0.03 g (ORX+V), 0.64 ± 0.02 g (ORX+EX), 1.54 ± 0.03 g (ORX+T), and 1.54 ± 0.04 g (ORX+TR) (see Figure 4-8). ORX+V and ORX+EX animals had a significant reduction in levator ani bulbocavernosus (LABC) mass 36.2% and 40%, respectively, compared to SHAM animals ($p<0.01$). However, ORX+TR and ORX+T animals resulted in significant myotrophic growth of the LABC compared to SHAM 44.5% and +44.1%, respectively. Moreover, ORX+TR and ORX+T treated animals experienced a similar increase 126% in LABC mass compared to both ORX+V and ORX+EX conditions ($p<0.01$). ORX+EX had a non- significant 6% reduction in LABC masses compared to ORX+V and treated groups ($p>0.05$). Similarly, androgen administered groups ORX+TR and ORX+ T had similar effects on LABC mass ($p>0.05$).

Treatment Effects on Prostate Mass

Androgen administration of testosterone enanthate significantly increased the mass of the prostate compared to control and orchiectomized groups. Trenbolone enathate exhibited a non-significant reduction in prostate mass compared to control conditions. Androgen ablation through orchiectomy and orchiectomy coupled with exercise resulted in significant reductions in prostate mass compared to controls. Prostate masses were the following: 0.231 ± 0.02 g (SHAM), 0.066 ± 0.02 g (ORX+V),

0.66 ± 0.01 g (ORX+EX), 0.513 ± 0.04 g (ORX+T), and 0.185 ± 0.02 g (ORX+TR) (see Figure 4-9). ORX+V and ORX+EX animals prostate masses were significantly smaller, 71.5% and 71.6%, respectively, at six weeks following surgery compared to SHAM ($p<0.01$). A non-significant reduction of 20% in prostate mass was observed for ORX+TR animals compared to SHAM ($p=0.12$). In contrast androgen treated ORX+T animals had prostate mass was significantly increased by 122% compared to SHAM ($p<0.01$). Androgen treatment with TREN resulted in prostate masses 180% vs. +181%, compared to the ORX+V and ORX+EX, respectively ($p<0.01$). Androgen treatment with T, significantly increased prostate mass (6-fold) compared to the ORX+V and ORX+EX groups, respectively ($p<0.01$). ORX+TR had significantly smaller prostate masses 64% compared to ORX+T ($p<0.01$). Weighted ladder climbing had no impact significant impact on prostate mass compared to the ORX+V treatment group ($p>0.05$).

Treatment Effects on Kidney Mass at Sacrifice

Androgen administration of testosterone and trenbolone enanthate maintained kidney mass at control levels. In contrast, orchiectomy and orchiectomy coupled with exercise resulted in significant reductions in kidney mass. Kidney masses were 1.12 ± 0.04g (SHAM), 0.97 ± 0.02 g (ORX+V), 0.96 ± 0.02 g (ORX+EX), 1.20 ± 0.04 g (ORX+T), and 1.15 ± 0.02 g (ORX+TR) (see Figure 4-10). After 6 weeks of ORX conditions, ORX+V kidney masses were significantly decreased 12.5% compared to SHAM ($p<0.05$). ORX+EX animals kidneys were significantly decreased 14.2% compared to SHAM conditions ($p<0.05$). Although not statistically significant both androgen treated groups ORX+T and ORX+TR group increased kidney mass by 7.5% vs. +3.3% compared to the SHAM group ($p<0.10$). ORX+TR significantly increased kidney mass 18.1% compared to ORX+V ($p<0.01$). Similarly, ORX+T treatment

increased kidney mass 22.9% above ORX+V conditions ($p<0.01$). Both ORX+TR and ORX+T increased kidney mass 20.4% vs. 25.2% compared to the ORX+EX group, respectively ($p<0.01$). There was no difference in kidney mass response between ORX+TR and ORX+T animals ($p>0.05$).

Treatment Effect on Hindlimb Muscles Mass

There was no significant effect of androgen administration of testosterone enanthate, trenbolone enanthate, orchiectomy, or orchiectomy coupled with exercise on hindlimb muscle mass. At sacrifice, semimembranosus values were 0.39 ± 0.01g (SHAM), 0.38 ± 0.01 g (ORX+V), 0.38 ± 0.01 g (ORX+EX), 0.38 ± 0.01 g (ORX+T), and 0.40 ± 0.01 g (ORX+TR) (see Figure 4-11). Plantaris masses were 0.44 ± 0.02 g (SHAM), 0.40 ± 0.02 g (ORX+V), 0.42 ± 0.01 g (ORX+EX), 0.40 ± 0.01 g (ORX+T), and 0.44 ± 0.02 g (ORX+TR) (see Figure 4-12). Soleus masses were 0.18 ± 0.01 g (SHAM), 0.16 ± 0.01 g (ORX+V), 0.16 ± 0.01 g (ORX+EX), 0.16 ± 0.01g (ORX+T), and 0.17± 0.01 g (ORX+TR) (see Figure 4-13). Flexor hallicus longus masses were 0.55 ± 0.03 g (SHAM), 0.52 ± 0.03 g (ORX+V), 0.50 ± 0.04 g (ORX+EX), 0.53 ± 0.02 g (ORX+T), and 0.52 ± 0.03 g (ORX+TR) (see Figure 4-14).

ORX+V treatment did not alter muscle wet weight of the flexor hallicus longus, semimembranosus, soleus, or plantaris compared to SHAM or ORX+EX conditions ($p>0.05$). Androgen treatment (ORX+TREN and ORX+T) did not alter muscle wet weight of the flexor hallicus longus, semimembranosus, soleus, or plantaris compared to ORX, ORX+EX, or SHAM ($p>0.05$). Interestingly, weighted ladder climbing (ORX+EX) did not increase wet muscle weights compared to any treatment conditions ($p>0.05$).

Grip Strength

Neither androgen administration nor progressive resistance training significantly improved grip strength values in the hypogonadal male rats. The grip strength values were 1.72 ± 0.07 kg (SHAM), 1.83 ± 0.11 kg (ORX), 1.76 ± 0.08 kg (ORX+EX), 1.94 ± 0.08 kg (ORX+T), and 1.83 ± 0.08 kg (ORX+TR) (see Figure 4-15). Group comparisons of forelimb strength found no difference between SHAM, ORX+V, ORX+EX, ORX+TREN or ORX+T ($p>0.05$).

Progressive Resistance Training (Weighted Ladder Climbing)

Orchiectomized male rats improved the total load (body weight+tail weight summed across climbs) carried over the course of the study. One week following ORX surgery, animals in the weighted ladder climbing group ascended the ladder with an average weekly load of 0.41 ± 0.01 kg (Week 1), 1.47 ± 0.13 kg (Week 2), 1.88 ± 0.18 kg (Week 3), 2.51 ± 0.37 kg (Week 4) and 3.00 ± 0.40 kg (Week 5) (see Figure 4-16). Load values increased significantly 631% from Week 1 to Week 5 of the training intervention ($p<0.01$). Training loads significantly increased 285% between Week 1 and Week 2 ($p<0.05$). However, training loads did not significantly increase between Week 2 and Week 3 ($p>0.05$) and tended to increase 33% between Week 2 and Week 4 ($p=0.054$). Training load was significantly increased between Week 1 and Week 3 of the study ($p<0.05$). Although, Week 2 training load was significantly increased 104% by Week 5 of the study ($p<0.01$). Similarly, training load did not significantly increase between Week 3 and Week 4 ($p>0.05$), however Week 3 training load was significantly increased 60% by Week 5 ($p<0.05$). Lastly, training load did not significantly increase between Week 4 and Week 5 of the intervention ($p>0.05$).

During week one of the study animals ascended the ladder carrying their bodyweight. In week two, the animals carried an average of 72.4 ± 12.2 g attached to their tail during their repeated ladder climbs. Weight increased during week three, with the animals 98.5 ± 12.7 g, and further increased the weight in week four to 137.0 ± 14.2 g. Lastly, tail weight increased by week five to 175.0 ± 22.0 g.

Treatment Effects on Femoral and Tibial Bone Mass and Length

There was no significant effect of administration of testosterone enanthate, trenbolone enanthate, orchiectomy, or orchiectomy coupled with exercise on femur or tibial mass or length. Femur weights were 1.01 ± 0.03 g (SHAM), 1.07 ± 0.02 g (ORX), 1.05 ± 0.02 g (ORX+EX), 1.08 ± 0.04 g (ORX+T), and 1.13 ± 0.04 g (ORX+TR). Femur lengths were 40.3 ± 0.36 mm (SHAM), 40.1 ± 0.23 mm (ORX), 39.9 ± 0.25 mm (ORX+EX), 40.0 ± 0.31 mm (ORX+T), and 40.5 ± 0.29 mm (ORX+TR). Tibia weights were 0.83 ± 0.03 g (SHAM), 0.83 ± 0.02 g (ORX), 0.82 ± 0.02 g (ORX+EX), 0.85 ± 0.03 g (ORX+T), and 0.86 ± 0.02 g (ORX+TR). Tibia lengths were 43.1 ± 0.45 mm (SHAM), 42.8 ± 0.33 mm (ORX), 42.8 ± 0.30 mm (ORX+EX), 42.6 ± 0.24 mm (ORX+T), and 43.4 ± 0.27 mm (ORX+TR) (see Figure 4-16 through 4-20). ORX+V conditions did not significantly decrease femoral bone mass or length ($p>0.05$). Androgen treatment (ORX+TREN and ORX+T) did not significantly increase femoral or tibial bone mass or length compared to other treatment conditions (SHAM, ORX+V, ORX+EX) ($p>0.05$). No differences existed between the response of ORX+TREN or ORX+T for femoral or tibial bone mass or length ($p>0.05$). Exercise training (ORX+EX) did not induce significant changes in femoral or tibial mass or length compared to any treatment following six weeks of the intervention ($p>0.05$).

Serum Osteocalcin at Sacrifice

Administration of testosterone and trenbolone enanthate resulted in significant reduction in circulating serum osteocalcin levels. In contrast, orchicectomy resulted in increased levels of circulating serum osteocalcin, whereas orchiectomy coupled with exercise maintained serum osteocalcin at a level similar to controls. At sacrifice serum osteocalcin values were 167.4 ± 11.4 ng/ml (SHAM), 255.5 ± 11.1 ng/ml (ORX), 199.6 ± 15.5 ng/ml (ORX+EX), 82.1 ± 7.13 ng/ml (ORX+T), and 101.1 ± 9.0 ng/ml (ORX+TR) (see Figure 4-21). Following six weeks of treatment, SHAM animals had significantly 52.7% lower levels of serum osteocalcin than ORX+V treated animals ($p<0.01$). ORX+EX, serum osteocalcin levels were 19% higher than SHAM animals, although these values did not reach statistical significance ($p>0.05$). ORX+TREN animals were lower 39.6% than SHAM ($p<0.05$). Also, ORX+T animals were lower 50.9% than SHAM ($p<0.05$). ORX+TR animals were lower 60.4% than OVX+V animals ($p<0.05$). Similarly, ORX+TR animals were also lower 49.4% than OVX+EX ($p<0.05$). The ORX+T treatment was significantly lower 67.9% compared to the ORX+V group ($p<0.05$). There was no significant difference between ORX+TREN or ORX+T treatment for serum osteocalcin ($p>0.05$). Serum osteocalcin levels were significantly lower 21.9% in ORX+EX animals compared to ORX+V ($p<0.05$).

Serum Trap 5b at Sacrifice

Androgen administration of testosterone and trenbolone enanthate resulted in significant reduction in Trap 5b levels. While orchiectomy did not significantly elevate Trap 5b above SHAM, Trap 5 b levels in ORX+V animals was significantly higher than in androgen treated animals. At sacrifice, serum Trap 5b values were 5.22 ± 0.38 U/L (SHAM), 5.94 ± 0.68 U/L (ORX), 5.89 ± 0.78 U/L (ORX+EX), 3.01 ± 0.17 U/L (ORX+T),

and 3.29 ± 0.30 U/L (ORX+TR) (see Figure 4-22). At six weeks following surgery, serum Trap 5b levels in SHAM animals were not significantly different than ORX+V, and ORX+EX animals ($p>0.05$). In SHAM animals Trab 5b levels were 37.0% higher than in ORX+TR animals ($p<0.01$). Similarly, SHAM animals had 42.3% higher serum Trap 5b levels compared to ORX+T treated animals ($p<0.01$). ORX+TR animals were significantly 40% lower compared to ORX+V and ORX+EX groups ($p<0.01$). ORX+T animals had significantly lower Trap 5b serum levels compared to both ORX+V and ORX+EX, ($p<0.01$). ORX+ TR and ORX+T had similar effects on lowering serum Trap 5b values compared to the other treatment groups($p>0.05$).

Treatment Effects on Bone Biomechanical Characteristics

Femoral Neck

Administration of trenbolone resulted in increased maximum load values compared to ORX+EX animals, and non-significantly increased maximum load values above ORX+V animals. Supraphysiologic testosterone enanthate tended to increase maximum load of the femoral neck compared to ORX+EX, and was not significantly stronger than ORX+V animals. At sacrifice femoral neck maximum load values in Newtons (N) were: 206.2 ± 3.13 (SHAM), 196.3 ± 3.72 (ORX+V), 178.9 ± 3.12 (ORX+EX), 212.9 ± 2.13 (ORX+T), and 227.8 ± 1.74 (ORX+TR) (see Figure 23-24). Six weeks following surgery, serum femoral neck maximum load in SHAM animals were not significantly different than any treatment group ($p>0.05$). ORX+TR animals had significantly stronger 22% femoral necks than ORX+EX ($p<0.01$), but only tended to increase 14% femoral neck maximum load values compared to ORX+V ($p=0.11$). Also, ORX+T tended to have higher maximum load values for the femoral neck compared to

ORX+EX 16% (p=0.08), but were not significantly different than ORX+V (p>0.05). There was no significant difference between ORX+T and ORX+TR treated animals (p>0.05).

Femoral Midshaft

Androgen administration and progressive resistance training had no significant effect on femoral midshaft maximum load. At sacrifice femoral neck maximum load values in Newtons (N) were: 172.5 ± 3.04 (SHAM), 161.1 ± 3.51 (ORX+V), 164.0 ± 1.77 (ORX+EX), 164.9 ± 2.85 (ORX+T), and 164.0 ± 2.34 (ORX+TR). A slight non-significant reduction in femoral midshaft maximum load was present for all ORX groups ranging between 4-7% (p>0.05).

Centralized and Internalized Nuclei of the FHL and Semimembransosus

The initial procedures froze the flexor hallicus and semimembranosus muscle tissue (n=6-7/group) directly in liquid nitrogen cooled isopentane. The direct immersion of O.C.T coated muscle tissue into liquid isopentane resulted in freeze artifact preventing the acquisition of acceptable histology samples. Procedures were adapted for the remaining animals (n=3-4/group) so that muscle tissue was frozen on an isopentane slurry. Although the number of animals initially proposed in the power analysis were unavailable for immunohistochemical analyses, a smaller sample was available for analysis. The small sample size points to potential trends for internalized nuclei in the ORX-EX, ORX+T and ORX+TR groups in the flexor hallicus longus, but not the semiembranosus. At sacrifice internalized nuclei counts (expressed per 100 fibers) of the FHL were as follows: 5.75 ± 1.8 nuclei (SHAM), 4.50 ± 2.0 nuclei (ORX), 12.0 ± 3.9 nuclei (ORX+EX), 7.83 ± 2.3 nuclei (ORX+T), and 10.0 ± 3.3 nuclei (ORX+TR). At sacrifice centralized nuclei counts (expressed per 100 fibers) of the FHL, 1.25 ± 0.63 nuclei (SHAM), 1.50 ± 1.5 nuclei (ORX), 1.83 ± 1.4 nuclei (ORX+EX), 2.17 ± 0.60 nuclei

(ORX+T), and 0.83 ± 0.83 nuclei (ORX+TR) (see Figure 4-25 and 27) Following six weeks of treatment, no significant group differences were detected for the presence of centralized nuclei or internalized nuclei in the flexor hallicus muscle group of these animals ($p>0.05$).

At sacrifice internalized nuclei counts (expressed per 100 fibers) of the SEMI were as follows: 6.25 ± 1.7 nuclei (SHAM), 5.83 ± 1.3 nuclei (ORX), 10.2 ± 5.4 nuclei (ORX+EX), 4.00 ± 0.58 nuclei (ORX+T), and 8.00 ± 0.29 nuclei (ORX+TR). At sacrifice, centralized nuclei counts (expressed per 100 fibers) of the SEMI, 0.88 ± 0.31 nuclei (SHAM), 2.50 ± 0.76 nuclei (ORX), 2.5 ± 1.3 nuclei (ORX+EX), 0.17± 0.17 nuclei (ORX+T), and 1.5 ± 0.76 nuclei (ORX+TR) (see Figure 26 and 28). Following six weeks of treatment, no significant group differences were detected for the presence of centralized nuclei or internalized nuclei in the semimembransosus muscle group of these animals ($p>0.05$).

Pax-7 Positive Nuclei of the FHL and Semimembranosus

Because of freeze artifacts only 3-4 samples/group were suitable for analysis. Treatment with androgens had no significant effects on the presence of Pax 7 positive nuclei in the flexor hallicus longus. Similarly, progressive resistance exercise did not increase Pax 7 positive nuclei populations in either muscle, and may have negatively impacted Pax 7 expression in the flexor hallicus longus.

Flexor Hallicus Longus

Treatment with androgens had no significant effects on the presence of Pax 7 positive nuclei in the flexor hallicus longus. Similarly, progressive resistance exercise did not effect Pax 7 positive nuclei populations in the primary mover of weighted ladder climbing the flexor hallicus longus. At sacrifice Pax-7 positive nuclei (expressed per 100

fibers) of the FHL were as follows: 14.3 ± 1.0 nuclei (SHAM), 11.3 ± 3.8 nuclei (ORX), 7.8 ± 2.4 nuclei (ORX+EX), 13.5 ± 2.0 nuclei (ORX+T), and 11.5 ± 5.5 nuclei (ORX+TR) (see Figure 4-29). At sacrifice Pax-7 positive nuclei counts (expressed as percentage of Pax-7 positive nuclei/ total myonuclei counts per 100 fibers) of the SEMI, 3.7 ± 0.43 % (SHAM), 2.3 ± 0.60 % (ORX), 3.3 ± 0.89% (ORX+EX), 4.1 ± 0.95 % (ORX+T), and 2.6 ± 0.92% (ORX+TR). No significant differences were detected between treatment groups for Pax7 positive nuclei of the FHL expressed per 100 fibers or as a percentage of total myonuclei per 100 fibers ($p>0.05$).

Semimembranosus

Treatment with androgens had no significant effects on the presence of Pax 7 positive nuclei in the semimembransus. Similarly, progressive resistance exercise did not effect Pax 7 positive nuclei populations. At sacrifice Pax-7 positive nuclei (expressed per 100 fibers) of the SEMI were as follows: 20.4 ± 6.2 nuclei (SHAM), 15.8 ± 2.9 nuclei (ORX), 21.0 ± 6.4 nuclei (ORX+EX), 44.2 ± 17.0 nuclei (ORX+T), and 42.0 ± 8.9 nuclei (ORX+TR) (see Figure 4-30). At sacrifice Pax-7 positive nuclei counts (expressed as percentage of Pax-7 positive nuclei/ total myonuclei counts per 100 fibers) of the SEMI, 1.9 ± 0.38 % (SHAM), 3.9 ± 0.72 % (ORX), 5.3 ± 1.6 % (ORX+EX), 4.1 ± 0.95 % (ORX+T), and 3.7 ± 0.64 % (ORX+TR). No significant differences were detected between treatment groups for Pax7 positive nuclei of the SEMI expressed per 100 fibers or as a percentage of total myonuclei per 100 fibers ($p>0.05$).

Treatment Effects on Cross-Sectional Area of the Flexor Hallicus Longus

Although the number of animals initially proposed in the power analysis were unavailable for immunohistochemical analyses; a small sample size identified potential trends of increased cross sectional area of Type IIa in ORX+EX animals participating in

weighted ladder climbing.Type IIbx fiber cross sectional appear to be preserved in ORX+T for the flexor hallicus longus, but appears to decline in ORX+EX treated animals (see Figure 4-31).

Treatment Effects on Cross Sectional Area of the Semimembranosus

Although the number of animals initially proposed in the power analysis were unavailable for immunohistochemical analyses; a small sample size identified potential trends in Type IIa fiber preservation in ORX+T and ORX+TR, whereas Type IIa fiber cross sectional area tends to decrease in ORX+EX animals. All treatement groups tend to provide partial protection to Type IIbx fiber cross sectional area compared to ORX (see Figure 4-32).

Treatment Effects on 5mm pQCT (Metaphysis)

Total Mineral Content, Total Density, Total Area, Trabecular Area, Cortical Content, Cortical Area

Group comparisons revealed no significant differences for the 5mm pQCT outcome variables of total mineral content, total density, total area, trabecular area, cortical content, and cortical area. Significant group differences were detected for the following variables: trabecular content, trabecular density, and cortical density. Total bone content values were 14.5 ± 0.60 mg/mm (SHAM), 13.2 ± 0.30 mg/mm (ORX), 13.6 ± 0.52 mg/mm (ORX+EX), 13.5 ± 0.37 mg/mm (ORX+T), and 13.9 ± 0.04 mg/mm (ORX+TR) (see Figure 4-33). Total femoral density values were 668.1 ± 10.74 mg/mm^2 (SHAM), 631.5 ± 7.56 mg/mm^2 (ORX), 662.4 ± 12.0 mg/mm^2 (ORX+EX), 672.2 ± 12.5 mg/mm^2 (ORX+T), and 669.1 ± 11.1 mg/mm^2 (ORX+TR) (see Figure 4-34). Total area values at the 5mm landmark of the femur were 21.8 ± 0.80 mm^2 (SHAM), 21.0 ± 0.52 mm^2 (ORX), 20.6 ± 0.72 mm^2 (ORX+EX), 20.0 ± 0.32 mm^2 (ORX+T), and 20.8 ± 0.43

mm^2 (ORX+TR). Trabecular area values at the 5mm landmark of the femur were 6.51 ± 0.24 mm^2 (SHAM), 6.29 ± 0.16 mm^2 (ORX), 6.17 ± 0.21 mm^2 (ORX+EX), 6.00 ± 0.10 mm^2 (ORX+T), and 6.22 ± 0.13 mm^2 (ORX+TR) (see Figure 4-35 through 38). Whereas, cortical bone content values at the 5mm landmark of the femur 9.60 ± 0.55 mg (SHAM), 8.50 ± 0.23 mg (ORX), 9.26 ± 0.48 mg (ORX+EX), 8.93 ± 0.38 mg (ORX+T), and 9.13 ± 0.43 mg (ORX+TR) (see Figure 4-39).

Trabecular Content (CNT_{TRB})

Trabecular content values at the 5mm landmark of the femur were 1.86 ± 0.09 mg/mm (SHAM), 1.41 ± 0.06 mg/mm (ORX+V), 1.49 ± 0.09 mg/mm (ORX+EX), 1.71 ± 0.09 mg/mm (ORX+T), and 1.77 ± 0.09 mg/mm (ORX+TR) (see Figure 4-35 through 38). Six weeks following surgery, femurs evaluated from ORX+V and ORX+EX animals had lower CNT_{TRB} 31.9% and 24.8% respectively, than SHAM animals ($p<0.05$). ORX+TR and ORX+T maintained CNT_{TRB} similar to SHAM conditions ($p>0.05$). ORX+TR femurs maintained 25.5% greater CNT_{TRB} than ORX+V femurs ($p<0.05$). Notably, ORX+TR animals maintained CNT_{TRB} at an 18.8% higher level than ORX+EX animals, however this was not statistically significant ($p=0.10$). A similar pattern was seen for CNT_{TRB}, with ORX+T animals retaining 15.6% greater content compared to ORX+V ($p<0.05$). Whereas, ORX+T maintained a 14.8% greater CNT_{TRB} when compared to ORX+EX, however this was not statistically significant ($p=0.09$). Weighted ladder climbing did not conserve CNT_{TRB} in excess of ORX+V conditions ($p>0.05$). There were no significant differences between ORX+T and ORX+TREN for CNT_{TRB} ($p>0.05$).

Trabecular Density ($TRAB_{DEN}$)

Trabecular density values at the 5mm landmark of the femur were 285.6 ± 8.74 mg/mm^2 (SHAM), 225.4 ± 8.92 mg/mm^2 (ORX+V), 242.0 ± 10.6 mg/mm^2 (ORX+EX), 285.1 ± 13.0 mg/mm^2 (ORX+T), and 283.4 ± 11.2 mg/mm^2 (ORX+TR) (see Figure 4-35 through 38). At the end of the study, femurs evaluated from ORX+V and ORX+EX animals had lower $TRAB_{DEN}$ 26.7% and -18.0% respectively, than SHAM animals ($p<0.05$). ORX+TR and ORX+T maintained $TRAB_{DEN}$ similar to SHAM conditions ($p>0.05$). ORX+TR femurs maintained 25.7% greater $TRAB_{DEN}$ than ORX+V femurs ($p<0.05$). Similarly, ORX+TR animals maintained $TRAB_{DEN}$ 17.1% higher than ORX+EX animals ($p<0.05$). Assessment of $TRAB_{DEN}$ found ORX+T animals retaining 26.4% greater density compared to ORX+V ($p<0.05$). Whereas, ORX+T maintained a 17.8% greater $TRAB_{DEN}$ when compared to ORX+EX ($p<0.05$). Weighted ladder climbing did not protect against losses in $TRAB_{DEN}$ compared to ORX+V conditions ($p>0.05$). There were no significant differences between ORX+T and ORX+TREN for $TRAB_{DEN}$ ($p>0.05$).

