Effects of Sex Steroids on Myocardial Anoxic Resistance (43537)

LOREN G. MARTIN,*,[1] GEORGE M. BRENNER,* KIRBY L. JAROLIM,† MARTIN W. BANSCHBACH,‡ DAVID L. COONS,[2] AND AMANDA K. WOLFE[3]

Departments of Physiology/Pharmacology, Anatomy,† and Biochemistry/Microbiology,‡ Oklahoma State University, College of Osteopathic Medicine, Tulsa, Oklahoma 74107-1898*

Abstract. **Eight treatment groups of CD strain castrate male or female rats were injected daily with cottonseed oil (sham), testosterone propionate (50 μg/100 g body wt), estradiol benzoate (7 μg/100 g body wt), or a combination of both steroids dissolved in cottonseed oil. These physiologic replacement dosages of sex steroids, determined by bioassay procedures, were injected in a 0.1-ml bolus of cottonseed oil daily (intraperitoneally) for 16 weeks. Myocardial anoxic resistance was quantified by means of an *in vitro* right ventricular strip preparation that evaluated the ability of the isolated right ventricle to maintain contractions in response to electrical pacing at 1 Hz after 10 min of anoxia. While this parameter was elevated 2- to 3-fold in the estrogen-treated groups of male and female castrates compared with the sham (oil)-injected groups, neither testosterone treatment alone nor combination steroid treatment produced anoxic resistance values that differed significantly from those of the sham-injected animals. Thus, although estrogen alone may afford anoxic protection to the myocardium, testosterone is able to abolish this hormone-induced protection.** [P.S.E.B.M. 1993, Vol 202]

Estrogens are known to exert a protective effect against ischemic heart disease in both premenopausal and postmenopausal women, but the mechanisms responsible for this effect remain to be fully elucidated. Recent studies have emphasized the favorable effect of estrogens on plasma lipids and high density lipoprotein cholesterol and have proposed that estrogens exert an antiatherogenic effect. However, this mechanism has been estimated to only account for about 25–50% of the protective effect of estrogens against the risk of coronary heart disease, with the remaining 50–75% provided through other, nonlipid-related mechanisms (1). Animal studies have shown that the female rat is less susceptible to myocardial necrosis induced by isoproterenol (2, 3), and the demonstration of estrogen and androgen receptors in the myocardium and the coronary blood vessels suggests the possibility that estrogens may act directly on the myocardium to either increase coronary blood flow and myocardial oxygenation or to augment the ability of the heart to maintain contractile tension during periods of ischemia (4–7). We have studied the latter possibility by determining the ability of right ventricular muscle, isolated from castrate male and female rats treated with replacement levels of sex steroids, to maintain contractile tension during a period of anoxia.

Materials and Methods

Animals and Treatments. Eight treatment groups of male or female castrate Charles River CD strain rats (1 month of age) were injected daily (intraperitoneally) for 16 weeks with 0.1 ml of cottonseed oil only or with 0.1 ml of oil containing estradiol benzoate (7 μg/100 g body wt), testosterone propionate (50 μg/100 g body wt), or a combination of both hormones at these dosages. Animals were weighed weekly and dosages were adjusted using the mean body weight of each group, employing stock solutions of the steroid hormones that were diluted to provide the appropriate dosage in 0.1

[1] To whom requests for reprints should be addressed at Department of Physiology/Pharmacology, Oklahoma State University College of Osteopathic Medicine, 1111 West 17th Street, Tulsa, OK 74107.

[2] Present address: Department of Special Education, Tulsa Public Schools, Tulsa, OK 74114.

[3] Present address: Department of Political Science, University of Florida, Gainesville, FL 32601.

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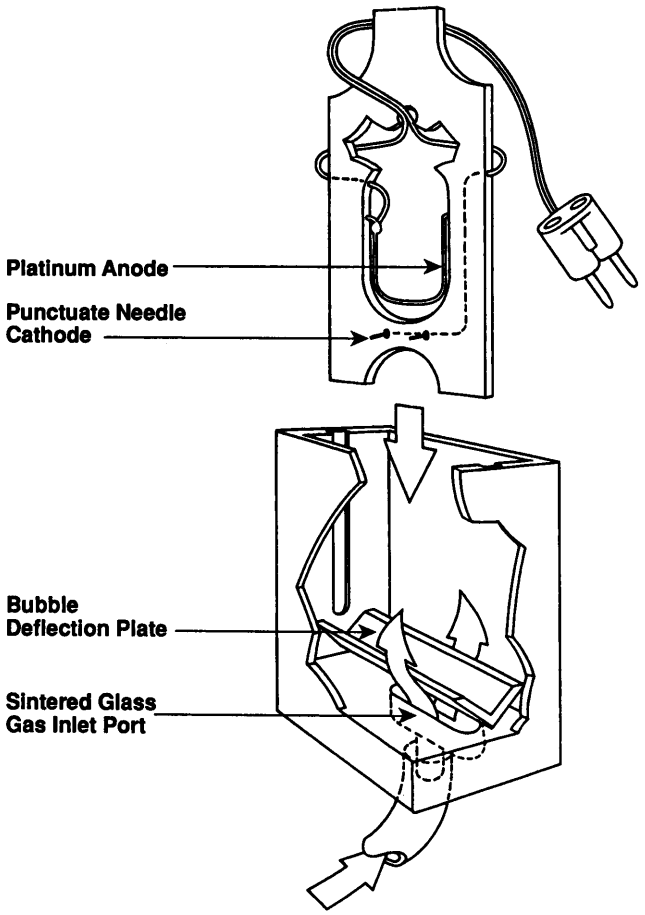