Cortical Density (CRT_{DEN})

Cortical density values at the 5mm landmark of the femur 960 ± 6.70 g (SHAM), 995.8 ± 7.92 mg/mm^2 (ORX), 999.6 ± 12.2 mg/mm^2 (ORX+EX), 972.7 ± 6.20 mg/mm^2 (ORX+T), and 974.8 ± 6.67 mg/mm^2 (ORX+TR) (see figure 4-40). At the conclusion of the study, femurs evaluated from ORX+V and ORX+EX animals had higher CRT_{DEN} 3.77% and +4.1% respectively, than SHAM animals ($p<0.01$). ORX+TR and ORX+T maintained CRT_{DEN} similar to SHAM conditions ($p>0.05$). Assessment of CRT_{DEN} found ORX+T animals with a tendency towards lower density 2.7% compared to ORX+EX ($p=0.08$). Weighted ladder climbing did not improve CRT_{DEN} compared to

ORX+V conditions (p>0.05). There were no differences between ORX+T and ORX+TREN for CRT_{DEN} (p>0.05).

Treatment Effects on 18 mm pQCT (Diaphysis)

No significant main effects were present at the 18mm femoral site for total mineral content, total density, total area, cortical content, cortical density, cortical area, cortical thickness, periosteal circumference and endosteal circumference (p>0.05) (see Figure 4-42 through 50).

Total Mineral Content, Total Density, Total Area

Total bone content values at the 18mm site were 11.8 ± 0.18 mg/mm (SHAM), 11.3 ± 0.24 mg/mm (ORX), 11.1 ± 0.23 mg/mm (ORX+EX), 11.4 ± 0.32 mg/mm (ORX+T), and 11.0 ± 0.25 mg/mm (ORX+TR). Total femoral density values at the 18 mm site were 985.9 ± 11.46 mg/mm^2 (SHAM), 992.5 ± 11.64 mg/mm^2 (ORX), 981.2 ± 13.08 mg/mm^2 (ORX+EX), 1010.0 ± 14.40 mg/mm^2 (ORX+T), and 993.48 ± 11.61 mg/mm^2 (ORX+TR). Total bone area values at the 18 mm landmark of the femur were 12.0 ± 0.19 mm^2 (SHAM), 11.4 ± 0.32 mm^2 (ORX), 11.4 ± 0.29 mm^2 (ORX+EX), 11.3 ± 0.27 mm^2 (ORX+T), and 11.1 ± 0.25 mm^2 (ORX+TR).

Cortical Content, Cortical Density, Cortical Area

Cortical bone content values at the 18 mm landmark of the femur 11.5 ± 0.17 mg/mm (SHAM), 11.0 ± 0.22 mg/mm (ORX), 10.9 ± 0.22 mg/mm (ORX+EX), 11.2 ± 0.30 mg/mm (ORX+T), and 9.13 ± 0.43 mg/mm (ORX+TR). Cortical bone density values at the 18 mm landmark of the femur 1390.6 ± 5.09 mg/mm^2 (SHAM), 1388.4 ± 5.40 mg/mm^2 (ORX), 1395.5 ± 5.10 mg/mm^2 (ORX+EX), 1396.6 ± 4.20 mg/mm^2 (ORX+T), and 1398.5 ± 5.39 mg/mm^2 (ORX+TR).Cortical bone area values at the 18

mm landmark of the femur 8.26 ± 0.12 mm^2 (SHAM), 7.91 ± 0.17 mm^2 (ORX), 7.78 ± 0.16 mm^2 (ORX+EX), 7.99 ± 0.21 mm^2 (ORX+T), and 7.71 ± 0.18 mm^2 (ORX+TR).

Cortical Thickness, Periosteal and Endosteal Circumference

Cortical bone thickness values at the 18 mm site were 0.87 ± 0.01 mm (SHAM), 0.85 ± 0.01 mm (ORX), 0.83 ± 0.01 mm (ORX+EX), 0.87 ± 0.02 mm (ORX+T), and 0.85 ± 0.01 mm (ORX+TR).Periosteal circumference values at the 18 mm landmark were 12.26 ± 0.10 mm (SHAM), 11.96 ± 0.17 mm (ORX), 11.94 ± 0.15 mm (ORX+EX), 11.92 ± 0.15 mm (ORX+T), and 11.81 ± 0.13 mm (ORX+TR). Endosteal circumference values at the 18mm landmark site were 6.82 ± 0.12 mm (SHAM), 6.60 ± 0.16 mm (ORX), 6.70 ± 0.16 mm (ORX+EX), 6.46 ± 0.12 mm (ORX+T), and 6.52 ± 0.11 mm (ORX+TR).

Intraskeletal Hormone Concentrations

Intraskeletal Testosterone

Tibial intraskeletal testosterone concentrations were 3.81 ± 1.07 ng/g (SHAM), 2.59 ± 0.72 ng/g (ORX), 2.59 ± 0.79 ng/g (ORX+EX), 11.4 ± 2.51 ng/g (ORX+T), and 2.80 ± 0.78 (ORX+TR) (see Figure 4-51). At the conclusion of the study, there were no significant differences between SHAM, ORX, ORX+EX and ORX+TR. ORX+T animals intraskeletal testosterone concentrations were 4 fold greater than all ORX conditions and 3 fold greater than SHAM conditions. ORX+T animals were significantly greater than all other treatment groups ($p<0.01$).

Intraskeletal DHT

Tibial intraskeletal dihydrotestosterone concentrations were 4013.2 ± 204.0 pg/g (SHAM), 3419.3 ± 290.1 pg/g (ORX), 3504.4 ± 214.9 pg/g (ORX+EX), 12413.1 ± 1177.5 pg/g (ORX+T), 3681.0 ± 164.1 pg/g (ORX+TR) (see Figure 4-52). At the conclusion of the study, there were no significant differences between SHAM, ORX, ORX+EX and

ORX+TR. ORX+T animals intraskeletal DHT concentrations were 3.3 fold greater than all ORX conditions and 3 fold greater than SHAM conditions. ORX+T animals were significantly greater than all other treatment groups ($p<0.01$).

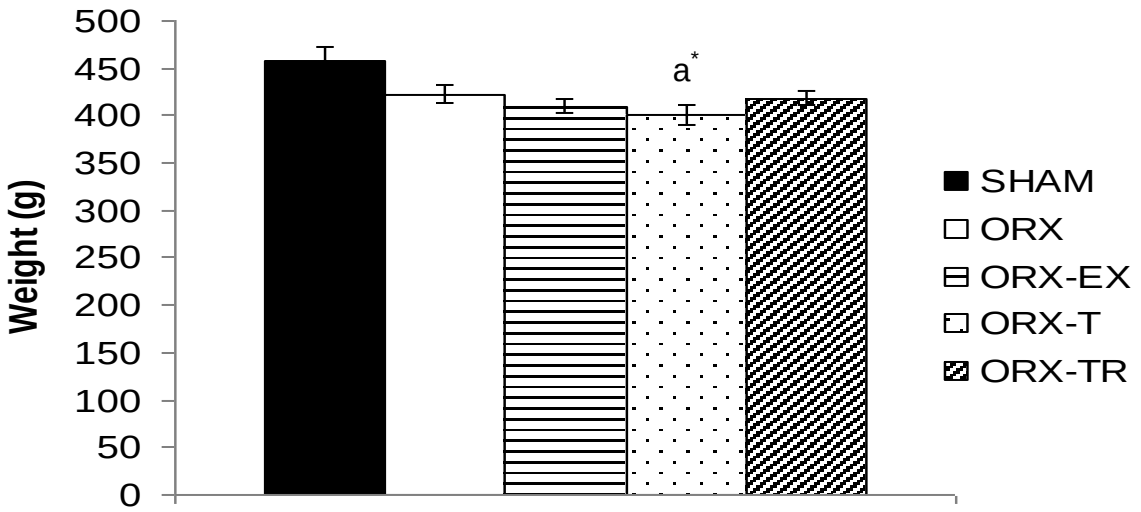

FIGURE 4-1. Effects of ORX+V, ORX+EX, testosterone-enanthate (TE), or trenbolone-enanthate (TR) on bodyweight at sacrifice. Values are Means ± SE, n = 9-10/group. Letters a-e indicate differences from respectively labeled groups at # $p < 0.05$ or * $p < 0.01$ (a = vs. SHAM, b = vs. ORX, c = vs.ORX + EX, d = vs. ORX + T, e = vs. ORX + TR).

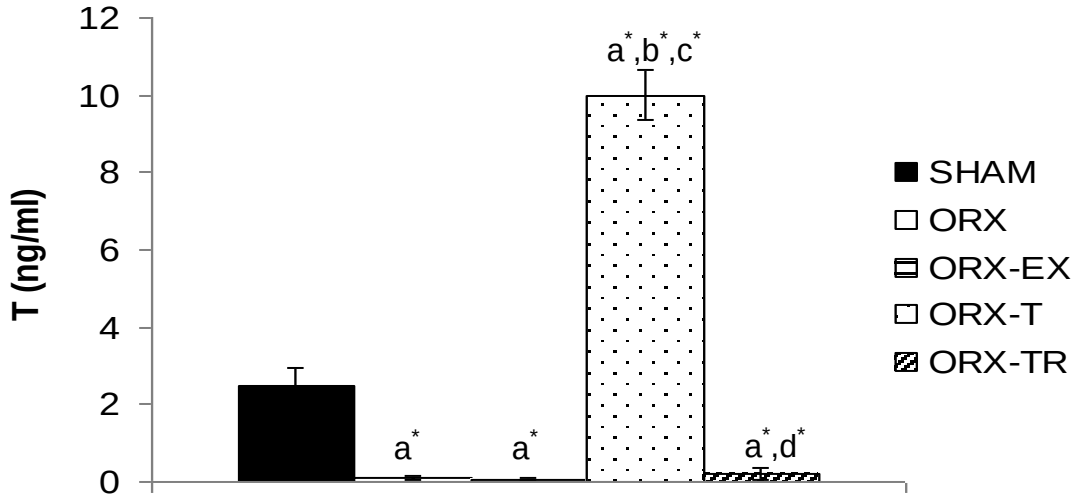

FIGURE 4-2. Effects of ORX+V, ORX+EX, testosterone-enanthate (TE), or trenbolone-enanthate (TR) on serum testosterone levels at sacrifice. Values are Means ± SE, n = 9-10/group. Letters a-e indicate differences from respectively labeled groups at # $p < 0.05$ or * $p < 0.01$ (a = vs. SHAM, b = vs. ORX, c = vs.ORX + EX, d = vs. ORX + T, e = vs. ORX + TR).

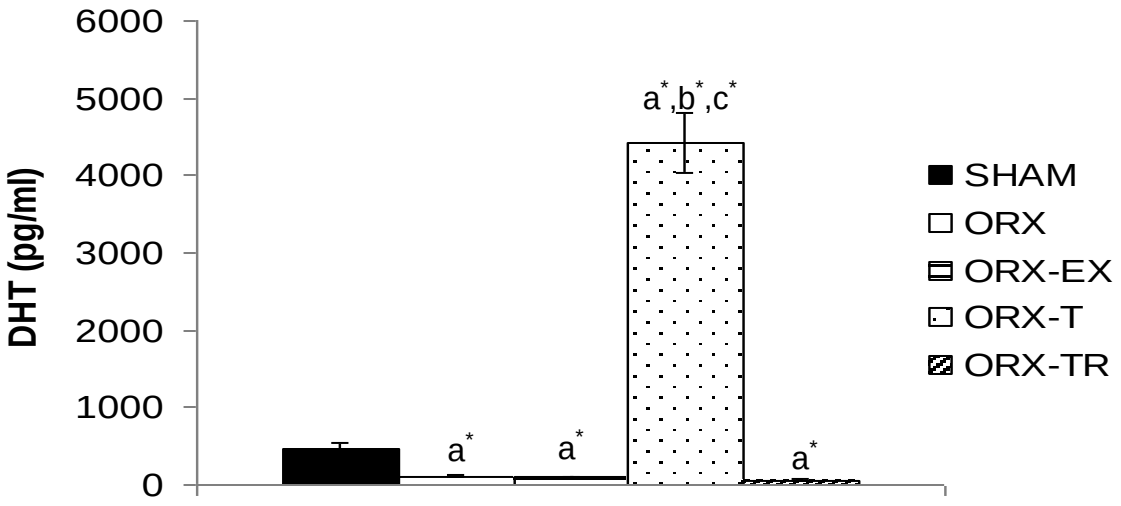

FIGURE 4-3. Effects of ORX+V, ORX+EX, testosterone-enanthate (TE), or trenbolone-enanthate (TR) on serum DHT at sacrifice. Values are Means ± SE, n = 9-10/group. Letters a-e indicate differences from respectively labeled groups at # $p < 0.05$ or * $p < 0.01$ (a = vs. SHAM, b = vs. ORX, c = vs.ORX + EX, d = vs. ORX + T, e = vs. ORX + TR).

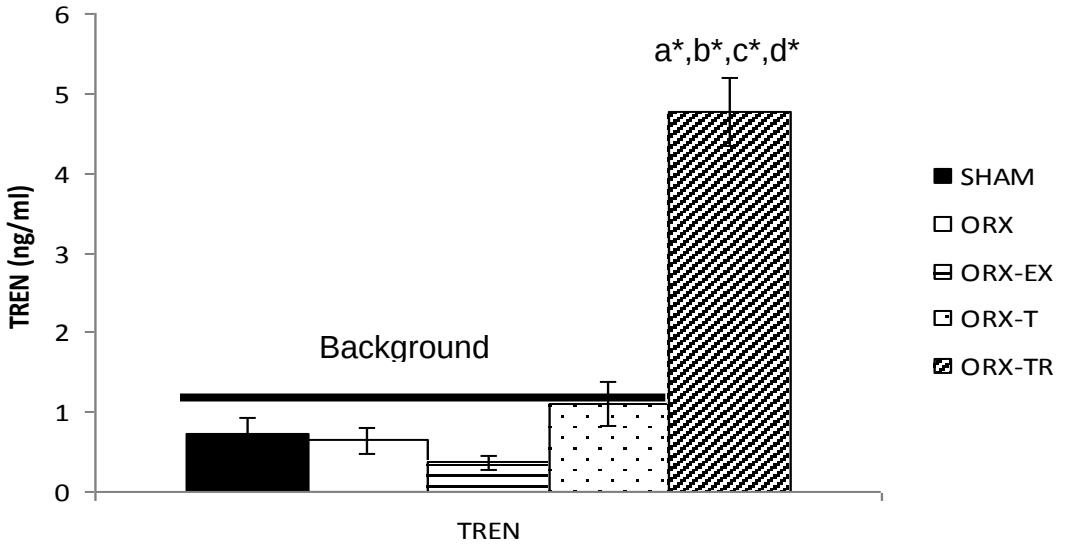

FIGURE 4-4. Effects of ORX+V, ORX+EX, testosterone-enanthate (TE), or trenbolone-enanthate (TR) on serum DHT at sacrifice. Values are Means ± SE, n = 9-10/group. Letters a-e indicate differences from respectively labeled groups at # $p < 0.05$ or * $p < 0.01$ (a = vs. SHAM, b = vs. ORX, c = vs.ORX + EX, d = vs. ORX + T, e = vs. ORX + TR).

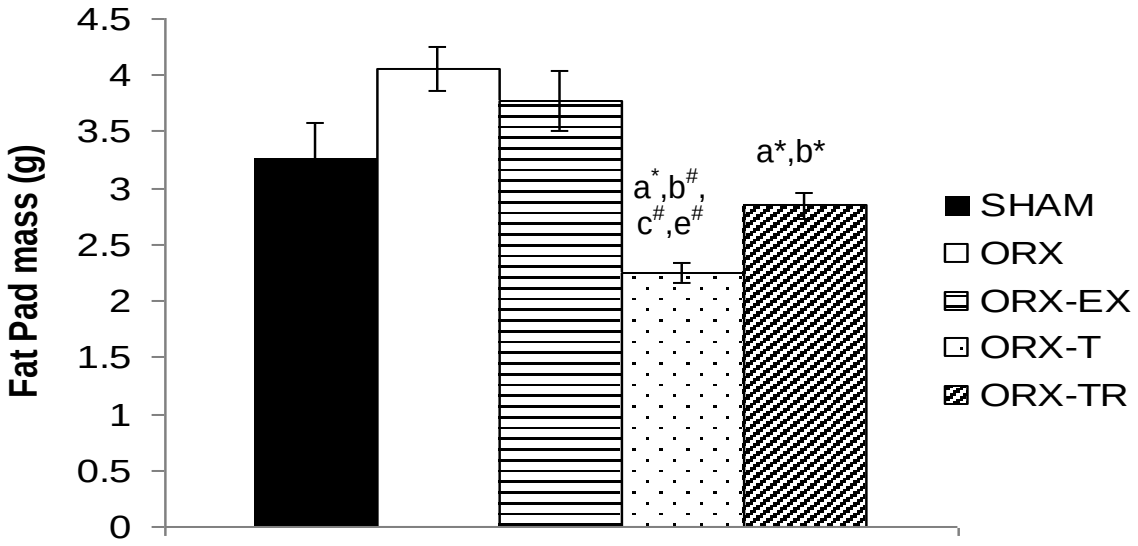

FIGURE 4-5. Effects of ORX+V, ORX+EX, testosterone-enanthate (TE), or trenbolone-enanthate (TR) on retroperitoneal fat pad mass at sacrifice. Values are Means ± SE, n = 9-10/group. Letters a-e indicate differences from respectively labeled groups at # $p < 0.05$ or * $p < 0.01$ (a = vs. SHAM, b = vs. ORX, c = vs.ORX + EX, d = vs. ORX + T, e = vs. ORX + TR).

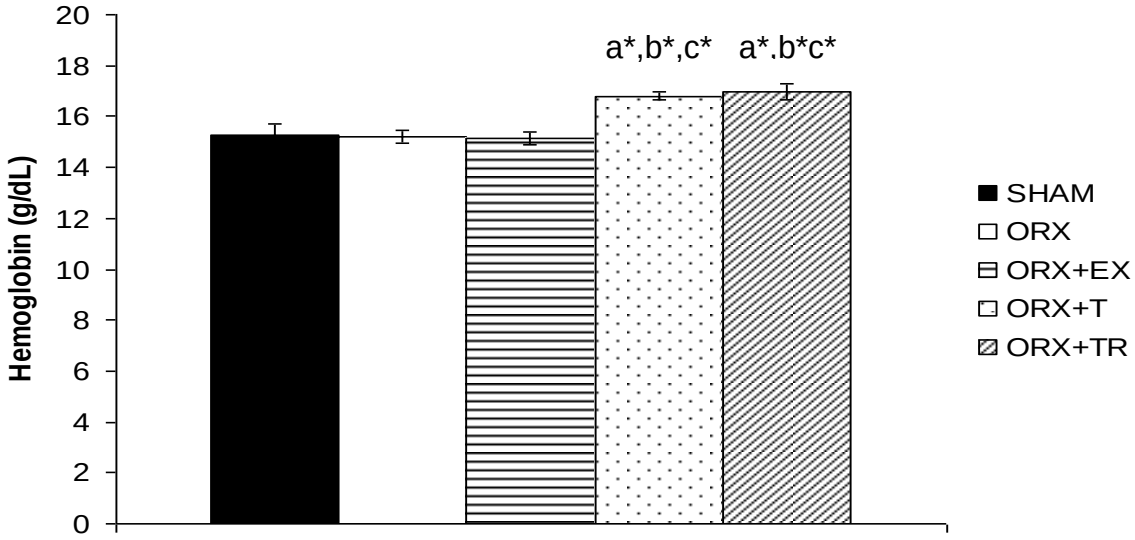

FIGURE 4-6. Effects of ORX+V, ORX+EX, testosterone-enanthate (TE), or trenbolone-enanthate (TR) on hemoglobin mass at sacrifice. Values are Means ± SE, n = 9-10/group. Letters a-e indicate differences from respectively labeled groups at # $p < 0.05$ or * $p < 0.01$ (a = vs. SHAM, b = vs. ORX, c = vs.ORX + EX, d = vs. ORX + T, e = vs. ORX + TR).

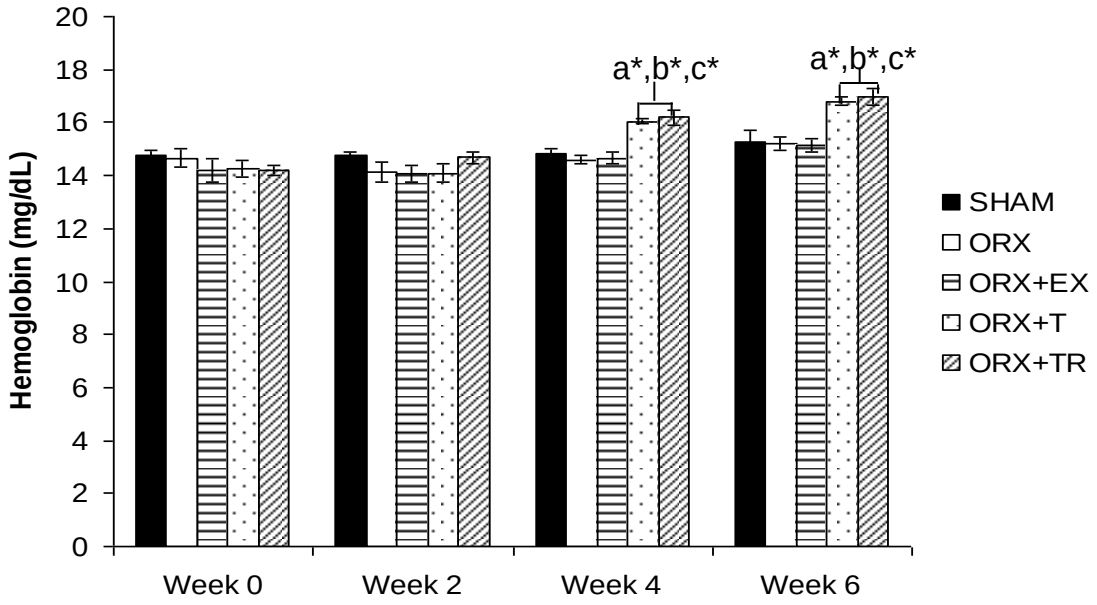

FIGURE 4-7. Effects of ORX+V, ORX+EX, testosterone-enanthate (TE), or trenbolone-enanthate (TR) time course of hemoglobin mass. Values are Means ± SE, n = 9-10/group. Letters a-e indicate differences from respectively labeled groups at $^{\#}$ $p < 0.05$ or * $p < 0.01$ (a = vs. SHAM, b = vs. ORX, c = vs.ORX + EX, d = vs. ORX + T, e = vs. ORX + TR).

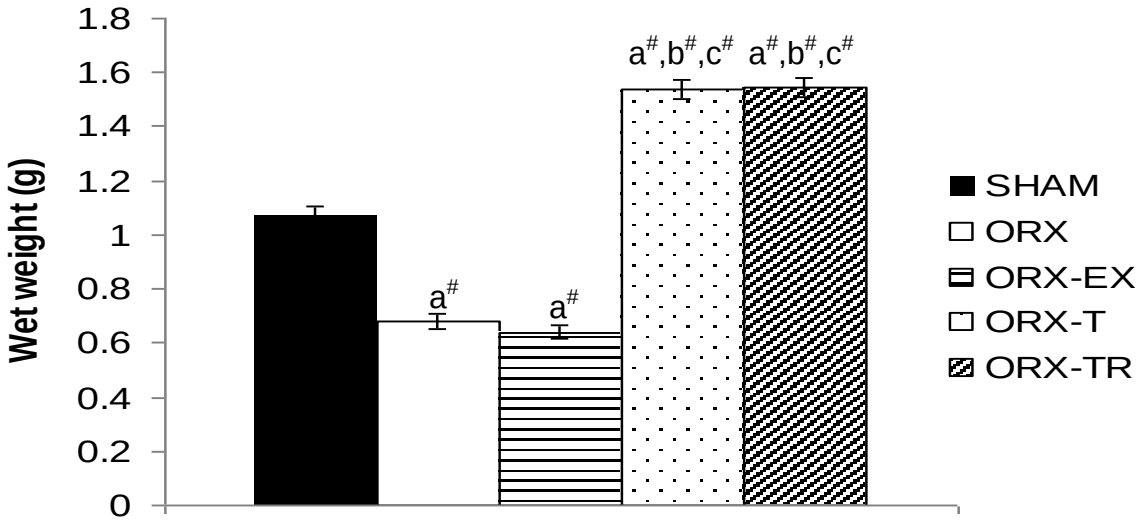

FIGURE 4-8. Effects of ORX+V, ORX+EX, testosterone-enanthate (TE), or trenbolone-enanthate (TR) on levator ani bulbocavernosus muscle mass at sacrifice. Values are Means ± SE, n = 9-10/group. Letters a-e indicate differences from respectively labeled groups at $^{\#}$ $p < 0.05$ or * $p < 0.01$ (a = vs. SHAM, b = vs. ORX, c = vs.ORX + EX, d = vs. ORX + T, e = vs. ORX + TR).

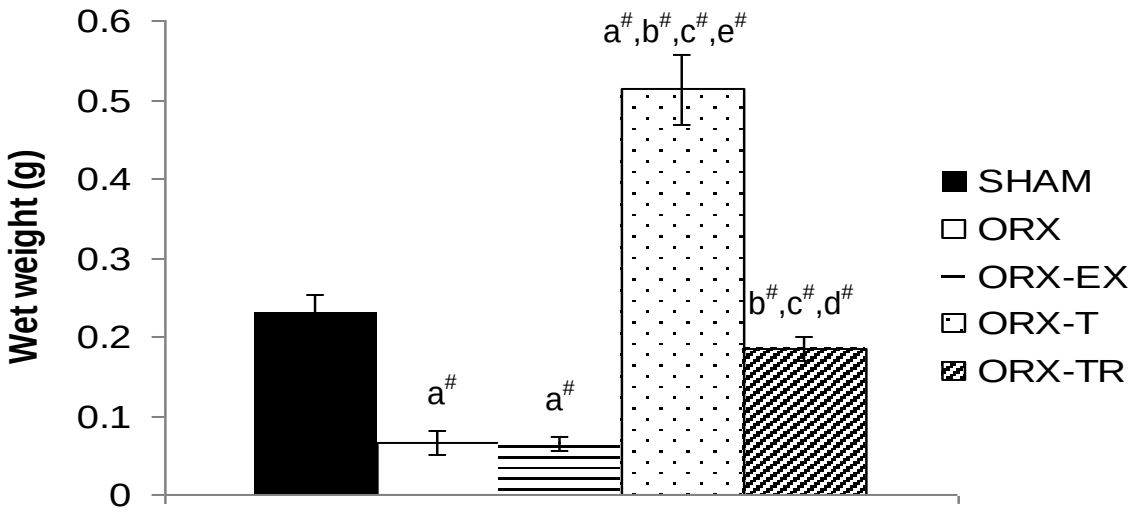

FIGURE 4-9. Effects of ORX+V, ORX+EX, testosterone-enanthate (TE), or trenbolone-enanthate (TR) on prostate mass at sacrifice. Values are Means ± SE, n = 9-10/group. Letters a-e indicate differences from respectively labeled groups at # $p < 0.05$ or * $p < 0.01$ (a = vs. SHAM, b = vs. ORX, c = vs.ORX + EX, d = vs. ORX + T, e = vs. ORX + TR).

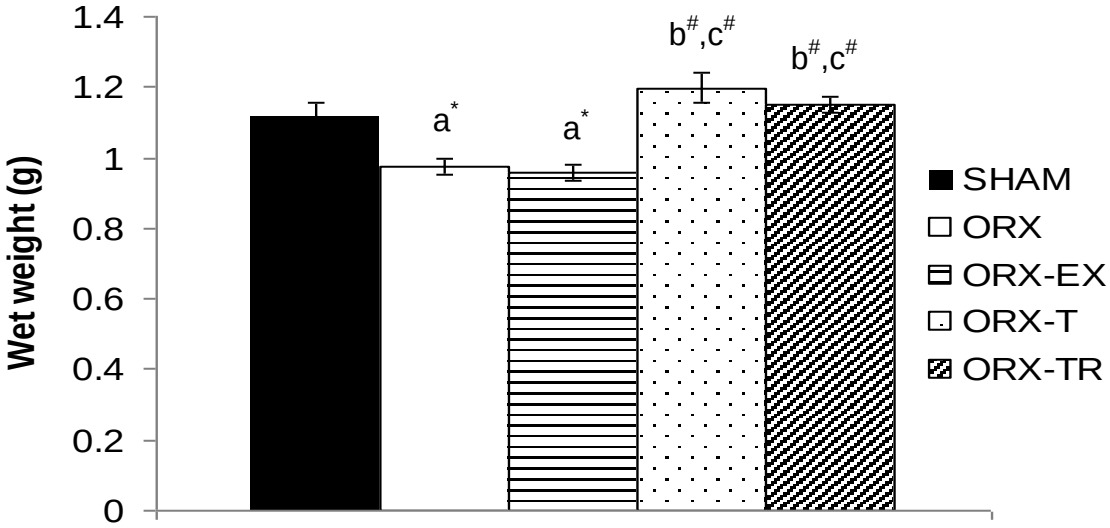

FIGURE 4-10. Effects of ORX+V, ORX+EX, testosterone-enanthate (TE), or trenbolone-enanthate (TR) on kidney mass at sacrifice. Values are Means ± SE, n = 9-10/group. Letters a-e indicate differences from respectively labeled groups at # $p < 0.05$ or * $p < 0.01$ (a = vs. SHAM, b = vs. ORX, c = vs.ORX + EX, d = vs. ORX + T, e = vs. ORX + TR).

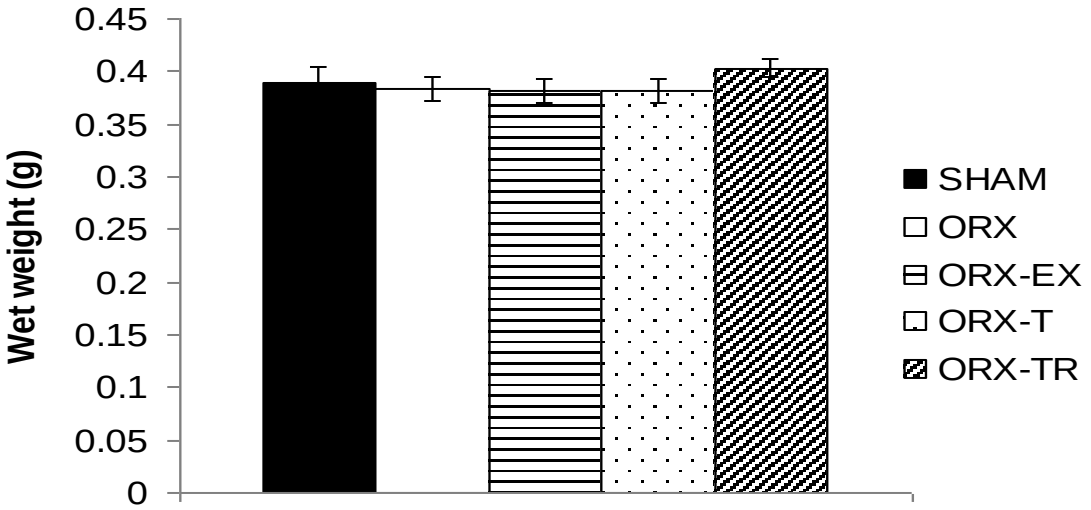

FIGURE 4-11. Effects of ORX+V, ORX+EX, testosterone-enanthate (TE), or trenbolone-enanthate (TR) on semimembranosus muscle mass at sacrifice. Values are Means ± SE, n = 9-10/group. Letters a-e indicate differences from respectively labeled groups at $^{\#}p < 0.05$ or * $p < 0.01$ (a = vs. SHAM, b = vs. ORX, c = vs.ORX + EX, d = vs. ORX + T, e = vs. ORX + TR).

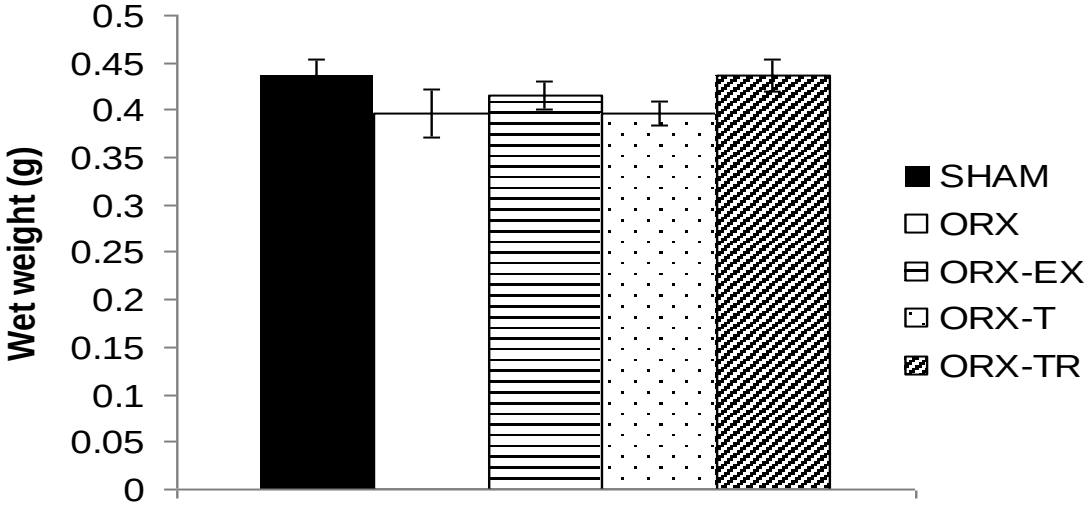

FIGURE 4-12. Effects of ORX+V, ORX+EX, testosterone-enanthate (TE), or trenbolone-enanthate (TR) on plantaris muscle mass at sacrifice. Values are Means ± SE, n = 9-10/group. Letters a-e indicate differences from respectively labeled groups at $^{\#}p < 0.05$ or * $p < 0.01$ (a = vs. SHAM, b = vs. ORX, c = vs.ORX + EX, d = vs. ORX + T, e = vs. ORX + TR).

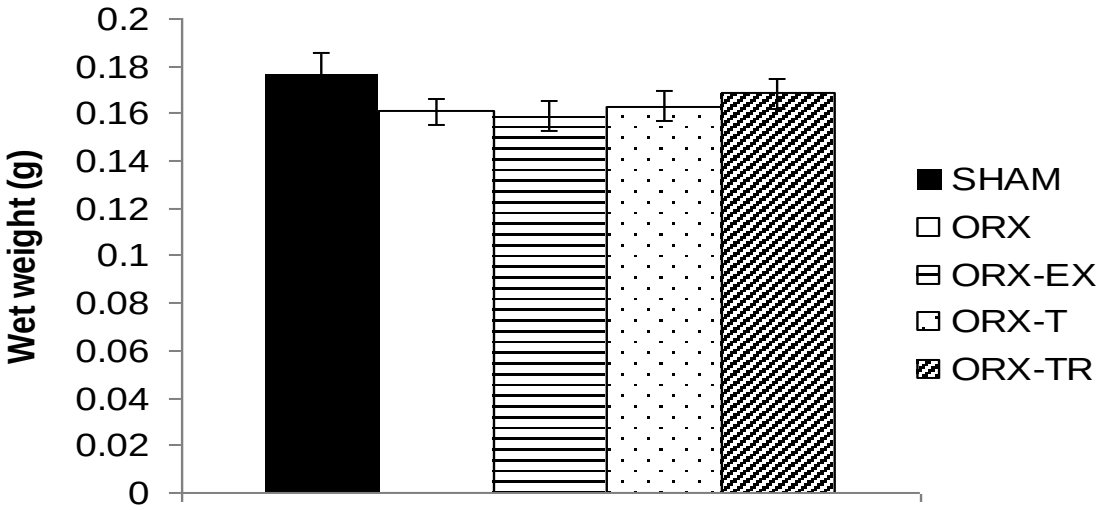

FIGURE 4-13. Effects of ORX+V, ORX+EX, testosterone-enanthate (TE), or trenbolone-enanthate (TR) on soleus muscle mass at sacrifice. Values are Means ± SE, n = 9-10/group. Letters a-e indicate differences from respectively labeled groups at # $p < 0.05$ or * $p < 0.01$ (a = vs. SHAM, b = vs. ORX, c = vs.ORX + EX, d = vs. ORX + T, e = vs. ORX + TR).

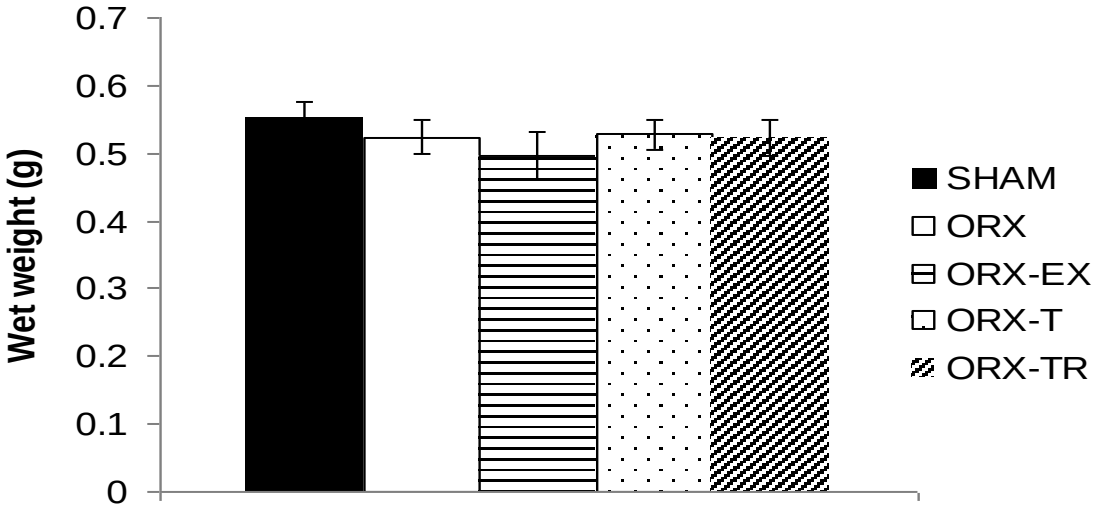

FIGURE 4-14. Effects of ORX+V, ORX+EX, testosterone-enanthate (TE), or trenbolone-enanthate (TR) on flexor hallicus longus muscle mass at sacrifice. Values are Means ± SE, n = 9-10/group. Letters a-e indicate differences from respectively labeled groups at # $p < 0.05$ or * $p < 0.01$ (a = vs. SHAM, b = vs. ORX, c = vs.ORX + EX, d = vs. ORX + T, e = vs. ORX + TR).

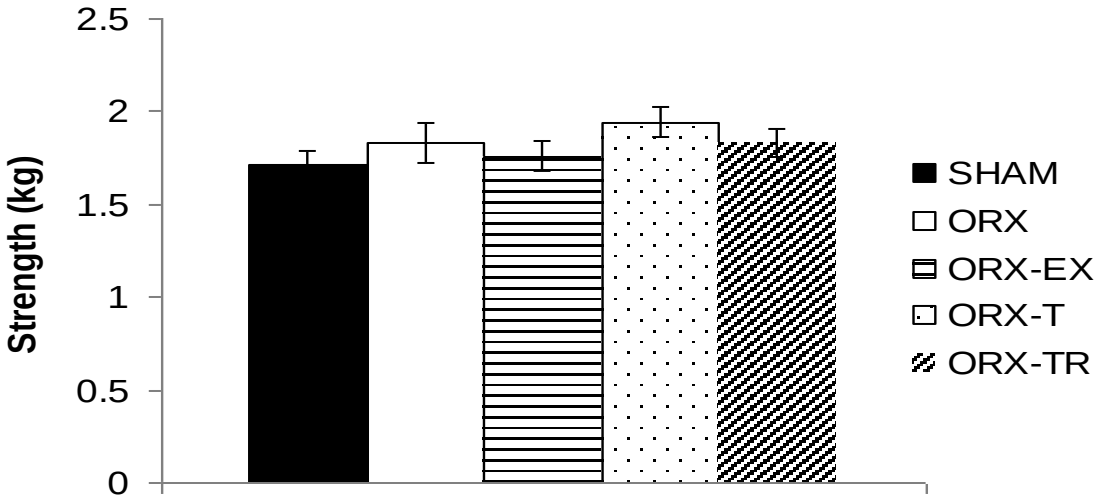

FIGURE 4-15. Effects of ORX+V, ORX+EX, testosterone-enanthate (TE), or trenbolone-enanthate (TR) on forelimb grip strength at sacrifice. Values are Means ± SE, n = 9-10/group. Letters a-e indicate differences from respectively labeled groups at # $p < 0.05$ or * $p < 0.01$ (a = vs. SHAM, b = vs. ORX, c = vs.ORX + EX, d = vs. ORX + T, e = vs. ORX + TR).

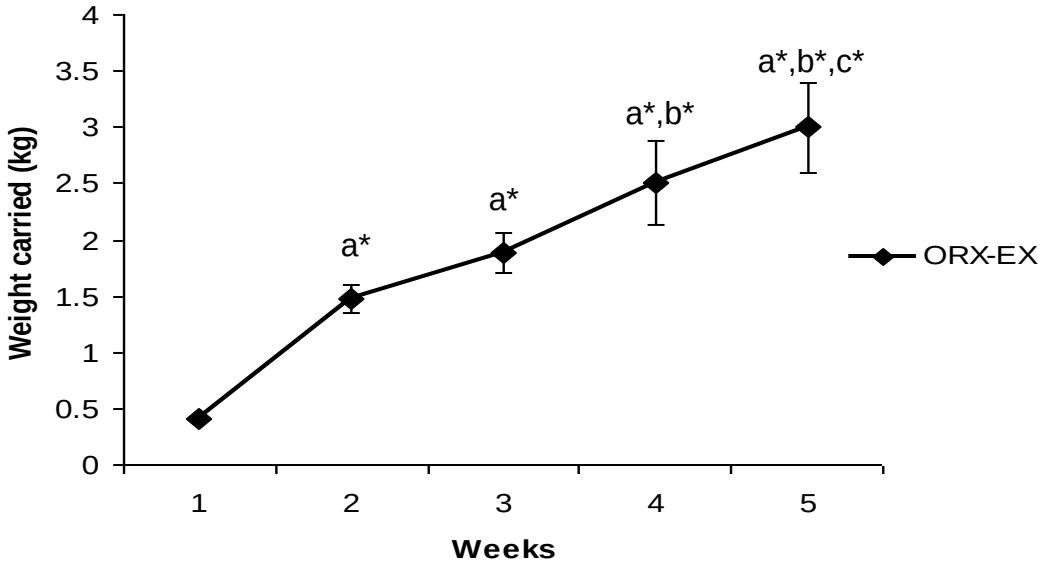

FIGURE 4-16. Total weekly training load carried by ORX+EX animals on a 1.1m ladder inclined at 85°. Values are Means ± SE, n = 10. Significant at # $p < 0.05$ or * $p < 0.01$ (a = vs. Week 1, b = vs. Week 2, c = vs Week 3+ EX, d = vs. Week 4, e = vs. Week 5.

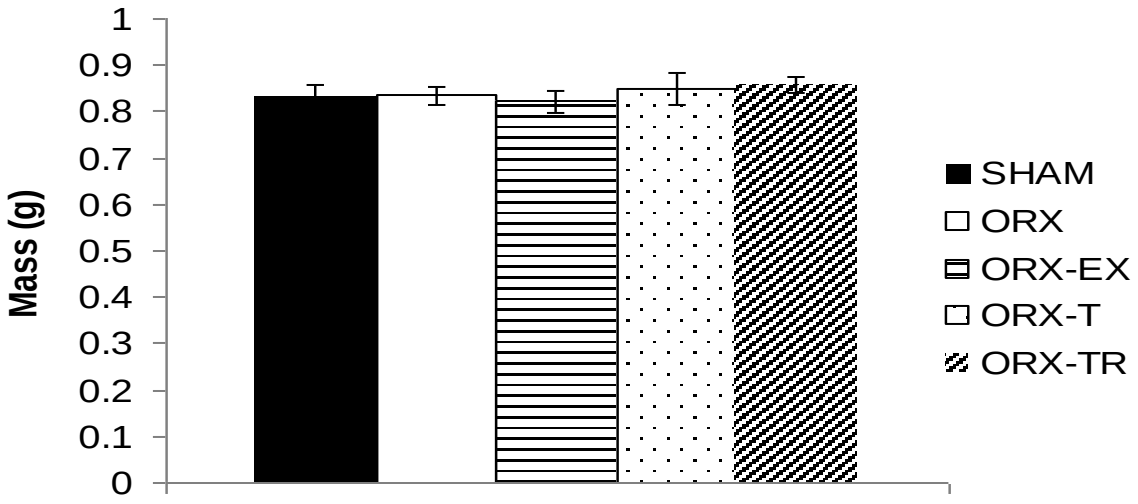

FIGURE 4-17. Effects of ORX+V, ORX+EX, testosterone-enanthate (TE), or trenbolone-enanthate (TR) on tibial mass at sacrifice. Values are Means ± SE, n = 9-10/group. Letters a-e indicate differences from respectively labeled groups at # $p < 0.05$ or * $p < 0.01$ (a = vs. SHAM, b = vs. ORX, c = vs.ORX + EX, d = vs. ORX + T, e = vs. ORX + TR).

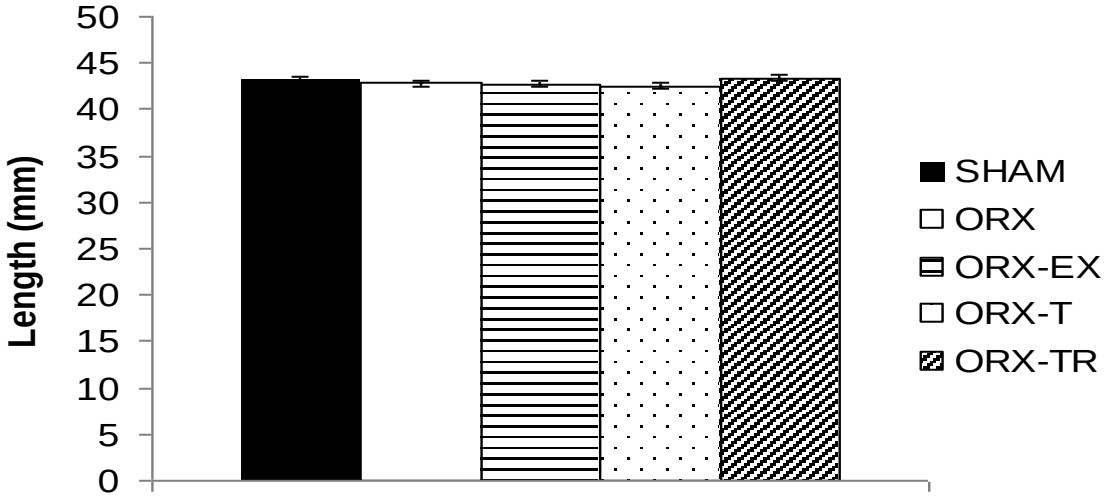

FIGURE 4-18. Effects of ORX+V, ORX+EX, testosterone-enanthate (TE), or trenbolone-enanthate (TR) on tibial length at sacrifice. Values are Means ± SE, n = 9-10/group. Letters a-e indicate differences from respectively labeled groups at # $p < 0.05$ or * $p < 0.01$ (a = vs. SHAM, b = vs. ORX, c = vs.ORX + EX, d = vs. ORX + T, e = vs. ORX + TR).

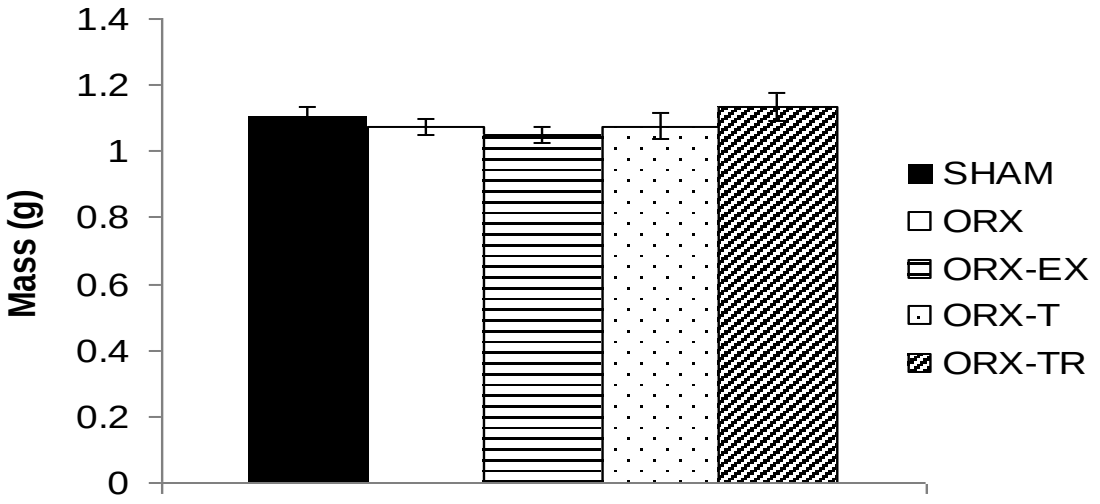

FIGURE 4-19. Effects of ORX+V, ORX+EX, testosterone-enanthate (TE), or trenbolone-enanthate (TR) on femoral mass at sacrifice. Values are Means ± SE, n = 9-10/group. Letters a-e indicate differences from respectively labeled groups at # $p < 0.05$ or * $p < 0.01$ (a = vs. SHAM, b = vs. ORX, c = vs.ORX + EX, d = vs. ORX + T, e = vs. ORX + TR).