Figure 1. Plexiglas muscle chamber used to conduct anoxic resistance tests.

ml of oil. The animals were maintained in the laboratory at 24 ± 2°C with lights on between 0600 and 1800 hr. Food (Purina Laboratory Chow) and tap water were provided *ad libitum.*

Right Ventricular Anoxic Resistance. After 16 weeks of daily injections, the animals were guillotined. As in our previous studies (8), the decapitated rat was quickly placed on a dissecting board, the thorax was opened by a ventral midline incision, and the ribs and musculature on the left side of the sternum were severed and separated with a wound retractor. The pulsating heart was quickly removed and placed in a beaker of well-aerated (95% O_2 and 5% CO_2) ice-cold Krebs-Ringer bicarbonate solution to remove the excess blood. The right ventricle was dissected away and was rapidly transferred into a Krebs-Ringer bicarbonate-filled muscle chamber (Fig. 1) that was also aerated by the 95% O_2 and 5% CO_2 mixture. After an initial stabilization period of 30 min, the aerating mixture was abruptly changed to 95% N_2 and 5% CO_2 as an anoxic test. During the entire test, the isolated right ventricle was kept at 29°C and was driven at a supramaximal stimulus strength (15 V, 7 msec) at a frequency of 1 Hz. The resting tension (preload) was adjusted during the preanoxic stabilization period to 1 g. The apical end of the excised ventricle was pinned and attached to an isometric strain gauge connected to a Grass polygraph. The ability to continuously generate evoked tension during anoxia was measured after 600 anoxic contractions (10 min) and was expressed as a percentage of the maximum control preanoxic tension developed by each ventricular preparation. Earlier studies have shown that the isolated right ventricle, under the conditions employed in this study, is stable for over 2 hr (9).

Sex Steroid Bioassay. In order to assess sex steroid dosage in relation to physiologic replacement levels, the trophic effects of the hormones on target tissues in male and female castrate animals were quantified. The uterine weight in female rats was used as an index of estrogen replacement and the seminal vesicle weight was used to assess testosterone replacement in male rats. The organ weights were expressed on a wet weight basis as mg/100 g body wt and were compared with organ weights of intact control animals of the same sex.

Data Analysis. Data are reported as treatment group means ± SE. The differences between treatment group mean values were analyzed by analysis of variance and Duncan's multiple range test with a $P < 0.05$ indicating a statistically significant difference between mean values.

Results

Anoxic Resistance. Contractile strengths were measured after 600 anoxic contractions (10 min) and were expressed as a percentage of each animal's control preanoxic strength (Figs. 2 and 3). Only castrate animals receiving the oil vehicle or replacement hormones were evaluated; in the intact animal, the concomitant interactions of numerous sex steroids and the fact that animals would be sampled at various times during the estrous cycle would make causal determinations nearly impossible. Values indicate treatment group means ± SE. As can be seen from Figure 2, exogenous estradiol significantly increased right ventricular anoxic resistance of the female castrate by approximately 125% above that of the sham (oil)-injected female castrate group. Exogenous estradiol also significantly elevated right ventricular anoxic resistance in the male castrate by 225% (Fig. 3). In contrast, chronic testosterone administration to castrates of either sex did not significantly modify right ventricular anoxic resistance when compared with the sham (oil)-injected control group of the same gender. However, when the castrate animals of either gender were treated with both estradiol and testosterone, the anoxic resistance of the right ventricle was reduced to the approximate low level of the sham (oil)-injected control value. This finding suggests that the simultaneous presence of both estradiol and testosterone, at the replacement levels employed in this study, prevents the estrogen-induced augmented myocardial