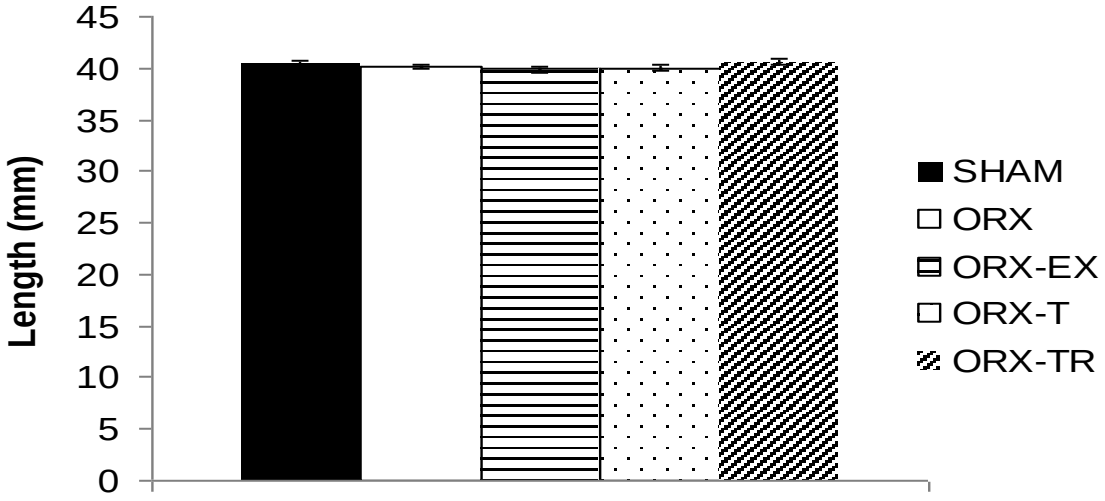

FIGURE 4-20. Effects of ORX+V, ORX+EX, testosterone-enanthate (TE), or trenbolone-enanthate (TR) on femoral length at sacrifice. Values are Means ± SE, n = 9-10/group. Letters a-e indicate differences from respectively labeled groups at # $p < 0.05$ or * $p < 0.01$ (a = vs. SHAM, b = vs. ORX, c = vs.ORX + EX, d = vs. ORX + T, e = vs. ORX + TR).

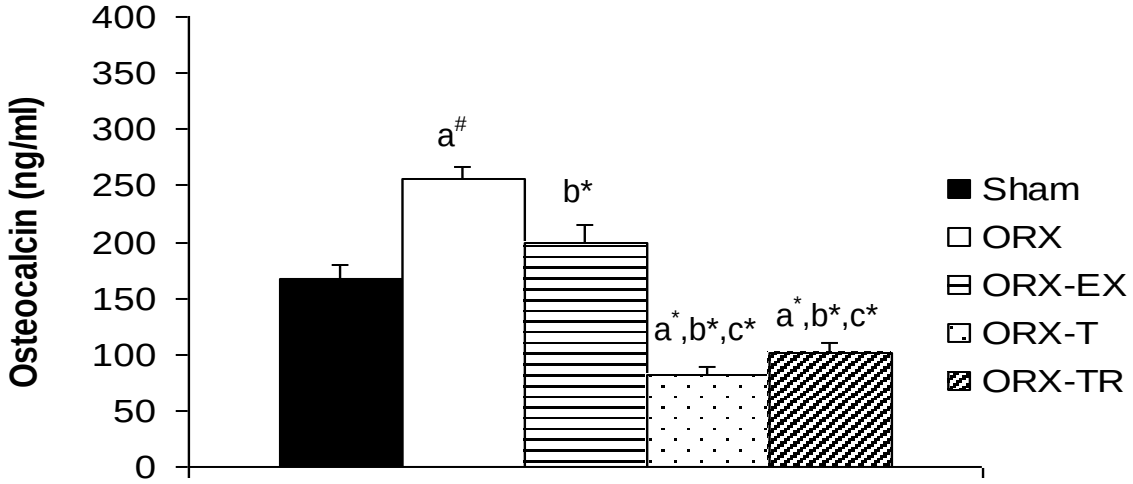

FIGURE 4-21. Effects of ORX+V, ORX+EX, testosterone-enanthate (TE), or trenbolone-enanthate (TR) on serum osteocalcin levels at sacrifice. Values are Means ± SE, n = 9-10/group. Letters a-e indicate differences from respectively labeled groups at # $p < 0.05$ or * $p < 0.01$ (a = vs. SHAM, b = vs. ORX, c = vs.ORX + EX, d = vs. ORX + T, e = vs. ORX + TR).

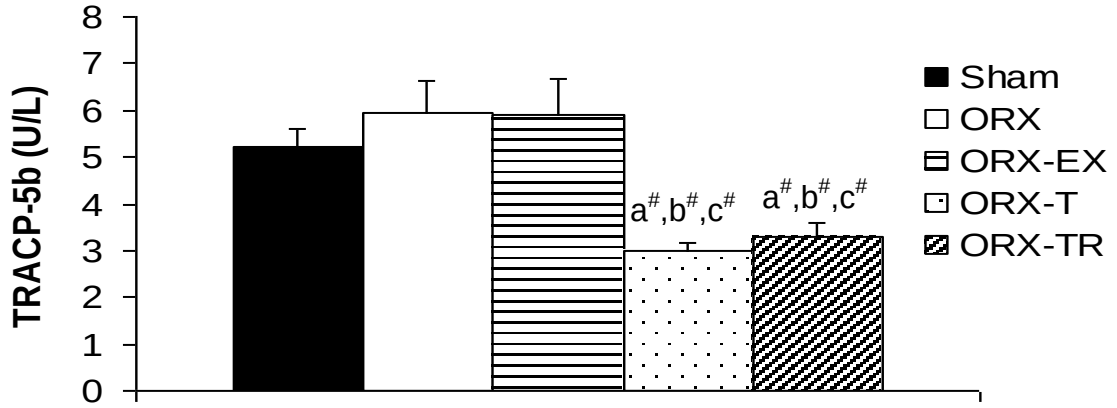

FIGURE 4-22. Effects of ORX+V, ORX+EX, testosterone-enanthate (TE), or trenbolone-enanthate (TR) on serum TRAP-5b levels at sacrifice. Values are Means ± SE, n = 9-10/group. Letters a-e indicate differences from respectively labeled groups at # $p < 0.05$ or * $p < 0.01$ (a = vs. SHAM, b = vs. ORX, c = vs.ORX + EX, d = vs. ORX + T, e = vs. ORX + TR).

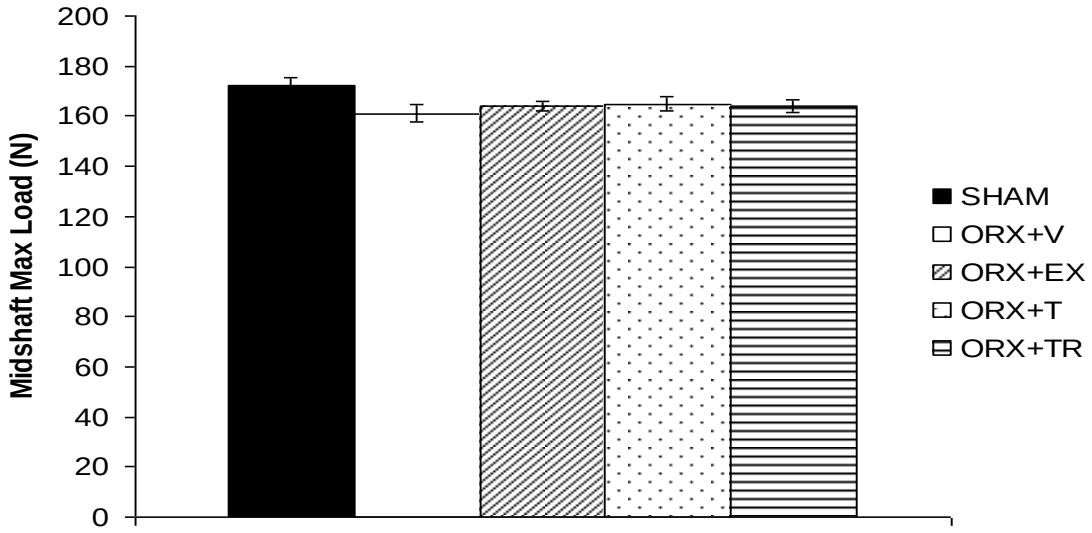

FIGURE 4-23. Effects of ORX+V, ORX+EX, testosterone-enanthate (TE), or trenbolone-enanthate (TR) on femoral midshaft maximum load. Values are Means ± SE, n = 9-10/group. Letters a-e indicate differences from respectively labeled groups at # $p < 0.05$ or * $p < 0.01$ (a = vs. SHAM, b = vs. ORX, c = vs.ORX + EX, d = vs. ORX + T, e = vs. ORX + TR).

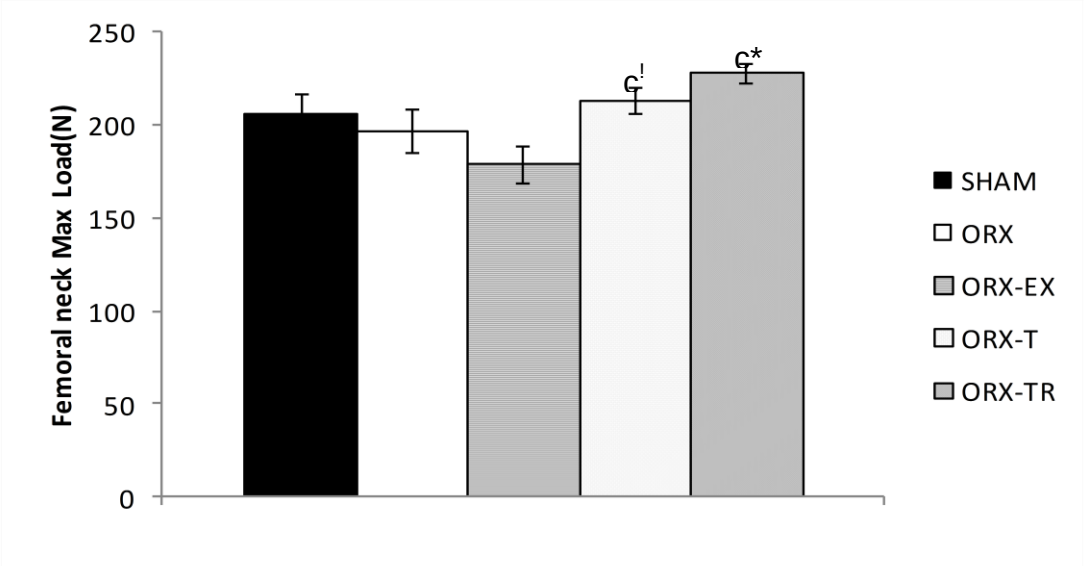

FIGURE 4-24. Effects of ORX+V, ORX+EX, testosterone-enanthate (TE), or trenbolone-enanthate (TR) on femoral neck maximum load. Values are Means ± SE, n = 8-10/group. Letters a-e indicate differences from respectively labeled groups at # $p < 0.05$ or * $p < 0.01$ (a = vs. SHAM, b = vs. ORX, c = vs.ORX + EX, d = vs. ORX + T, e = vs. ORX + TR).

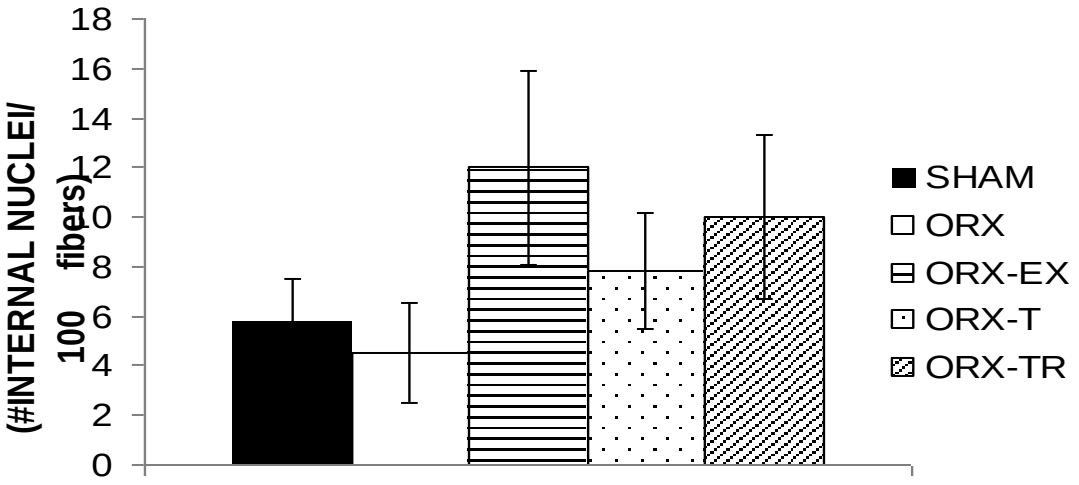

FIGURE 4-25. Effects of ORX+V, ORX+EX, testosterone-enanthate (TE), or trenbolone-enanthate (TR) on internalized nuclei of the FHL. Values are Means ± SE, n = 8-10/group. Letters a-e indicate differences from respectively labeled groups at # $p < 0.05$ or * $p < 0.01$ (a = vs. SHAM, b = vs. ORX, c = vs. ORX + EX, d = vs. ORX + T, e = vs. ORX + TR).

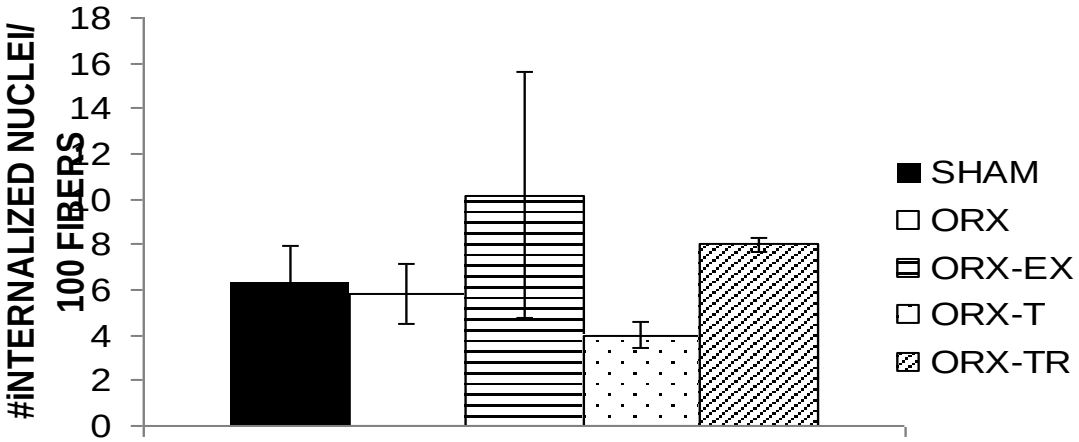

FIGURE 4-26. Effects of ORX+V, ORX+EX, testosterone-enanthate (TE), or trenbolone-enanthate (TR) on internalized nuclei of the semimembranosus. Values are Means ± SE, n = 8-10/group. Letters a-e indicate differences from respectively labeled groups at # $p < 0.05$ or * $p < 0.01$ (a = vs. SHAM, b = vs. ORX, c = vs. ORX + EX, d = vs. ORX + T, e = vs. ORX + TR).

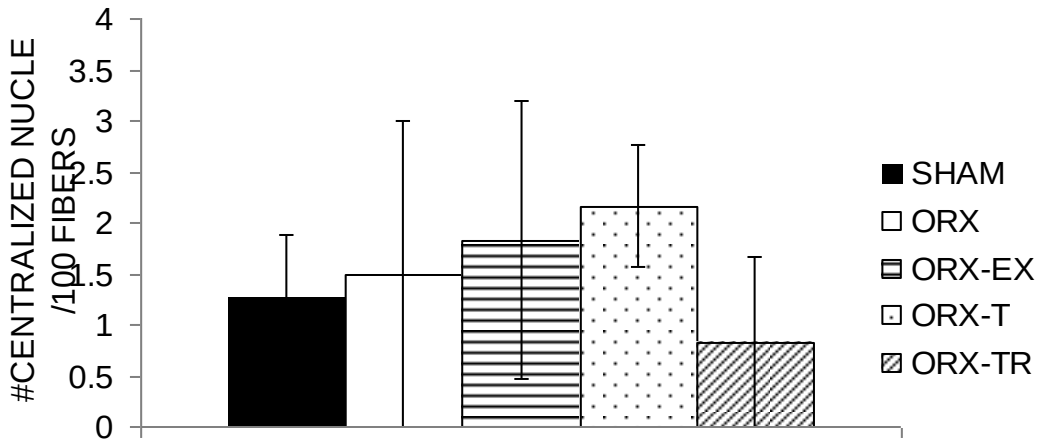

FIGURE 4-27. Effects of ORX+V, ORX+EX, testosterone-enanthate (TE), or trenbolone-enanthate (TR) on centralized nuclei of the FHL. Values are Means ± SE, n = 8-10/group. Letters a-e indicate differences from respectively labeled groups at $^{\#}$ $p < 0.05$ or * $p < 0.01$ (a = vs. SHAM, b = vs. ORX, c = vs. ORX + EX, d = vs. ORX + T, e = vs. ORX + TR).

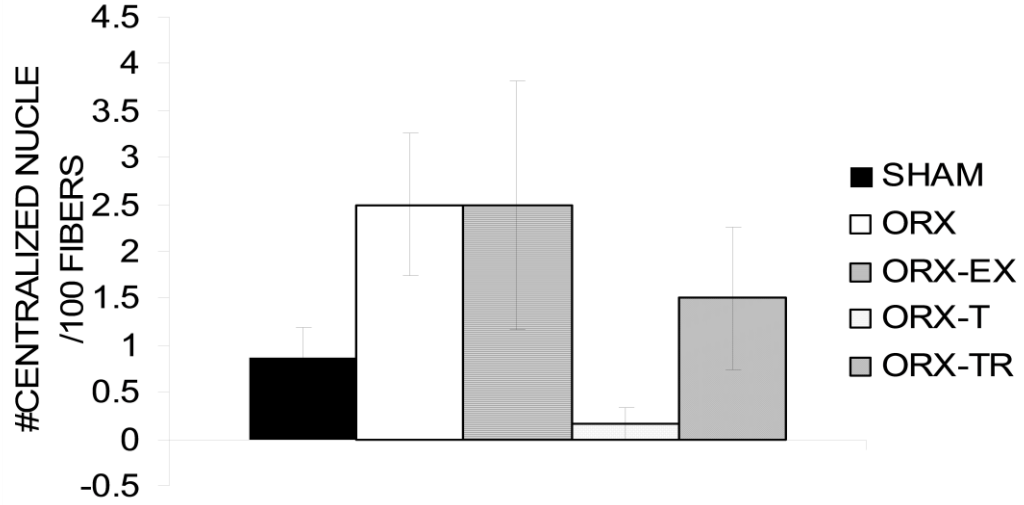

FIGURE 4-28. Effects of ORX+V, ORX+EX, testosterone-enanthate (TE), or trenbolone-enanthate (TR) on centralized nuclei of the semimembranosus. Values are Means ± SE, n = 8-10/group. Letters a-e indicate differences from respectively labeled groups at $^{\#}$ $p < 0.05$ or * $p < 0.01$ (a = vs. SHAM, b = vs. ORX, c = vs. ORX + EX, d = vs. ORX + T, e = vs. ORX + TR).

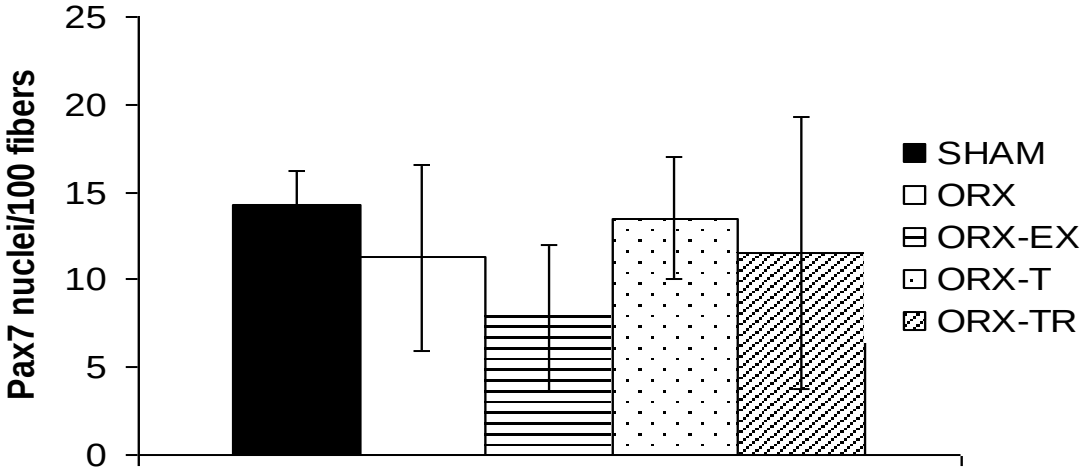

FIGURE 4-29. Effects of ORX+V, ORX+EX, testosterone-enanthate (TE), or trenbolone-enanthate (TR) on Pax 7+ nuclei expressed per 100 fibers of the flexor hallicus longus. Values are Means ± SE, n = 4/group. Letters a-e indicate differences from respectively labeled groups at $^{\#}p < 0.05$ or * $p < 0.01$ (a = vs. SHAM, b = vs. ORX, c = vs.ORX + EX, d = vs. ORX + T, e = vs. ORX + TR).

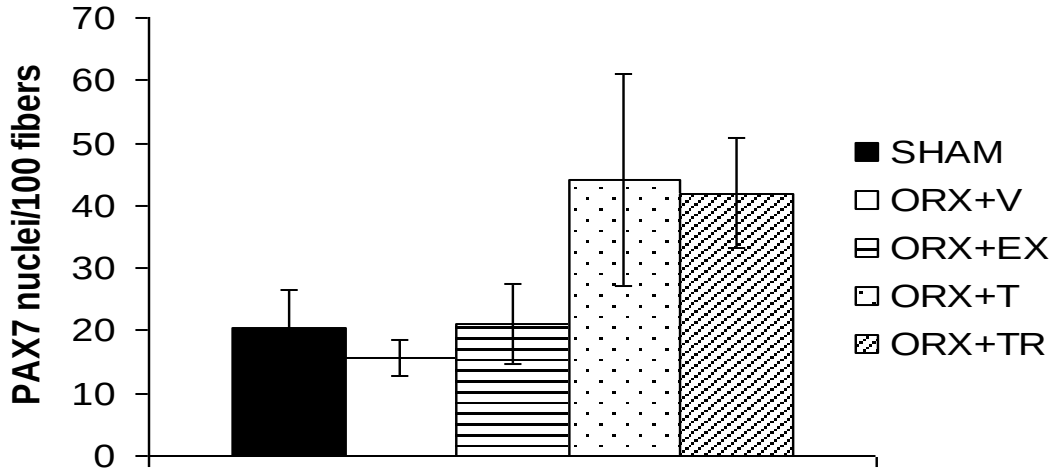

FIGURE 4-30. Effects of ORX+V, ORX+EX, testosterone-enanthate (TE), or trenbolone-enanthate (TR) on Pax 7+ nuclei expressed per 100 fibers of the semimembranosus. Values are Means ± SE, n = 4/group. Letters a-e indicate differences from respectively labeled groups at $^{\#}p < 0.05$ or * $p < 0.01$ (a = vs. SHAM, b = vs. ORX, c = vs.ORX + EX, d = vs. ORX + T, e = vs. ORX + TR).

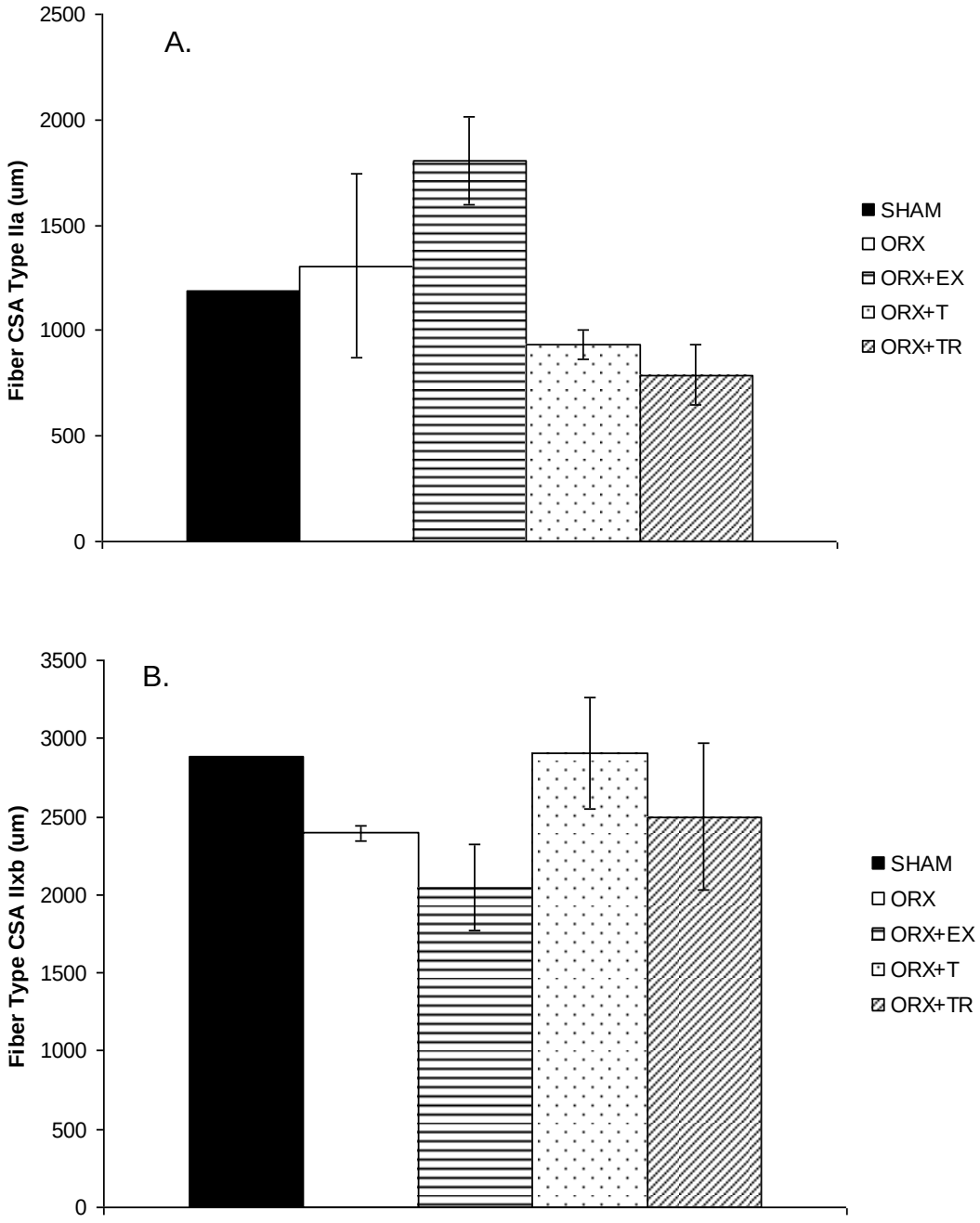

FIGURE 4-31. Effects of ORX+V, ORX+EX, testosterone-enanthate (TE), or trenbolone-enanthate (TR) on Type IIa fibers of the flexor hallicus longus. Values are Means ± SE, n = 1-4/group. (A=Type IIa FIBERS); (B=Type IIbx)

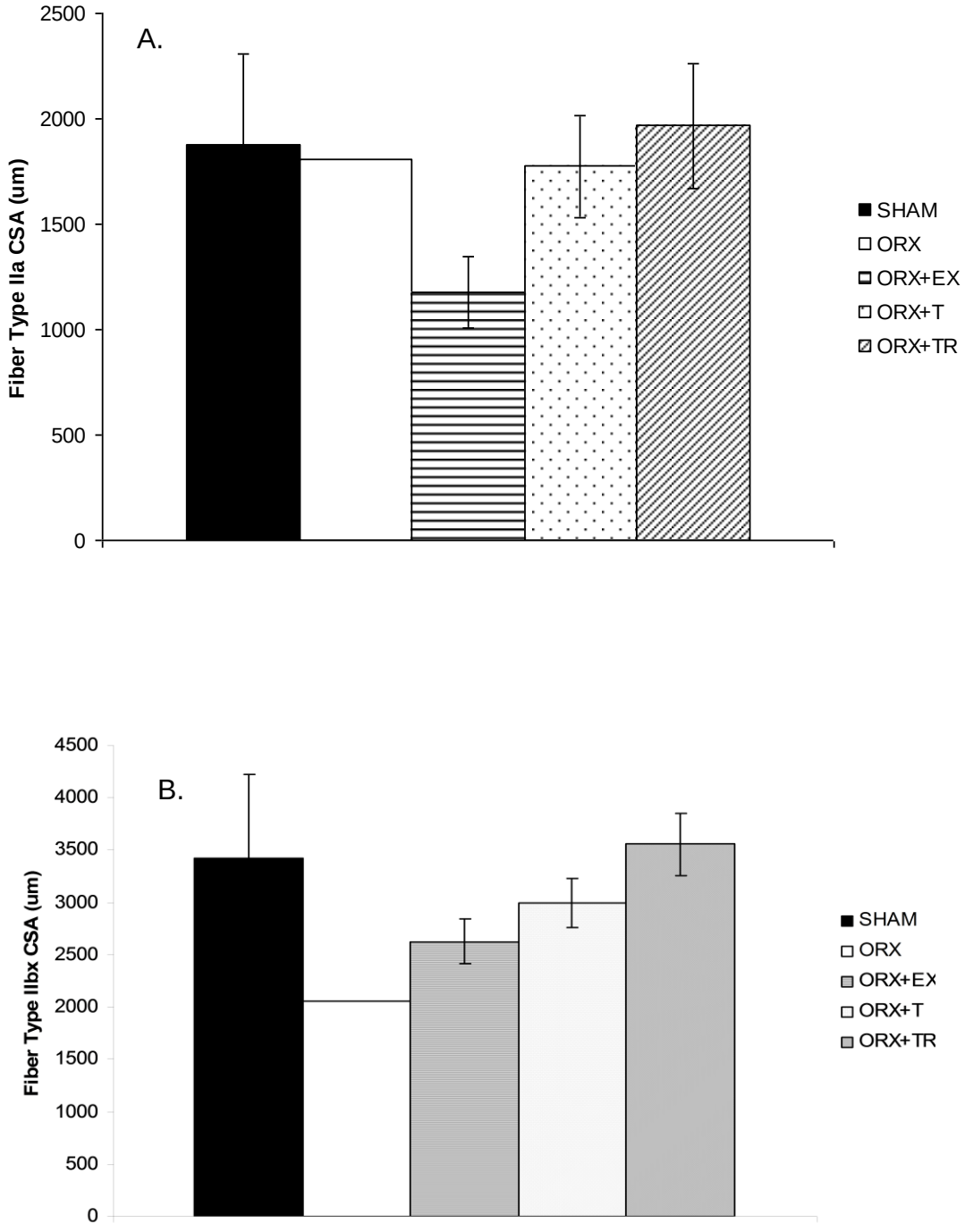

FIGURE 4-32. Effects of ORX+V, ORX+EX, testosterone-enanthate (TE), or trenbolone-enanthate (TR) on Type IIa fibers of the flexor hallicus longus. Values are Means ± SE, n = 1-4/group. (A=Type IIa FIBERS); (B=Type IIbx)

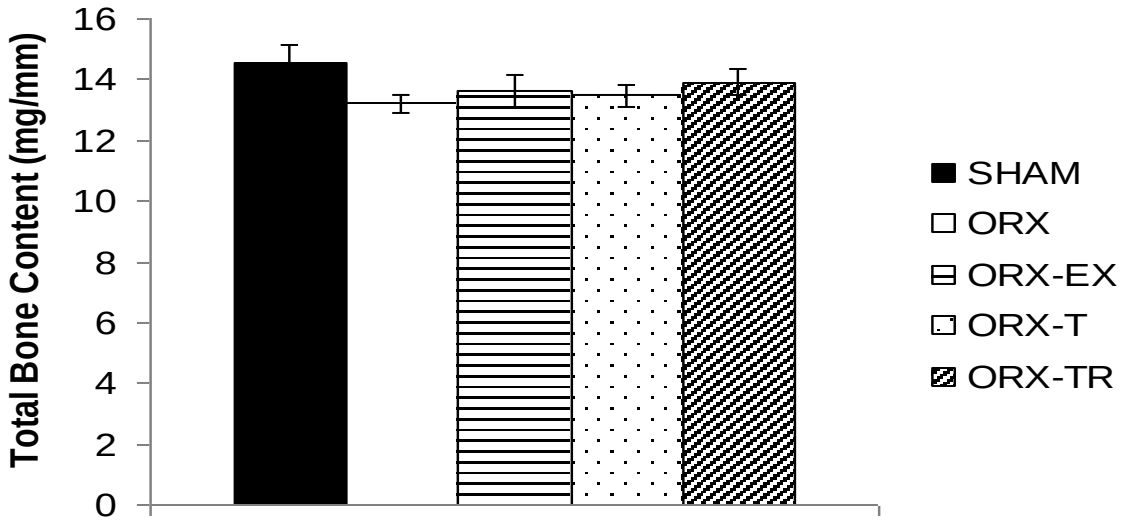

FIGURE 4-33. Effects of ORX+V, ORX+EX, testosterone-enanthate (TE), or trenbolone-enanthate (TR) on femoral total bone content at 5mm. Values are Means ± SE, n = 9-10/group. Letters a-e indicate differences from respectively labeled groups at # $p < 0.05$ or * $p < 0.01$ (a = vs. SHAM, b = vs. ORX, c = vs.ORX + EX, d = vs. ORX + T, e = vs. ORX + TR).

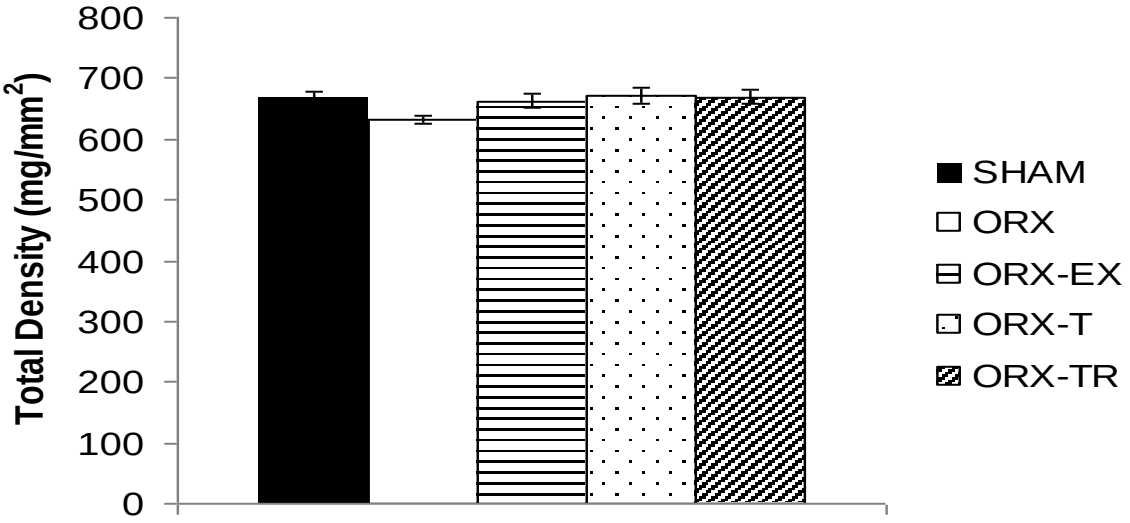

FIGURE 4-34. Effects of ORX+V, ORX+EX, testosterone-enanthate (TE), or trenbolone-enanthate (TR) on femoral total density at 5mm. Values are Means ± SE, n = 9-10/group. Letters a-e indicate differences from respectively labeled groups at # $p < 0.05$ or * $p < 0.01$ (a = vs. SHAM, b = vs. ORX, c = vs.ORX + EX, d = vs. ORX + T, e = vs. ORX + TR).

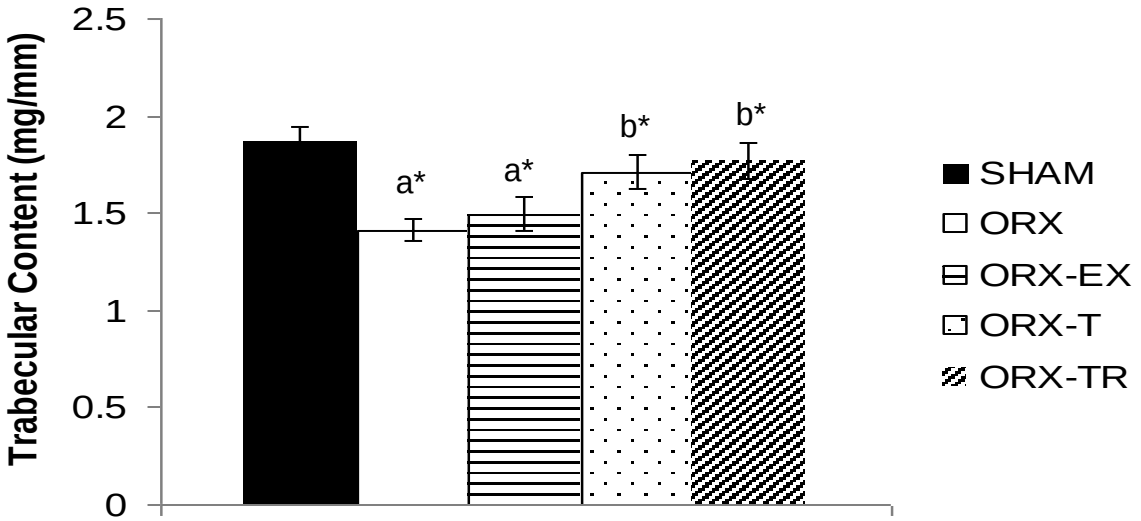

FIGURE 4-35. Effects of ORX+V, ORX+EX, testosterone-enanthate (TE), or trenbolone-enanthate (TR) on femoral trabecular content at 5mm. Values are Means ± SE, n = 9-10/group. Letters a-e indicate differences from respectively labeled groups at # $p < 0.05$ or * $p < 0.01$ (a = vs. SHAM, b = vs. ORX, c = vs.ORX + EX, d = vs. ORX + T, e = vs. ORX + TR).

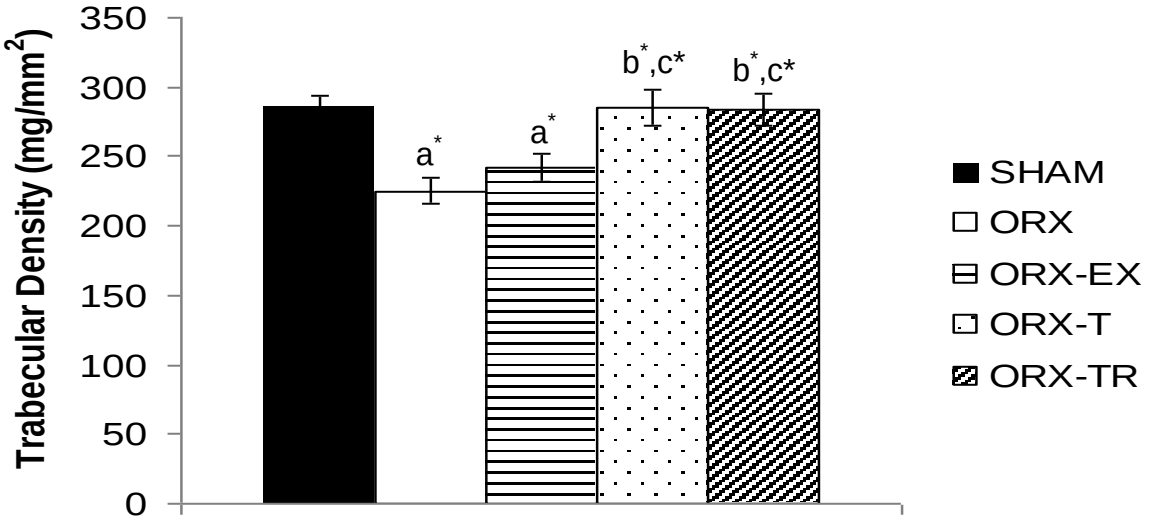

FIGURE 4-36. Effects of ORX+V, ORX+EX, testosterone-enanthate (TE), or trenbolone-enanthate (TR) on trabecular density at 5mm. Values are Means ± SE, n = 9-10/group. Letters a-e indicate differences from respectively labeled groups at # $p < 0.05$ or * $p < 0.01$ (a = vs. SHAM, b = vs. ORX, c = vs.ORX + EX, d = vs. ORX + T, e = vs. ORX + TR).

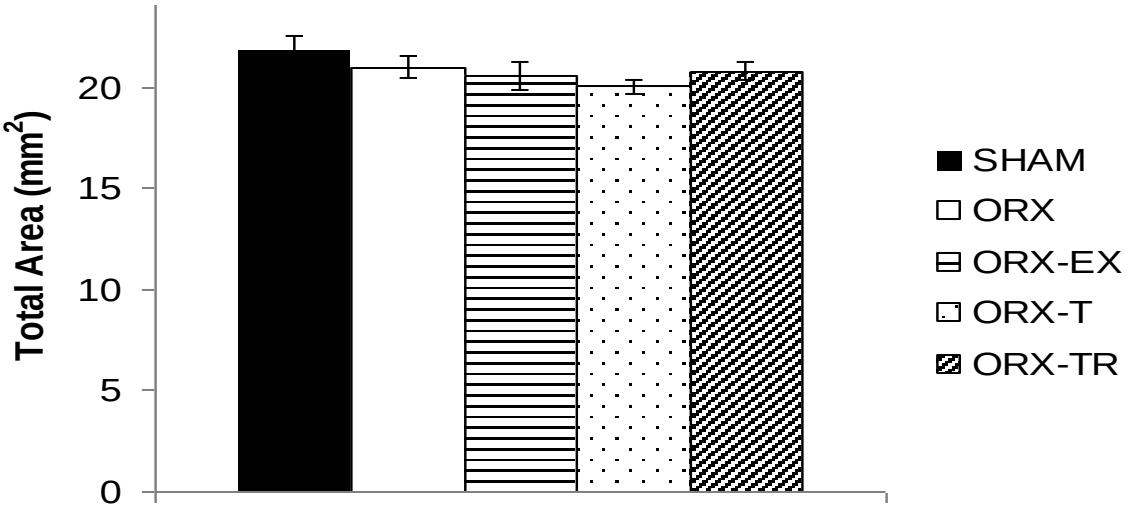

FIGURE 4-37. Effects of ORX+V, ORX+EX, testosterone-enanthate (TE), or trenbolone-enanthate (TR) on femoral total area at 5mm. Values are Means ± SE, n = 9-10/group. Letters a-e indicate differences from respectively labeled groups at # $p < 0.05$ or * $p < 0.01$ (a = vs. SHAM, b = vs. ORX, c = vs.ORX + EX, d = vs. ORX + T, e = vs. ORX + TR).

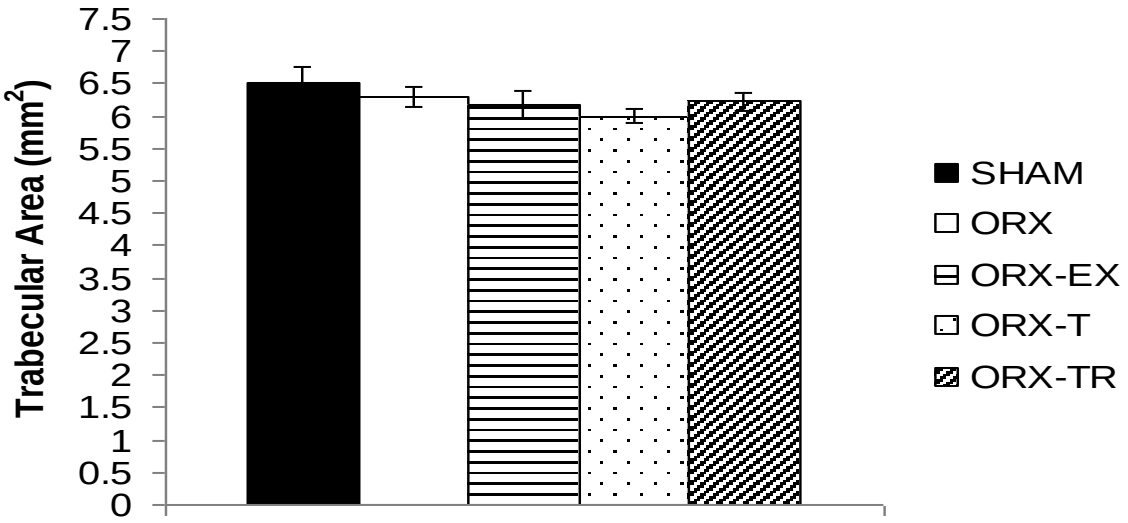

FIGURE 4-38. Effects of ORX+V, ORX+EX, testosterone-enanthate (TE), or trenbolone-enanthate (TR) on trabecular bone area at 5mm. Values are Means ± SE, n = 9-10/group. Letters a-e indicate differences from respectively labeled groups at # $p < 0.05$ or * $p < 0.01$ (a = vs. SHAM, b = vs. ORX, c = vs.ORX + EX, d = vs. ORX + T, e = vs. ORX + TR).

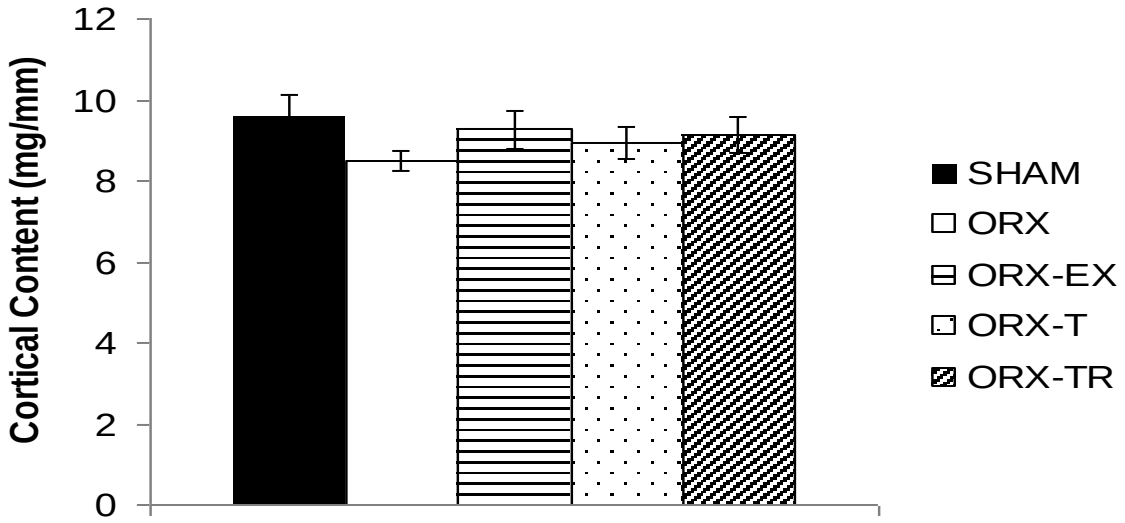

FIGURE 4-39. Effects of ORX+V, ORX+EX, testosterone-enanthate (TE), or trenbolone-enanthate (TR) on cortical bone content at 5mm. Values are Means ± SE, n = 9-10/group. Letters a-e indicate differences from respectively labeled groups at # $p < 0.05$ or * $p < 0.01$ (a = vs. SHAM, b = vs. ORX, c = vs.ORX + EX, d = vs. ORX + T, e = vs. ORX + TR).

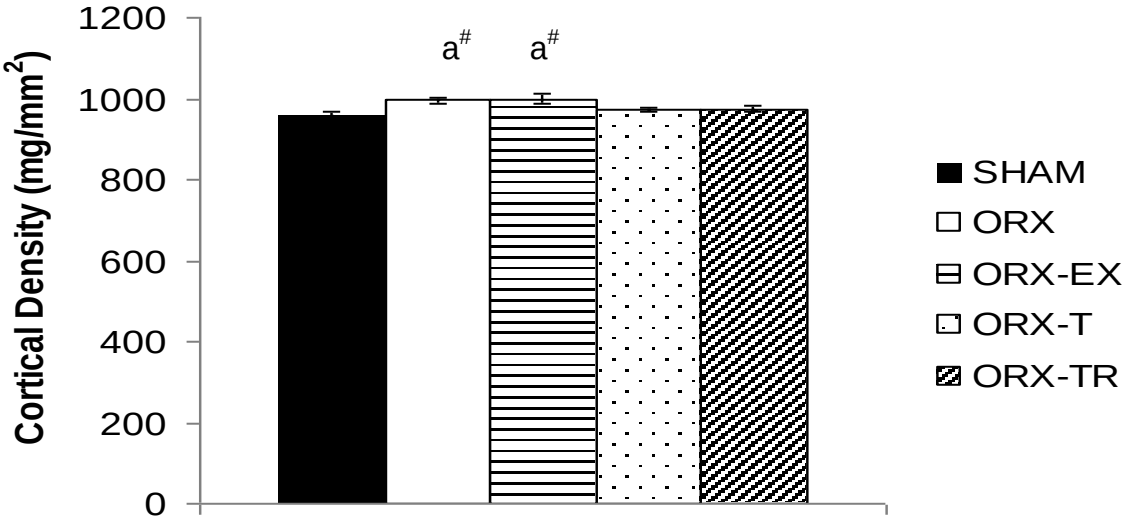

FIGURE 4-40. Effects of ORX+V, ORX+EX, testosterone-enanthate (TE), or trenbolone-enanthate (TR) on cortical bone density at 5mm. Values are Means ± SE, n = 9-10/group. Letters a-e indicate differences from respectively labeled groups at # $p < 0.05$ or * $p < 0.01$ (a = vs. SHAM, b = vs. ORX, c = vs.ORX + EX, d = vs. ORX + T, e = vs. ORX + TR).

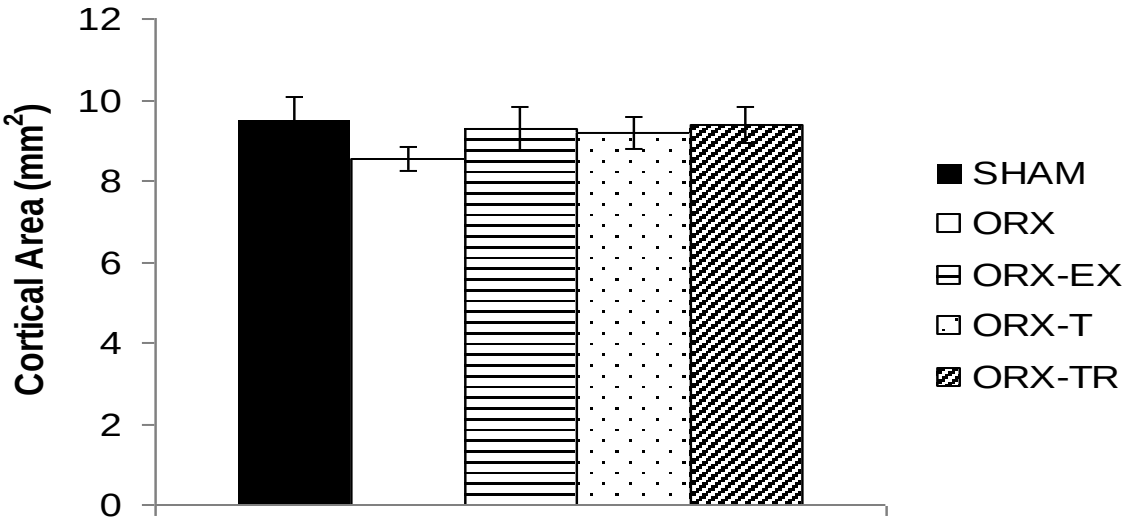

FIGURE 4-41. Effects of ORX+V, ORX+EX, testosterone-enanthate (TE), or trenbolone-enanthate (TR) on cortical bone area at 5mm. Values are Means ± SE, n = 9-10/group. Letters a-e indicate differences from respectively labeled groups at # $p < 0.05$ or * $p < 0.01$ (a = vs. SHAM, b = vs. ORX, c = vs.ORX + EX, d = vs. ORX + T, e = vs. ORX + TR).

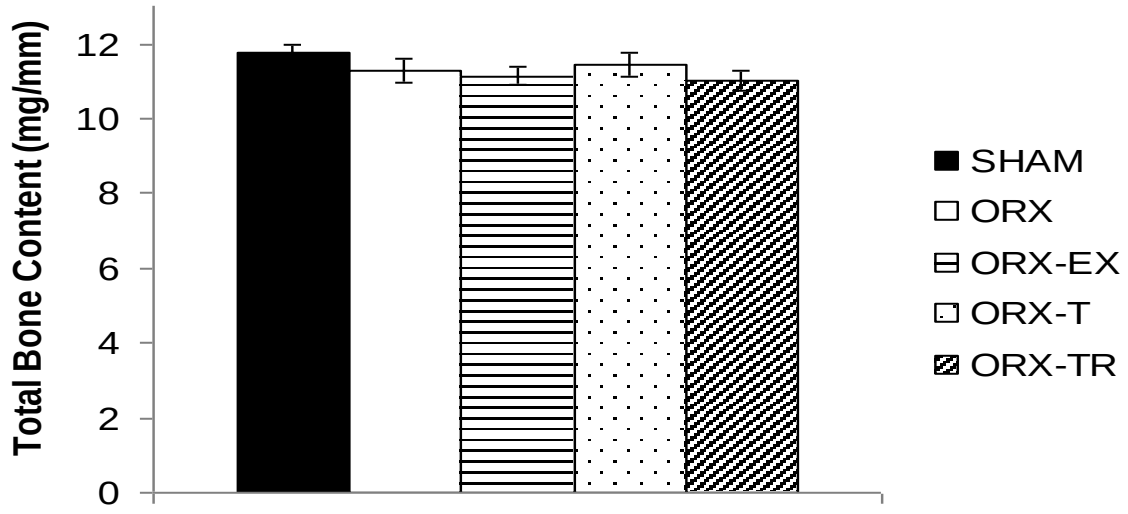

FIGURE 4-42. Effects of ORX+V, ORX+EX, testosterone-enanthate (TE), or trenbolone-enanthate (TR) on total bone content at 18mm. Values are Means ± SE, n = 9-10/group. Letters a-e indicate differences from respectively labeled groups at #$p < 0.05$ or * $p < 0.01$ (a = vs. SHAM, b = vs. ORX, c = vs.ORX + EX, d = vs. ORX + T, e = vs. ORX + TR).

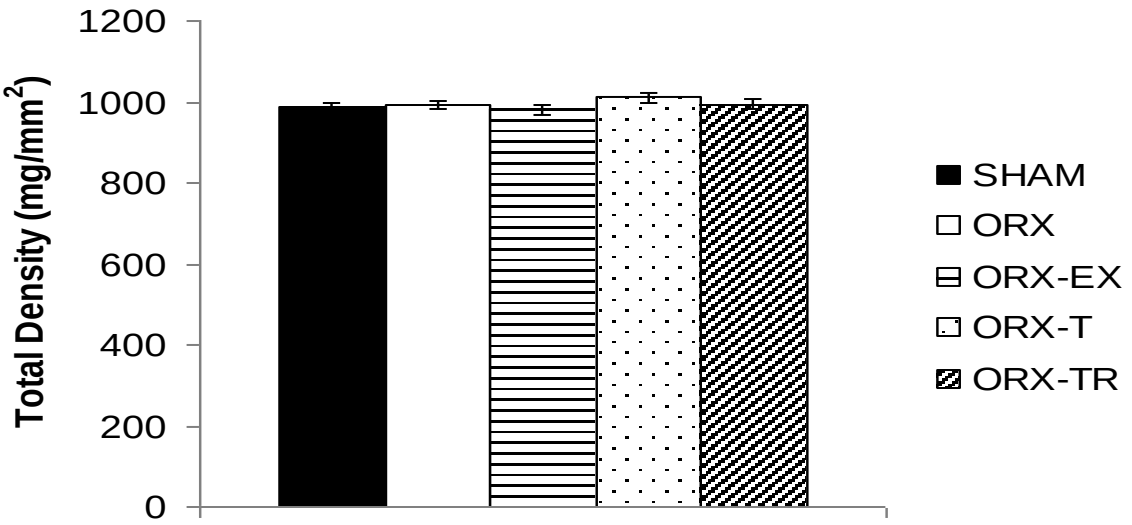

FIGURE 4-43. Effects of ORX+V, ORX+EX, testosterone-enanthate (TE), or trenbolone-enanthate (TR) on total bone density at 18mm. Values are Means ± SE, n = 9-10/group. Letters a-e indicate differences from respectively labeled groups at # $p < 0.05$ or * $p < 0.01$ (a = vs. SHAM, b = vs. ORX, c = vs.ORX + EX, d = vs. ORX + T, e = vs. ORX + TR).

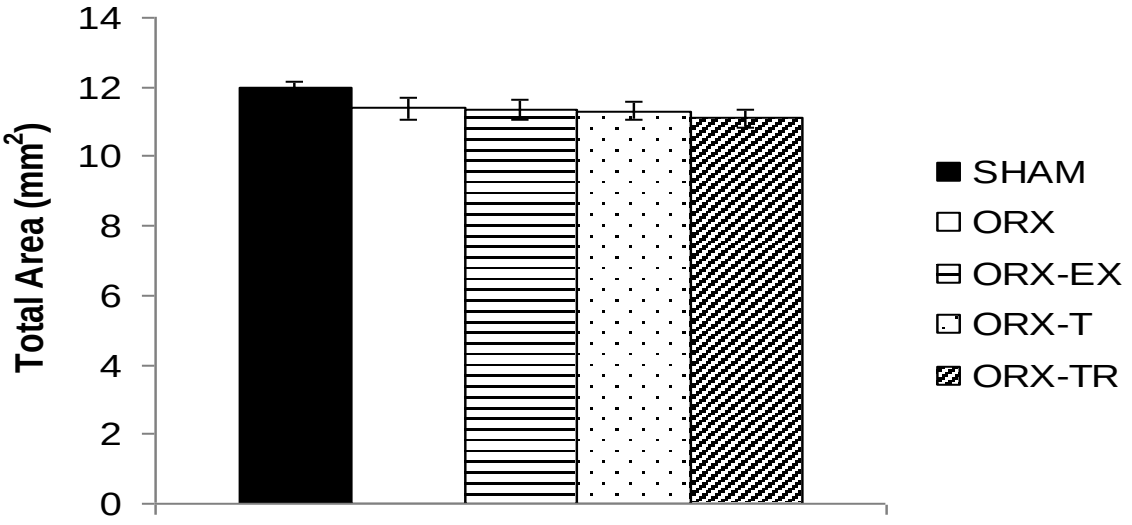

FIGURE 4-44. Effects of ORX+V, ORX+EX, testosterone-enanthate (TE), or trenbolone-enanthate (TR) on total bone area at 18mm. Values are Means ± SE, n = 9-10/group. Letters a-e indicate differences from respectively labeled groups at $p < 0.05$ or * $p < 0.01$ (a = vs. SHAM, b = vs. ORX, c = vs.ORX + EX, d = vs. ORX + T, e = vs. ORX + TR).

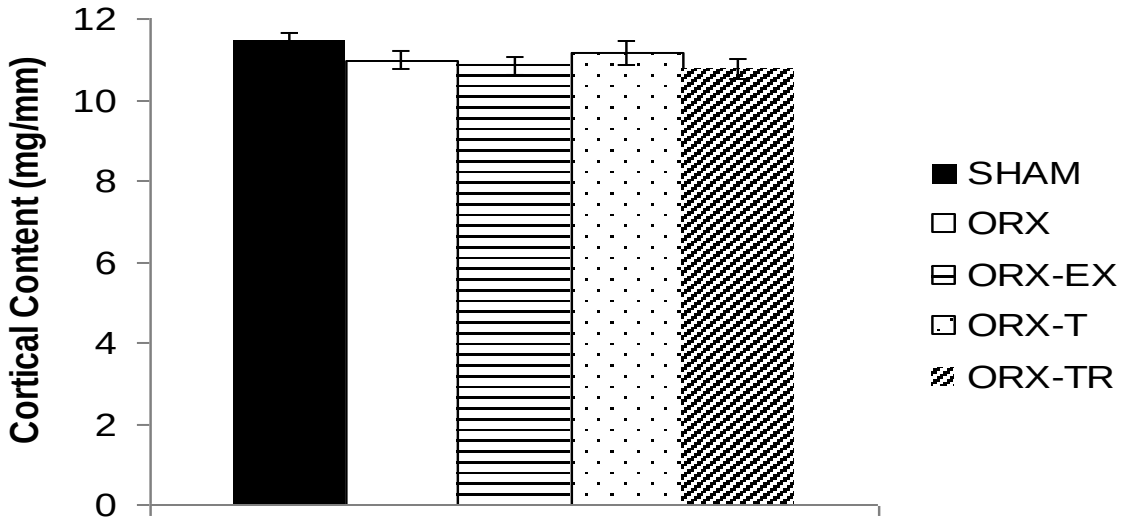

FIGURE 4-45. Effects of ORX+V, ORX+EX, testosterone-enanthate (TE), or trenbolone-enanthate (TR) on cortical bone content at 18mm. Values are Means ± SE, n = 9-10/group. Letters a-e indicate differences from respectively labeled groups at # $p < 0.05$ or * $p < 0.01$ (a = vs. SHAM, b = vs. ORX, c = vs.ORX + EX, d = vs. ORX + T, e = vs. ORX + TR).

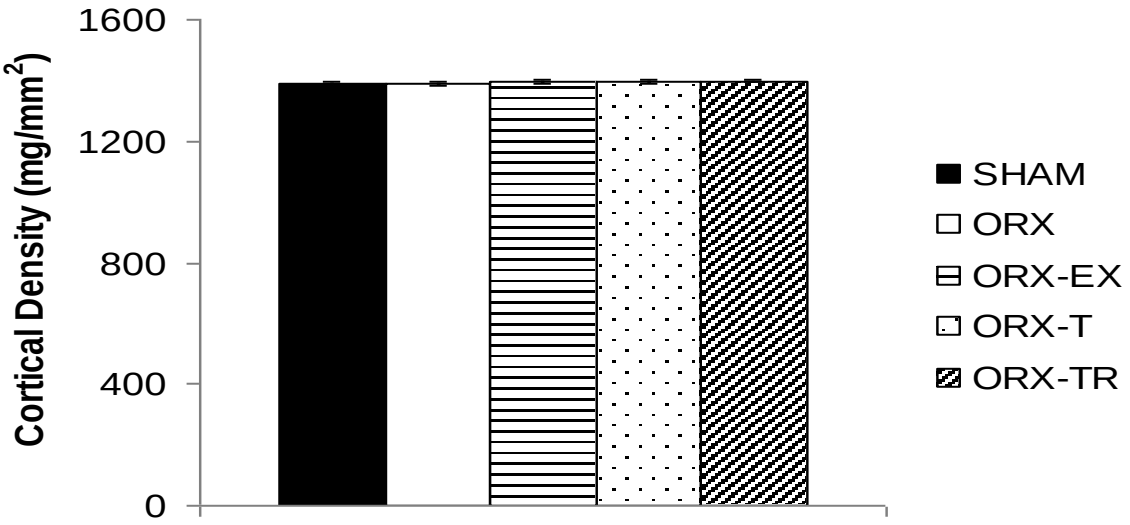

FIGURE 4-46. Effects of ORX+V, ORX+EX, testosterone-enanthate (TE), or trenbolone-enanthate (TR) on cortical bone density at 18mm. Values are Means ± SE, n = 9-10/group. Letters a-e indicate differences from respectively labeled groups at # $p < 0.05$ or * $p < 0.01$ (a = vs. SHAM, b = vs. ORX, c = vs.ORX + EX, d = vs. ORX + T, e = vs. ORX + TR).

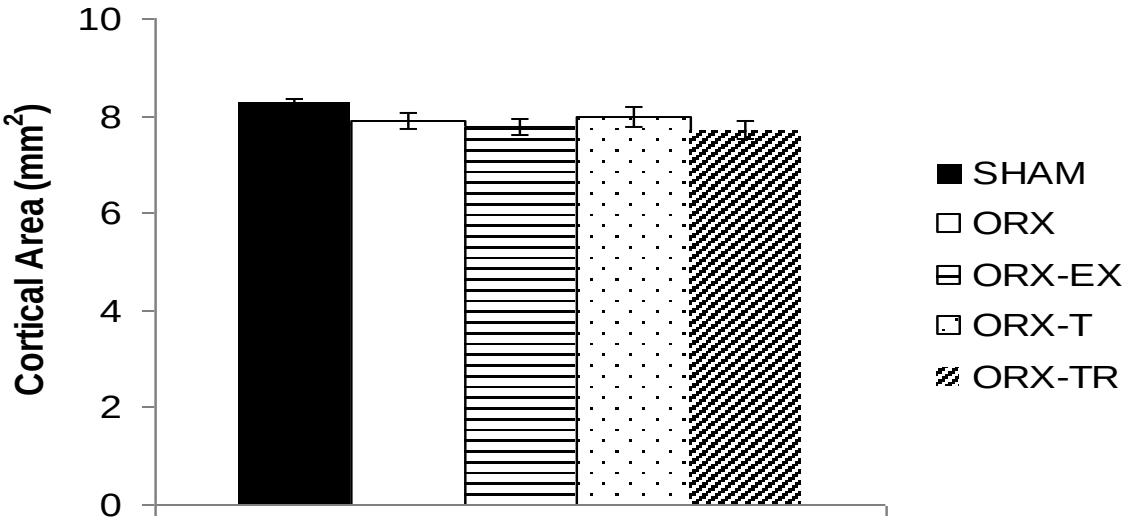

FIGURE 4-47. Effects of ORX+V, ORX+EX, testosterone-enanthate (TE), or trenbolone-enanthate (TR) on cortical bone area at 18mm. Values are Means ± SE, n = 9-10/group. Letters a-e indicate differences from respectively labeled groups at # $p < 0.05$ or * $p < 0.01$ (a = vs. SHAM, b = vs. ORX, c = vs.ORX + EX, d = vs. ORX + T, e = vs. ORX + TR).

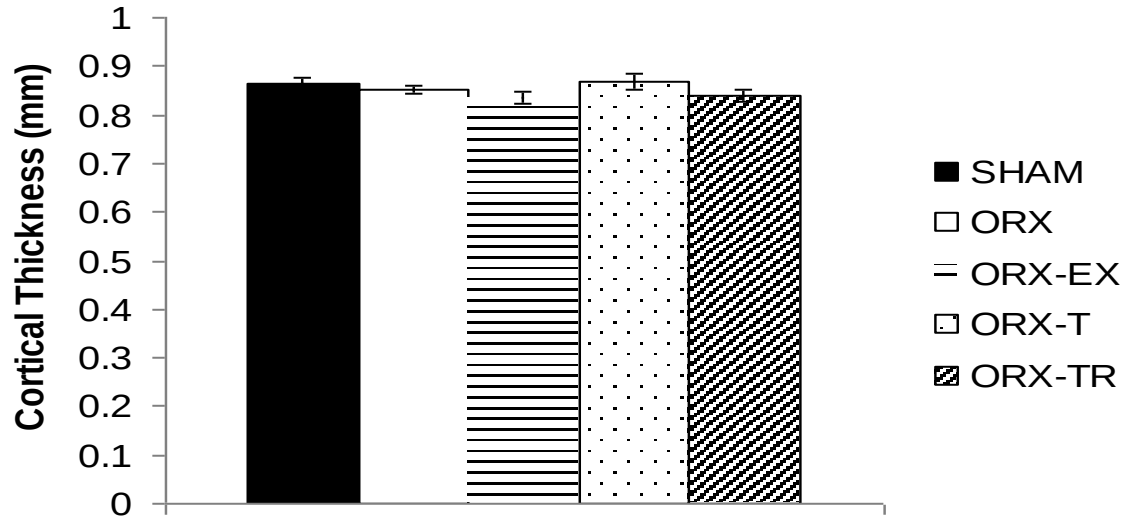

FIGURE 4-48. Effects of ORX+V, ORX+EX, testosterone-enanthate (TE), or trenbolone-enanthate (TR) on cortical bone thickness. Values are Means ± SE, n = 9-10/group. Letters a-e indicate differences from respectively labeled groups at # $p < 0.05$ or * $p < 0.01$ (a = vs. SHAM, b = vs. ORX, c = vs.ORX + EX, d = vs. ORX + T, e = vs. ORX + TR).

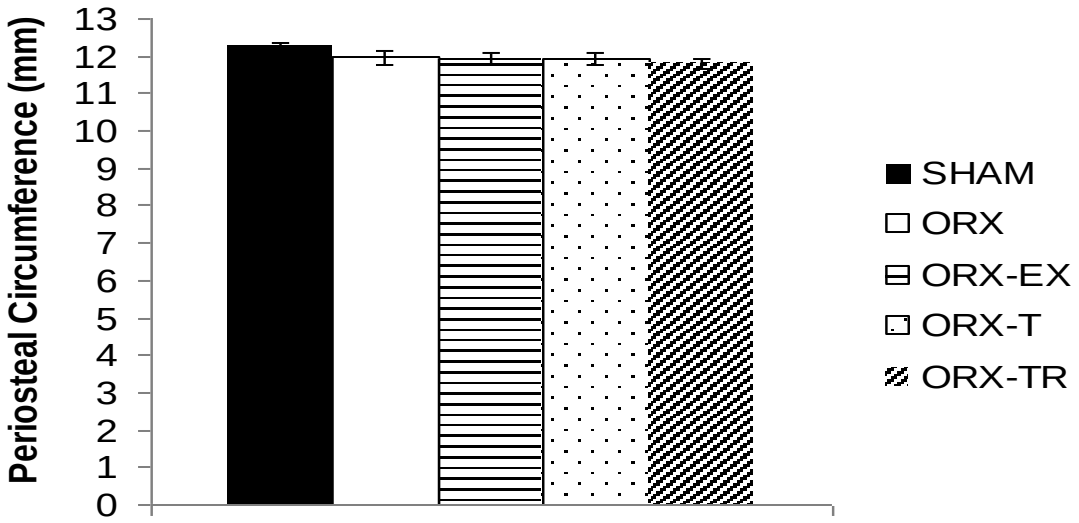

FIGURE 4-49. Effects of ORX+V, ORX+EX, testosterone-enanthate (TE), or trenbolone-enanthate (TR) on periosteal bone circumference. Values are Means ± SE, n = 9-10/group. Letters a-e indicate differences from respectively labeled groups at $^{\#}p < 0.05$ or * $p < 0.01$ (a = vs. SHAM, b = vs. ORX, c = vs.ORX + EX, d = vs. ORX + T, e = vs. ORX + TR).

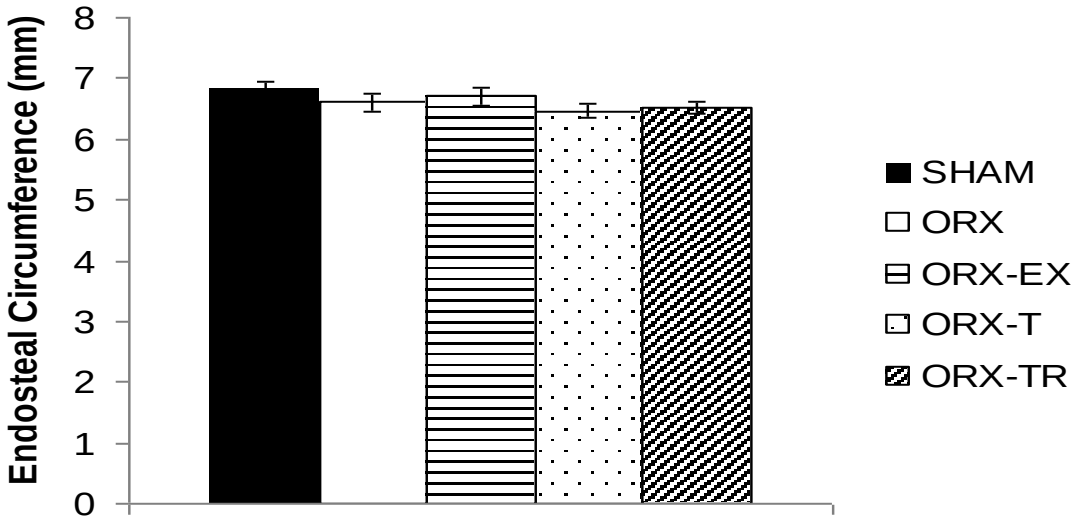

FIGURE 4-50. Effects of ORX+V, ORX+EX, testosterone-enanthate (TE), or trenbolone-enanthate (TR) on endosteal bone circumference. Values are Means ± SE, n = 9-10/group. Letters a-e indicate differences from respectively labeled groups at $^{\#}p < 0.05$ or * $p < 0.01$ (a = vs. SHAM, b = vs. ORX, c = vs.ORX + EX, d = vs. ORX + T, e = vs. ORX + TR).

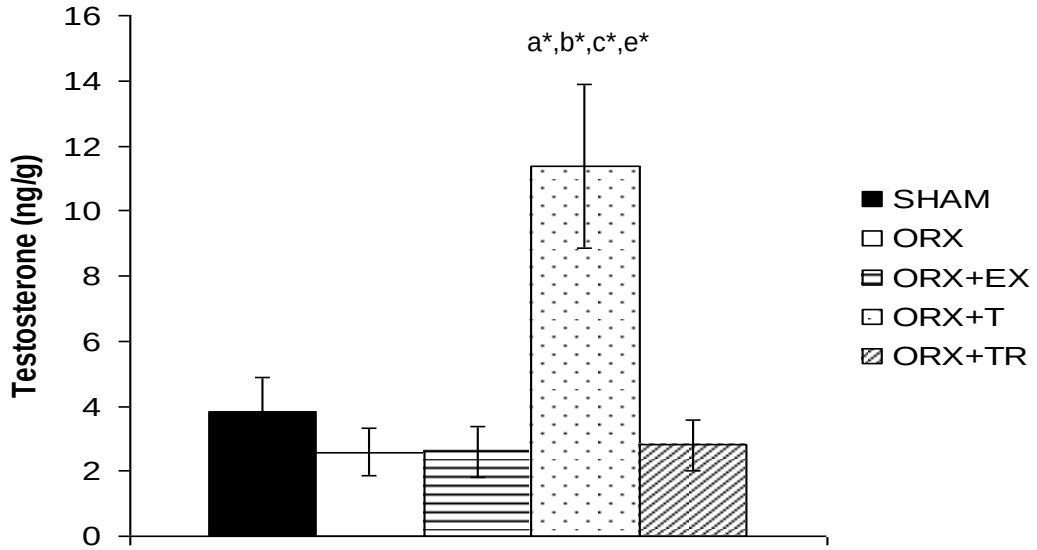

FIGURE 4-51. Effects of ORX+V, ORX+EX, testosterone-enanthate (TE), or trenbolone-enanthate (TR) on intraskeletal testosterone concentrations. Values are Means ± SE, n = 9-10/group. Letters a-e indicate differences from respectively labeled groups at # $p < 0.05$ or * $p < 0.01$ (a = vs. SHAM, b = vs. ORX, c = vs.ORX + EX, d = vs. ORX + T, e = vs. ORX + TR).

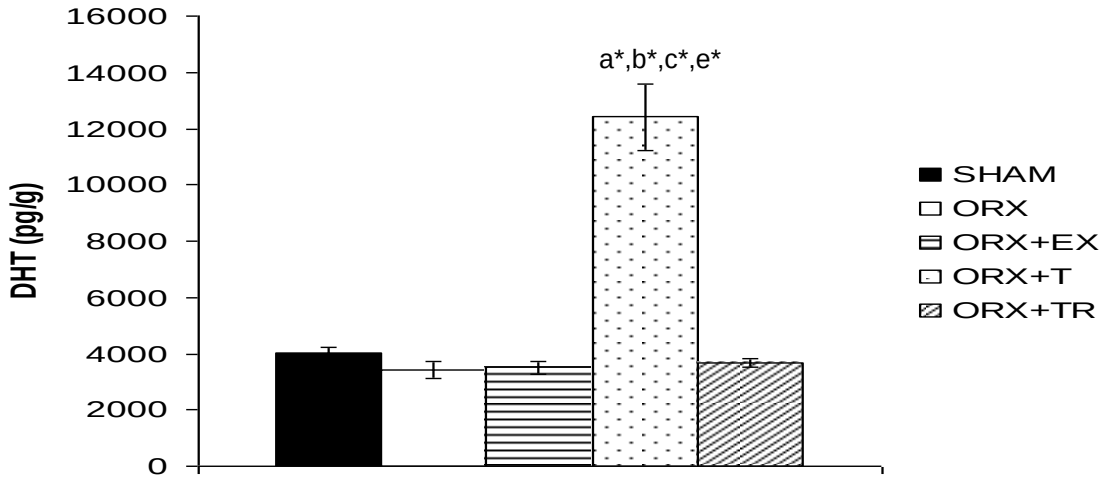

FIGURE 4-52. Effects of ORX+V, ORX+EX, testosterone-enanthate (TE), or trenbolone-enanthate (TR) on intraskeletal dihydrotestosterone concentrations. Values are Means ± SE, n = 9-10/group. Letters a-e indicate differences from respectively labeled groups at # $p < 0.05$ or * $p < 0.01$ (a = vs. SHAM, b = vs. ORX, c = vs.ORX + EX, d = vs. ORX + T, e = vs. ORX + TR).

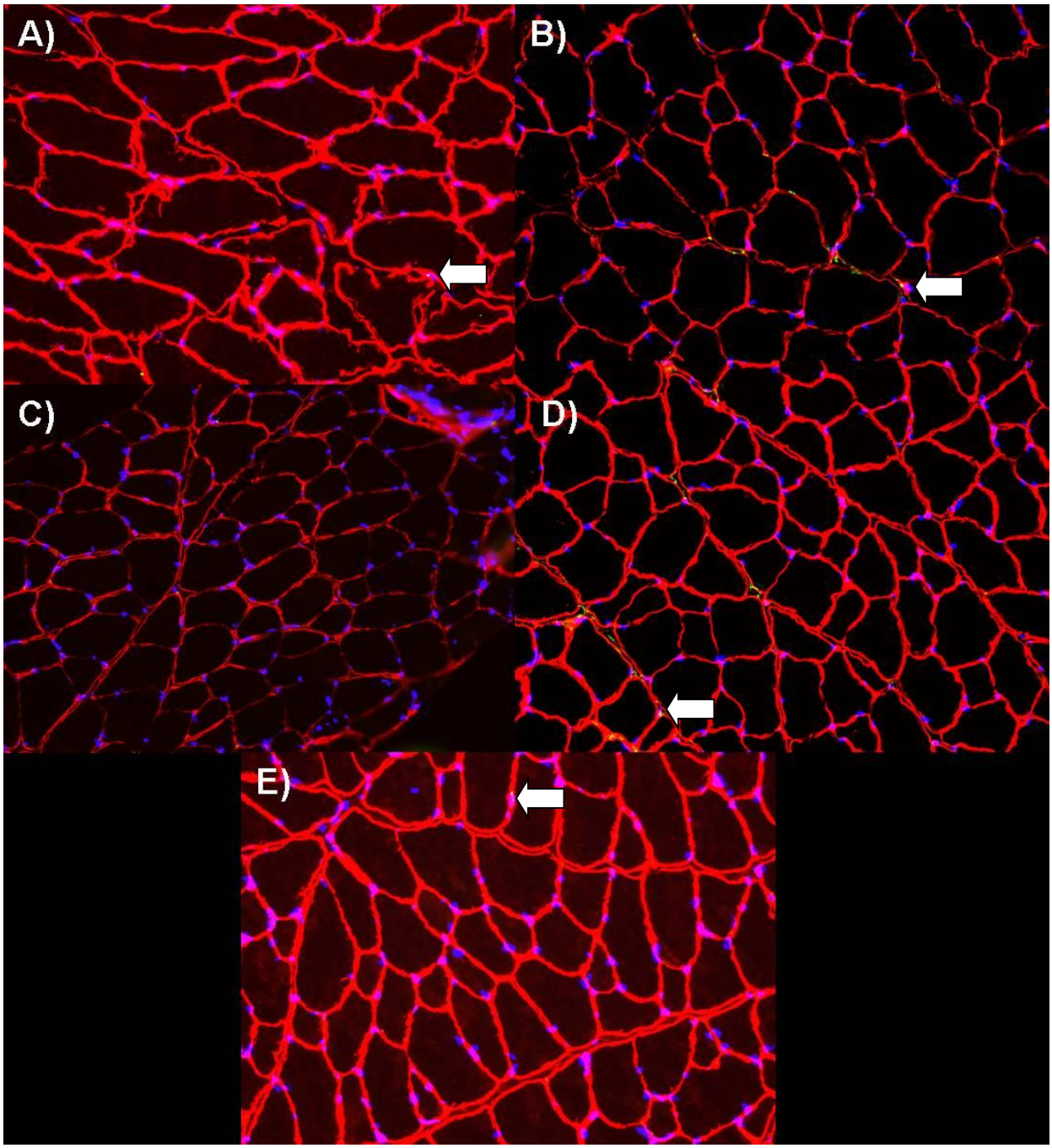

FIGURE 4-53. Effects of ORX+V, ORX+EX, testosterone-enanthate (TE), or trenbolone-enanthate (TR) on Pax 7 expression in the flexor hallicus longus. A) = SHAM, B)=ORX, C)=ORX+EX, D)=ORX+T, E)=ORX+TR. White Arrows Denote Pax 7+ Cells.

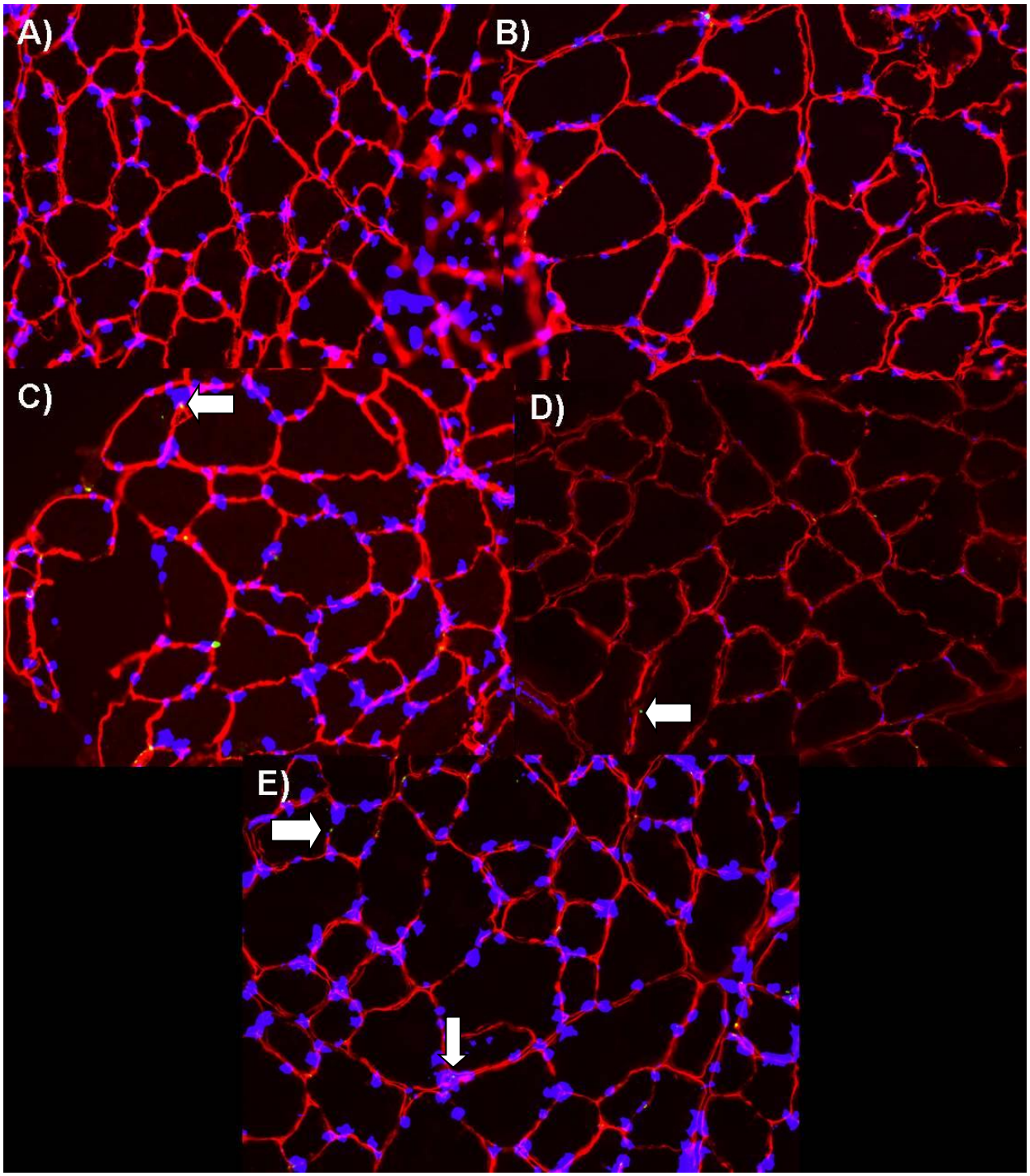

FIGURE 4-54. Effects of ORX+V, ORX+EX, testosterone-enanthate (TE), or trenbolone-enanthate (TR) on Pax 7 expression in the semimembranosus. A) = SHAM, B)=ORX, C)=ORX+EX, D)=ORX+T, E)=ORX+TR. White Arrows Denote Pax 7+ Cells.

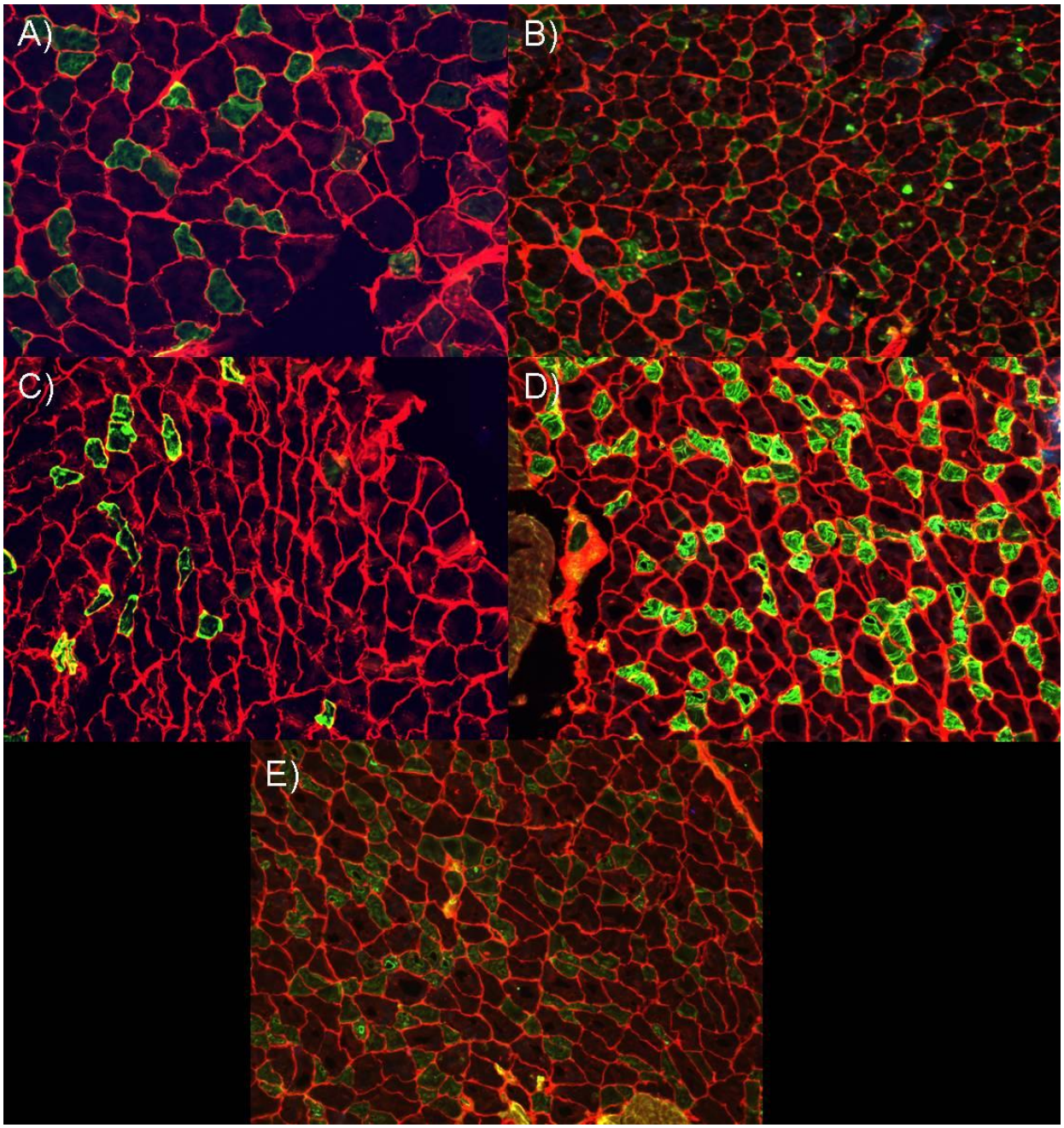

FIGURE 4-55. Effects of ORX+V, ORX+EX, testosterone-enanthate (TE), or trenbolone-enanthate (TR) on fiber type percentage and muscle cross sectional area of the flexor hallicus longus. A) = SHAM, B)=ORX, C)=ORX+EX, D)=ORX+T, E)=ORX+TR. Type 1 Fibers=Blue; Type Iia Fibers=Green; Type Iix/B=Black

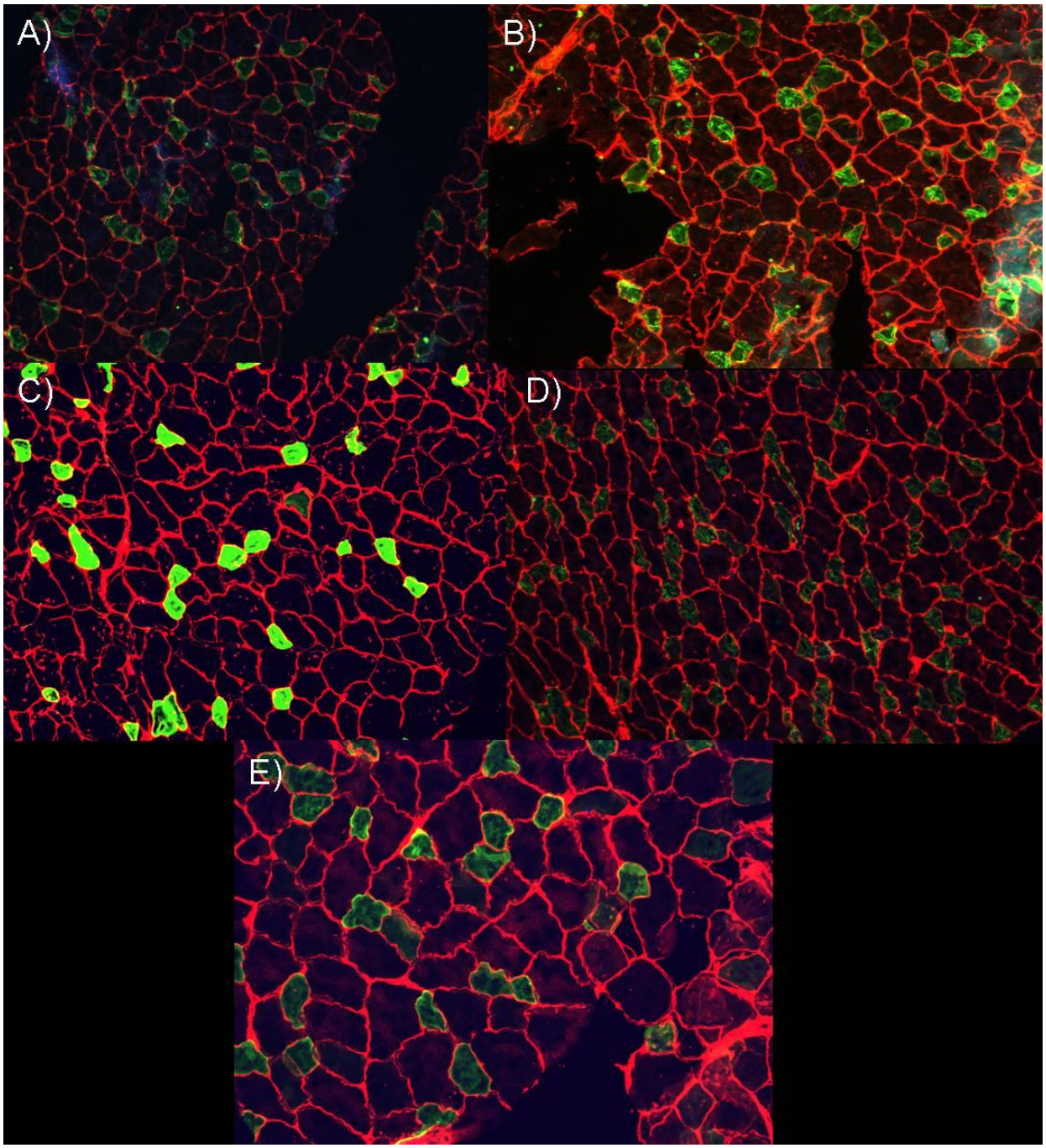

FIGURE 4-56. Effects of ORX+V, ORX+EX, testosterone-enanthate (TE), or trenbolone-enanthate (TR) on fiber type percentage and muscle cross sectional area of the semimembranosus. A) = SHAM, B)=ORX, C)=ORX+EX, D)=ORX+T, E)=ORX+TR. Type 1 Fibers=Blue; Type IIa Fibers=Green; Type IIx/B=Black

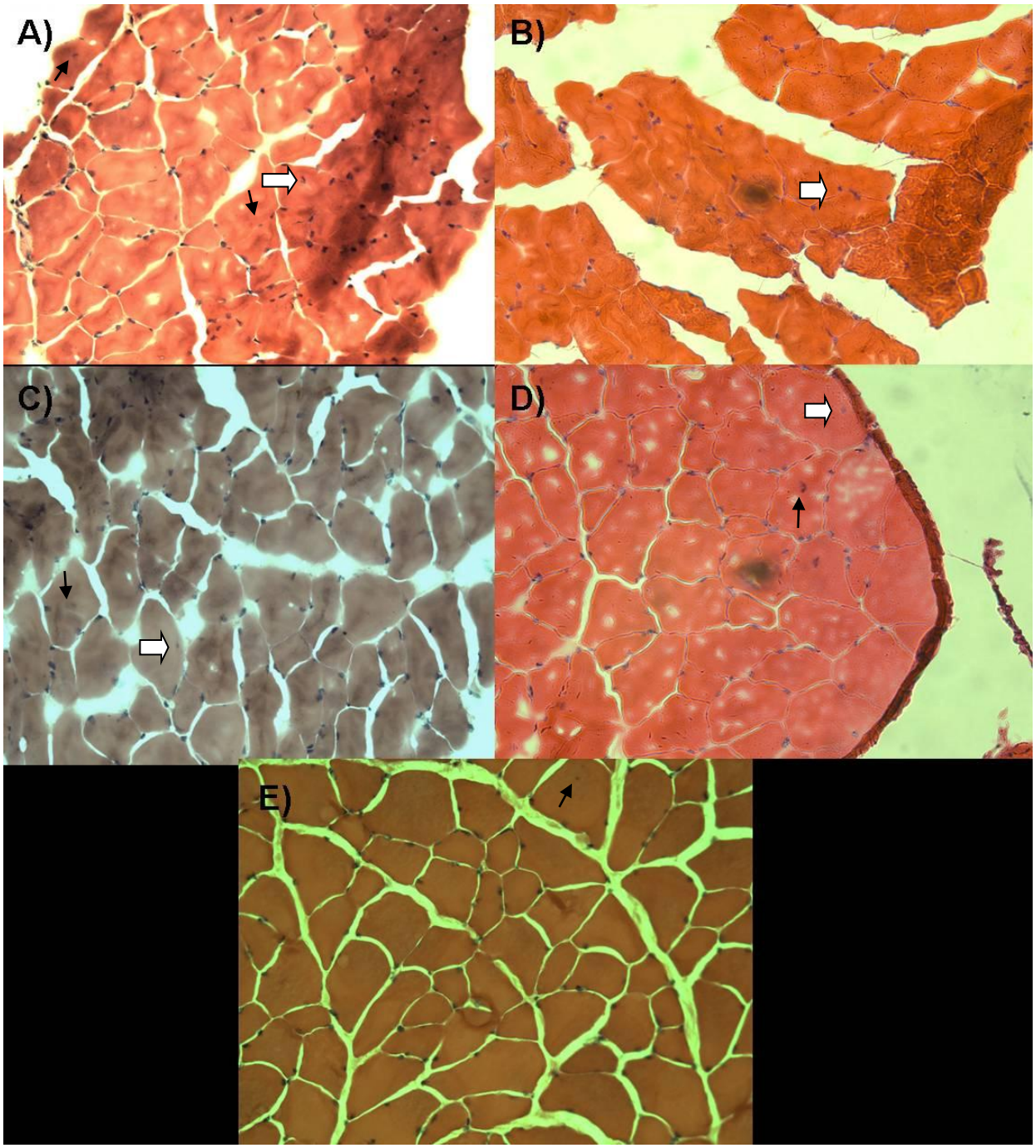

FIGURE 4-57. Effects of ORX+V, ORX+EX, testosterone-enanthate (TE), or trenbolone-enanthate (TR) on centralized and internalized nuclei following hematoxylin and eosin staining in the flexor hallicus longus. A) = SHAM, B)=ORX, C)=ORX+EX, D)=ORX+T, E)=ORX+TR. White Block Arrow=Internalized; Black Arrow=Centralized

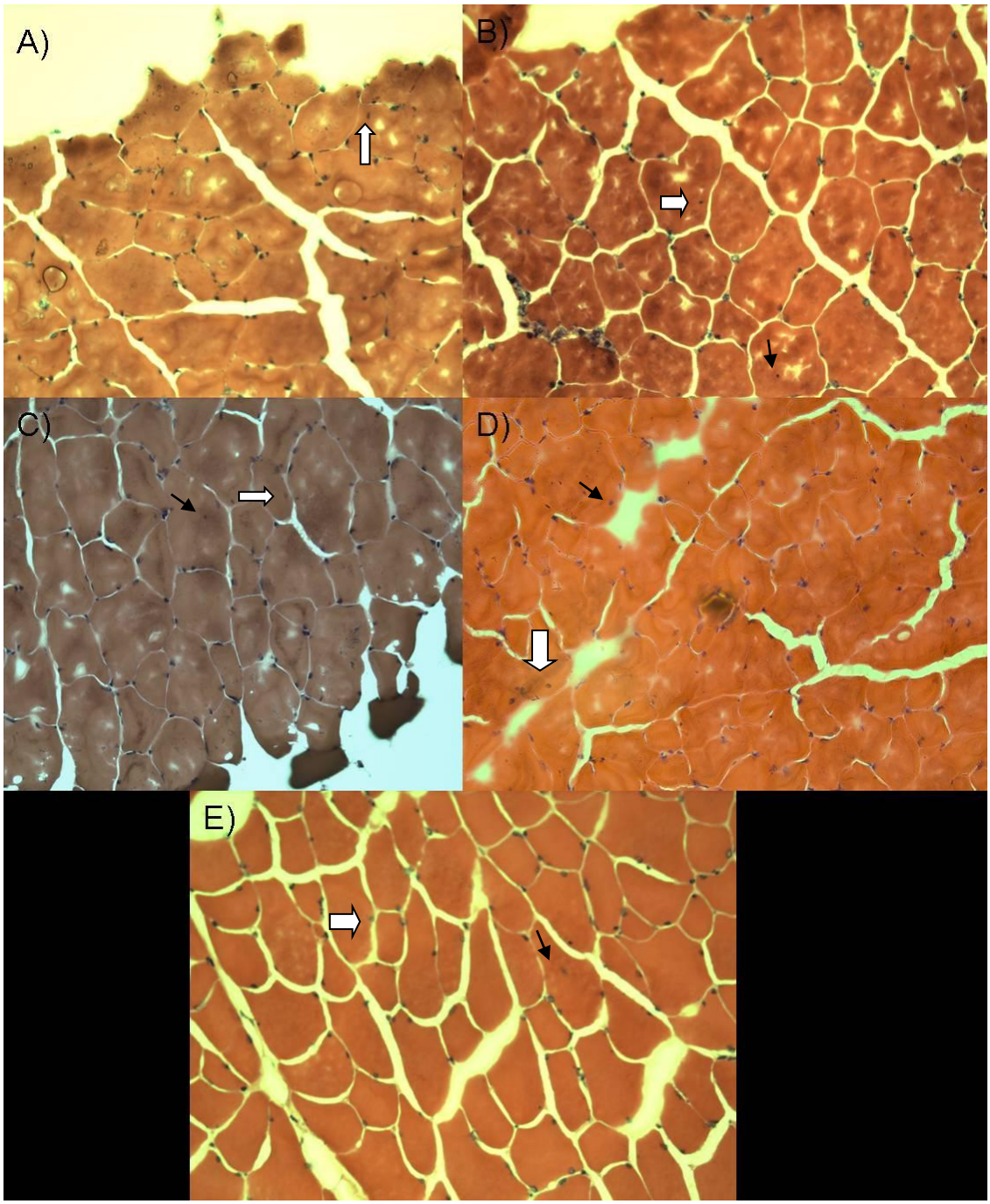

FIGURE 4-58. Effects of ORX+V, ORX+EX, testosterone-enanthate (TE), or trenbolone-enanthate (TR) on centralized and internalized nuclei following hematoxylin and eosin staining in the semimembranosus. A) = SHAM, B)=ORX, C)=ORX+EX, D)=ORX+T, E)=ORX+TR. White Block Arrow=Internalized; Black Arrow=Centralized

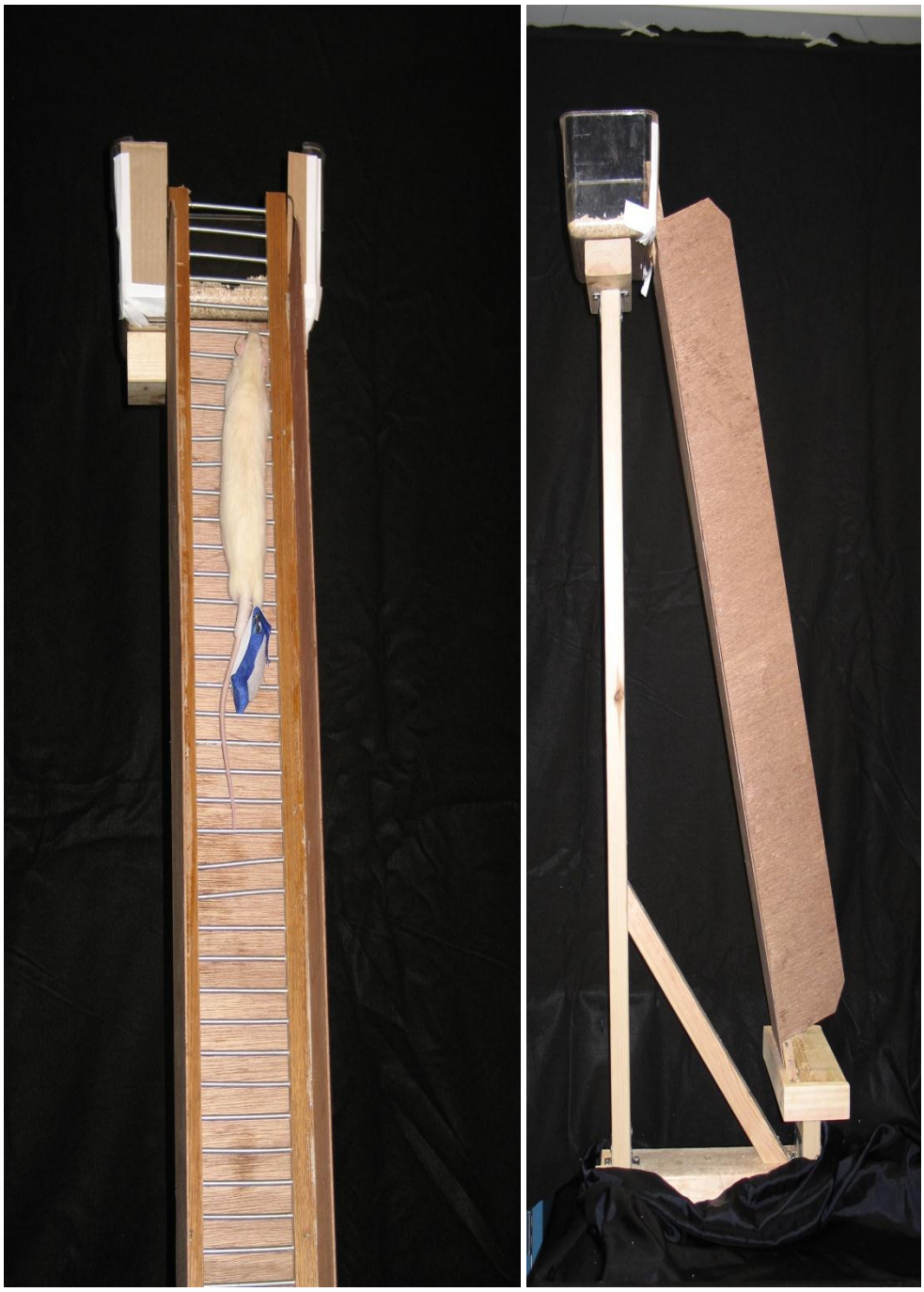

FIGURE 4-59. Weighted ladder climbing on a 1m ladder inclined to 85°.

CHAPTER 5
DISCUSSION

Overview of Principle Findings

In this experiment we studied the anabolic effects of weighted ladder climbing, testosterone enanthate and trenbolone enanthate on serum hormone levels of T and DHT, muscular hypertrophy and histology, bone, adiposity, and hemoglobin in orchiectomized 10 month-old Fisher-Brown Norway rats. Testosterone enanthate and trenbolone enanthate were effective at improving LABC muscle mass, reducing adiposity and improving hemoglobin levels in these animals. Specifically, testosterone enanthate and trenbolone enanthate reduced bone turnover through their effects on markers of bone osteoclast activity (i.e., indirect resorption measure) and formation through lowered serum levels of Trap5b and osteocalcin. Similarly, ORX+T and ORX+TR animals prevented ORX-induced deficits in trabecular bone content, trabecular bone density, and cortical bone density at the femoral metaphysis. Treatment with testosterone enanthate and resulted in elevated levels of intraskeletal testosterone and DHT. Opposing effects of the individual androgens were seen in the prostate as testosterone resulted in large increases in prostate mass, whereas trenbolone administration tended to decrease prostate mass. While testosterone elevated intraskeletal T and DHT; elevated levels of trenbolone were not detectable with current methodologies. However, the trenbolone group, increased maximal load of the femoral neck, while testosterone did not suggesting that trenbolone exerts a bone protective effect similar to testosterone. In the androgen responsive kidney tissue, both androgen treated groups elevated kidney mass compared to the ORX treatment groups. Whereas,

progressive resistance exercise training without the presence of testosterone failed to induce positive changes in muscle mass, adiposity, Pax 7, and hemoglobin.

Anabolic and Androgenic Effects of Testosterone and Trenbolone Enanthate and Weighted Ladder Climbing on Muscle, Bone and Prostate

Background

In the aging male population, dysregulation of supportive anabolic pathways in muscle and bone may lead to decreased independence, increased risk of falls, fracture risk, and higher rates of morbidity and mortality (18, 52, 68). The role of testosterone in the preservation of bone and muscle has been reported in both human (6, 9, 32, 64, 74, 125, 135-137) and animal studies (8, 11, 30, 40). However, administration of testosterone does cause prostate enlargement, polycythemia and other adverse events, even at replacement dosages (9, 138). In the current study, we provided our orchiectomized animals with a weekly supraphysiologic dose of testosterone which elevated serum testosterone ~9 fold over SHAM. In an effort to minimize prostate growth an additional group of animals were also treated with trenbolone enanthate, a synthetic analog of testosterone with reported SARM-like effects (40). A recent review by Yarrow and colleagues, reports 17beta-hydroxyestra-4,9,11-trien-3-one (trenbolone) has a full spectrum on anabolic effects in muscle and bone, but shows reduced androgenic and estrogenic activity originating from reduced 5-alpha reductase and aromatase conversion to DHT and estradiol (41), respectively.

Anabolic Effects on Skeletal Muscle

Once weekly testosterone enanthate or trenbolone enanthate injection yielded higher levels of serum hormones in circulation. At the conclusion of the study, T treated animals, serum T was elevated (9-fold), whereas circulating DHT increased to levels

(15-fold) above SHAM. In TR treated animals levels of circulating T and DHT fell to the levels of ORX treated animals. In T treated animals, the higher circulating levels of T and DHT may be implicated in the large increases seen in the LABC muscle mass. Both T and DHT are capable of binding to the androgen receptor and may increase muscular hypertrophy through direct or indirect mechanisms. However, anabolic effects on the soleus, plantaris, semimembranosus, and flexor hallicus longus were not evident in the current study for either of the androgen treated groups.

Trenbolone treated animals increased LABC muscle mass to a similar degree as testosterone treated animal, without increased serum levels of T, and DHT. Trenbolone is capable of binding to the androgen receptor with three times the affinity of T, specific binding to the androgen receptor may partially explain the hypertrophic response in the LABC. However, the exercise intervention failed to maintain or hypertrophy the LABC, muscle while the soleus, plantaris, semimembranosus, and flexor hallicus longus muscles remained unchanged throughout the study.

These results are similar to other published studies examining the responsiveness of skeletal muscle tissues to anabolic agents. The hypertrophy recorded for the LABC in T and TR treated animals is similar to results found in younger animals in our laboratory (11, 40). The responsiveness of the LABC to androgens can partially be explained by its 3x greater androgen receptor density compared to other skeletal muscles in the rat with approximately 75% of the levator ani muscle fibers expressing androgen receptors (42). Since TR treated animals have low circulating levels of T and DHT, hypertrophic responses may be directly related to binding of TR to the androgen receptor—since TR binds with a 3x greater affinity than T and with the

same affinity as DHT (35). However, this doesn't preclude other unknown hypertrophic mechanisms possibly induced by trenbolone or its metabolites possibly having additional inhibitory actions on glucocorticoids. Interestingly, whole body progressive resistance training in ORX induced males did not maintain LABC muscle mass, nor did it cause hypertrophy in the primary muscle involved in ladder climbing the flexor hallicus longus. Whereas other studies in intact animals found significant hypertrophy to this muscle in intact males over a similar time course (62). In the hypogonadal state, resistance training may not be able to induce hypertrophic responses without adequate hormonal support.

Anabolic Effects on Bone

Hypogonadism in elderly men is associated with a greater risk of fractures (15, 68), loss of functional independence, and nearly double the one year mortality risk compared to women (18). Borst and colleagues identified that the Fisher F344 and Brown Norway rats were models for displaying high turnover osteopenia following ORX (60). Increases in catabolic bone and muscle effects are evident in bone as early as two weeks following hormone ablation in younger rodents (60). In the current study, we had no changes between any groups in gross measures of tibial and femoral bone mass or length. The current study also examined changes in cortical and trabecular bone utilizing pQCT. In this study animals were aged ten months, prior to study initiation, which generally denotes a mature skeleton and the large changes in length and mass seen in younger animals was not expected.

Six weeks following orchiectomy, significant loss to cortical bone mass occurs. However, previous studies with testosterone undecanoate and testosterone enanthate attenuated changes in high turnover osteopenia in younger animals. Testosterone

administration resulted in reductions of both serum osteocalcin and Trap 5b concentrations levels to below that of SHAM and ORX animals. Similar results were found by Yarrow and colleagues in three month old animals receiving the same dosage of testosterone during a four week long intervention (11, 40). Testosterone treated animals also had higher trabecular content, trabecular density and cortical density compared to both ORX groups at the 5mm evaluation site following six weeks of the intervention. Testosterone treated animals also tended to have stronger femoral neck strength, a common fracture site in hypogondal elderly men. Similarly, TR treated animals had significantly elevated trabecular content, trabecular density, and cortical density at the 5mm evaluation site compared to ORX controls. Both androgens were able to maintain trabecular content, trabecular density and cortical density at the 5mm site at the level of SHAM animals, preventing the high turnover osteopenia experienced by the ORX animals. Trenbolone treated animals had stronger femoral neck strength values compared to ORX+EX animals and tended to have increased femoral neck strength compared to ORX+V controls. Progressive resistance training without optimal hormone support may lead to impairments in bone formation, which warrants further attention.

Intraskeletal Testosterone and DHT Concentrations

Administration of 7.0 mg·week^{-1} of testosterone enanthate resulted in a four fold increase in intraskeletal T concentration above ORX conditions, and 3 fold above SHAM conditions. Yarrow and colleagues evaluated three month old Fischer 344 rats after 28 days of treatment and reported similar concentrations for both the SHAM and ORX values of intraskeletal T to our ten month old rats evaluated after 42 days of treatment. In contrast, three month old rats administered 7.0 mg·week^{-1} of testosterone enanthate

had intraskeletal concentrations of 27.4 ng/g; whereas we observed intraskeletal T concentration of 11.4 ng/g. Notably, the administration of trenbolone enanthate did not impact intraskeletal levels of T. Future studies should examine the time-course of T depletion, the responsiveness to testosterone replacement and its effects on bone morphology and strength.

Administration of 7.0 mg·week^{-1} resulted in elevated levels of serum DHT of 3 fold above SHAM and ORX conditions. Yarrow and colleagues reported elevations in intraskeletal DHT concentrations of 11,554 pg/g following weekly dosage of 7.0 mg of testosterone enanthate for 28 days. In the current study, we observed DHT concentrations of 12,413 pg/g in ten month old rodents following a 42 day treatment period. Trenbolone administration did not alter intraskeletal DHT concentration. In conclusion, older animals appear to have a greater conversion of T to DHT in bone. Intraskeletal androgen levels appear to be conserved following 42 days of orchiectomy.

Anabolic Effects on the Prostate

Benign prostate hyperplasia is a commonly reported side effect of administration of testosterone (6, 9, 12, 53, 136). Several large clinical trials have exclusionary criteria for prostate symptomology and track its growth over the course of a study. In an ongoing study, Borst and colleagues have administered a 5 alpha reductase inhibitor in tandem with testosterone replacement therapy in an attempt to minimize prostate growth (8, 30).

Similar to results from human testosterone replacement trials and supraphysiological administration of testosterone in younger orchiectomized rodents (40) prostate mass was significantly increased by superphysiological testosterone in our 10 month old orchidectimized male rats. Testosterone treatment resulted in prostate

masses nearly double SHAM controls, and greater than 6 fold increase in mass than ORX treatment conditions. Whereas, TR treatment non-significantly reduced prostate mass by 20% compared to SHAM, and were 64% smaller than T treated animals at sacrifice.

Anabolic Effects on Hemoglobin

Anemia is a common blood disorder defined as a low number of red blood cells. Anemic disorders number in the hundreds and can be a result of stomach ulcers, medications, cancers, iron deficiency, vitamin B12 and folate deficiencies. The reported prevalence 8.1% of anemia in a sample of 2905 men of anemia in the aging population and therapies able to improve hemoglobin concentrations without serious side effects would benefit oxygen carrying capacity and energy levels in the aging population (154). Administration of testosterone elevates red blood cell concentrations in some individuals to pathological levels, increasing risks of serious adverse events and requiring stringent screening criteria for study inclusion. In the present study, we found elevations in blood hemoglobin concentrations following both T and TR administration in 10 month old orchiectomized male rats. While both drugs increased hemoglobin concentrations, the process took till the fourth week of the experiment to elevate concentrations above SHAM conditions. A similar increase in circulating hemoglobin concentrations has been seen by Yarrow and colleagues (unpublished) in younger orchiectomized rats also occurring at the four week time period. Since TR exerted a similar response to T while suppressing T and DHT; TR may induce elevations in hemoglobin concentrations through direct binding to the androgen receptor. Some possible mechanisms through which TR may induce hemoglobin alterations are through upregulation of erythropoietin, inhibiting hepcidin production (139), enhancing red blood cell integrity, direct effects on

bone marrow or through another unknown mechanism. Progressive resistance training exerted no effect on hemoglobin concentrations during the experimental period.

Effects of Testosterone and Trenbolone Enanthate and Weighted Ladder Climbing on Serum markers of T, DHT and TR

Serum Testosterone at Sacrifice

At sacrifice, nadir (6 days after the last injection) values of T were evaluated in all groups. Administration of 7.0mg·week^{-1} of testosterone enanthate into the quadriceps muscle group resulted in a significant elevation of serum T in middle aged rodents. Testosterone administered animals serum T values were greater than SHAM animals and ORX greater than TR treated animals. Similar superphysiological levels have been reported by Yarrow and colleagues in a 4 week study using 3 month old rats (40).

Serum DHT at Sacrifice

At sacrifice, nadir (6 days after the last injection) values of DHT were evaluated in all groups. Administration of 7.0mg·week^{-1} of testosterone enanthate into the quadriceps muscle group resulted in a significant elevation of serum DHT in middle aged rodents. Testosterone administered animals serum DHT values were greater than SHAM, ORX and greater ORX+TR treated animals. Similar levels of serum DHT have been reported by Yarrow and colleagues in a 4 week study using 3 month old rats (40).

Serum TR at Sacrifice

At sacrifice, nadir (6 days removed from last injection) values of TR were evaluated in all groups. Administration of 1.0mg·week^{-1} of trenbolone enanthate into the quadriceps muscle group resulted in a significant elevation of serum TR in middle aged rodents. Trenbolone administered animals serumTR values were greater than SHAM,

ORX and TR treated animals. Similar levels of TR have been reported by Yarrow and colleagues in a 4 week study using 3 month old rats (40).

Effects of Testosterone and Trenbolone Enanthate and Weighted Ladder Climbing on Retroperitoneal Fat Pad and Kidney Mass

Retroperitoneal Fat Pad Mass

Increasing adiposity is associated with cardiovascular risk, insulin resistance, diabetes, fatigue and the metabolic syndrome. Orchiectomized rodents exhibited an increase in visceral adiposity during the experimental period. Notably, castrated male rats engaging in weighted ladder climbing three times a week, also increased visceral adiposity stores during the experimental period. In contrast, animals treated with supraphysiologic levels of T, experienced significant decreases in visceral adiposity during the study. Similar effects have been shown by Yarrow and colleagues in younger animals (40). Trenbolone administered animals reduced visceral adiposity stores below the levels of SHAM and ORX conditions. The lipolytic effect seen from the androgen group could be attributable to binding of the androgen receptor, increased physical or foraging activity.

Kidney Mass at Sacrifice

Borst and colleagues have demonstrated increased kidney mass following androgen administration in younger rodents. Both T and TR treated animals conserved kidney mass at SHAM levels compared to ORX treated animals. ORX treated animals reduction in kidney mass of similar reductions have been shown in younger animals (40). Disease states also show a reduction in kidney mass and function. Although kidney mass was reduced, changes in renal clearance and physiologic function were not assessed in the present investigation.

Effects of Testosterone and Trenbolone Enanthate and Weighted Ladder Climbing on Centralized and Internalized Myonuclei

Sinha-Hikim and colleagues previously reported an increase in satellite cell number in humans receiving a “superphysiologic” dosage of 600mg/ week, with no significant changes in satellite cell populations following doses replacing testosterone at levels consistent with a eugonadal state (64). In the present study, muscle fibers were evaluated in a small number of animals treated with a superphysiologic dose of testosterone and trenbolone. Although not significant, the presence of centralized and internalized nuclei in these treated animals may indicate the possibility of muscle regeneration and renewal. In contrast, the exercising group without androgen replacement experienced a trend towards slightly declining populations of centralized and internalized nuclei. Trenbolone and testosterone treatment may initiate or commit myonuclear populations for regeneration, or renewal, however due to the small populations sampled these results must be interpreted with caution.

Effects of Testosterone and Trenbolone Enanthate and Weighted Ladder Climbing on Pax7+ Nuclei in the Semimembranosus and Flexor Hallicus Longus

Thompson and colleagues produced one of the seminal articles evaluating the induction of cellular modifications to satellite cells following trenbolone administration (35). Similarly, preliminary findings from the current investigation seem to suggest trenbolone may possibly induce Pax 7 activation of satellite cells in castrated male rats. Similarly, superphysiologic doses of testosterone tended to have increases in satellite cell Pax 7+ nuclei. Interestingly, weighted ladder climbing in castrated male rats seems to result in a reduction in Pax 7+ nuclei number. It has been reported that the functional importance of Pax 7+ may diminish after 21 days of age, and may be critical for early stage muscle development. Resistance training induces post-exercise GH, and IGF-1

secretion that may partially induce satellite cell activation, under normal hormonal control. The exact impact of reduced satellite cell number on the organism, and potential for recovery, renewal, and regeneration throughout out the lifespan or following injury during the hypogonadal state warrants further investigation.

Effects of ORX on Weighted Ladder Climbing

Hornberger and colleagues demonstrated an increase in weighted ladder climbing weight, muscular hypertrophy, and specific tension over a period of 8 weeks in intact animals without changes to myosin heavy chain concentrations or short term energy substrates of ATP, ADP and creatinine phosphate (62). In the present study, hypogonadal animals participating in weighted ladder climbing improved weight carried throughout the study, however weight carried was significantly lower than our previous experiments with intact animals (unpublished observations). ORX animals increased the weight carried without the concomitant muscular hypertrophy reported by Hornberger and colleagues. Although not statistically significant, the primary muscle of movement (i.e., flexor hallicus longus) during the climbing activity experienced a reduction in satellite cell number, central and internalized nuclei, with a minor loss in hindlimb muscle wet weight. These preliminary findings may assist in the development of optimized resistance training programs for hypogonadal individuals.

Effects of Weighted Ladder Climbing, Testosterone Enanthate, and Trenbolone Enanthate on Muscle Fiber Cross-sectional Area of the Flexor Hallicus Longus

Weighted ladder climbing may promote increases in Type IIa cross sectional area in the flexor hallicus longus, whereas androgen treatments do not appear to preserve Type IIa cross sectional area compared to ORX. Similar results have been observed with a 23% increase in muscle mass following eight weeks of weighted ladder climbing

in intact rodents (61, 62). In Type IIbx, testosterone appears to partially attenuate the loss in cross sectional area following ORX, while weighted ladder climbing appears to reduce cross sectional area in these fibers. Type I muscle fibers represented less than 0.5% of muscle cross sectional area and were excluded from analysis. In conclusion, weighted ladder climbing and testosterone administration may exert differing effects on the preservation of Type II muscle fibers of the FHL following ORX, however these results must be interpreted cautiously due to the small number of animals sampled.

Effects of Weighted Ladder Climbing, Testosterone Enanthate, and Trenbolone Enanthate on Muscle Fiber Cross-sectional Area of the Flexor Hallicus Longus

Weighted ladder climbing appeared to reduce muscle cross sectional area of the semimembranosus compared to ORX. The Type IIa fibers of the semimembranosus appear to be partially resistant to the effects of ORX. Type IIbx fibers appear to be decreased following ORX, and this decrement is partially prevented by trenbolone administration. Type I muscle fibers represented less than 0.5% of muscle cross sectional area and were excluded from analysis. Future research should examine the individual and combined effects of androgen administration and progressive resistance training on slow and fast twitch muscle fibers.

Conclusion

In conclusion, we have examined the impact of two anabolic and androgenic agents on skeletal muscle, bone, intraskeletal horomone concentrations, and adipose tissue in middle-aged ORX male rats. The present investigation determined that intramuscular injections of testosterone, and trenbolone enanthate result in elevations in plasma concentrations of both substances. In contrast to testosterone, trenbolone administration decreased DHT and testosterone. Interestingly, both substances caused

significant hypertrophy the LABC complex, however trenbolone administration resulted in prostate reduction compared to a doubling of prostate mass in testosterone treated animals. Notably, progressive resistance exercise failed to prevent the typical pattern of osteopenia and sarcopenia seen in the castrated male rat model. Similarly, preliminary evidence suggests that progressive resistance training in the absence of normal levels of testosterone impair skeletal muscle satellite cell disposition, mechanisms involved in skeletal muscle hypertrophy, and markers of bone health. Administration of testosterone enanthate elevates intraskeletal concentration of testosterone and dihydrotestosteronealthough the clinical implications of intraskeletal androgen levels are unknown. Future work is planned to evaluate the intraskeletal presence of trenbolone and estrogen concentration and their association with pQCT and biomechanical strength measurements. Future investigations should be directed at elucidating the mechanisms behind testosterone and trenbolone enanthate’s positive effects on exercise performance, hemoglobin, muscular hypertrophy, and bone health.

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BIOGRAPHICAL SKETCH

Sean C McCoy was born in Trenton, New Jersey and moved to Collegeville, Pennsylvania where he worked for Areufit, Inc, a health and wellness promotions company, while he completed a Bachelor of Science degree in Exercise and Sports Science in 1999. He then went on to coach intercollegiate Men's and Women's Swimming and Diving at Frostburg State University and graduated with a Master of Science degree in Health and Human Performance in 2001. Sean's interest in skeletal muscle lipid metabolism led him to begin his doctoral work under Dr. Lesley White researching intramyocellular lipid on the 3T magnetic resonance imaging machine. In 2004, Dr. White changed her research focus to the effects of resistance training on multiple sclerosis and Sean began his second chapter of academic endeavors. During this period of inadequate funding and academic support, Sean worked for the Florida Department of Corrections at Florida State Prison as a correctional officer. Following Dr. White not being offered tenure at the University of Florida, Sean followed her to the University of Georgia to complete his dissertation. After one year of no progress at the University of Georgia, Sean returned to the University of Florida under Dr. Stephen Borst and focused on the anabolic properties of testosterone and trenbolone to attenuate sarcopenia and osteopenia in men with hypogonadism. Upon completion of his Ph.D. Sean will be working on anabolic agents in patients with multiple sclerosis.