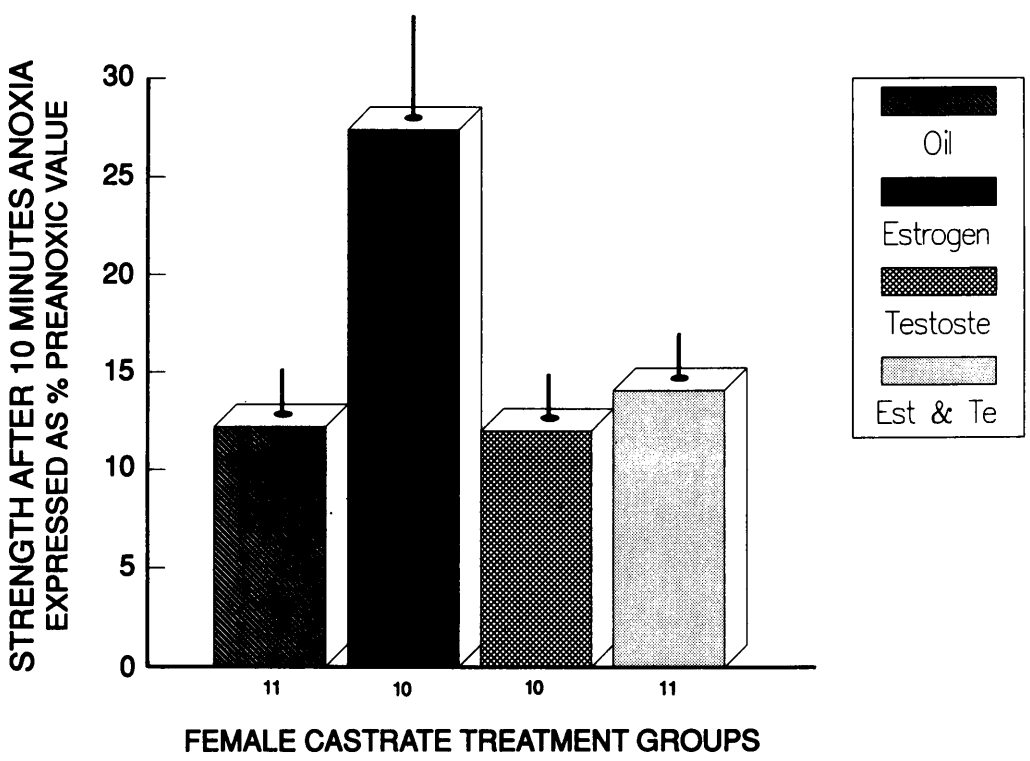

Figure 2. Sex steroid influence upon right ventricular anoxic resistance in the female castrate treatment groups. Bars represent group means ± 1 SE. Numbers below bars represent group *n* values.

anoxic resistance seen in the estradiol-treated male or female castrate animal.

Hormone Bioassay. In order to determine the physiologic relevance of these studies, bioassay data of steroid-sensitive tissues were examined on the basis of tissue wet wt/100 g body wt. As can be seen from Figure 4, the uterine weights of estradiol-replaced female castrates did not differ significantly from those of intact female control animals. Similarly, in the male groups, seminal vesicle weights did not differ significantly between the testosterone-replaced castrate group and the intact male control group (Fig. 5). These bioassay data indicate that the levels of exogenously administered sex steroids are at near-physiologic levels.

Discussion

Although it has long been recognized that the premenopausal human female demonstrates a much lower incidence of fatal myocardial infarction than does the male, only recently has the study by Stampfer *et al.* (10) provided clear and convincing epidemiologic data that women who have undergone either natural menopause or surgical bilateral oophorectomy and have received replacement estrogen are protected specifically from coronary heart disease and generally experience a reduced mortality from cardiovascular disease. Many of the earlier conflicting reports concerning estrogen's effects upon the cardiovascular system, i.e., increased risk of ischemic heart disease in pre-1980 reports and decreased risk of ischemic heart disease and cardiovascular mortality in more recent studies, may be explained by the more recent chronic use of lower, more physiologic dosages of shorter-lived replacement estrogens (11). Although the mechanism of this protective effect may include a favorable effect on plasma lipids with an increased level of high density lipoprotein cholesterol, this mechanism has been estimated to account for only 25–50% of the overall protective effects of estrogen against ischemic heart disease, with the remaining 50–75% of the protection mediated through nonlipid-related mechanisms (1).

Animal studies have shown that the myocardium of the male rat is more susceptible to the necrogenic actions of isoproterenol than is the heart of the female rat (2, 3). Although it has been suggested that the male animal's increased susceptibility to cardiac damage might result from the fact that the male rat demonstrates a greater body weight at any given age than does the female, the finding that the female Japanese quail, which is consistently heavier than the male at any age, also demonstrates a greater resistance to isoproterenol-induced cardiac necrosis seems to refute this hypothesis (12). This latter study, employing an avian model,

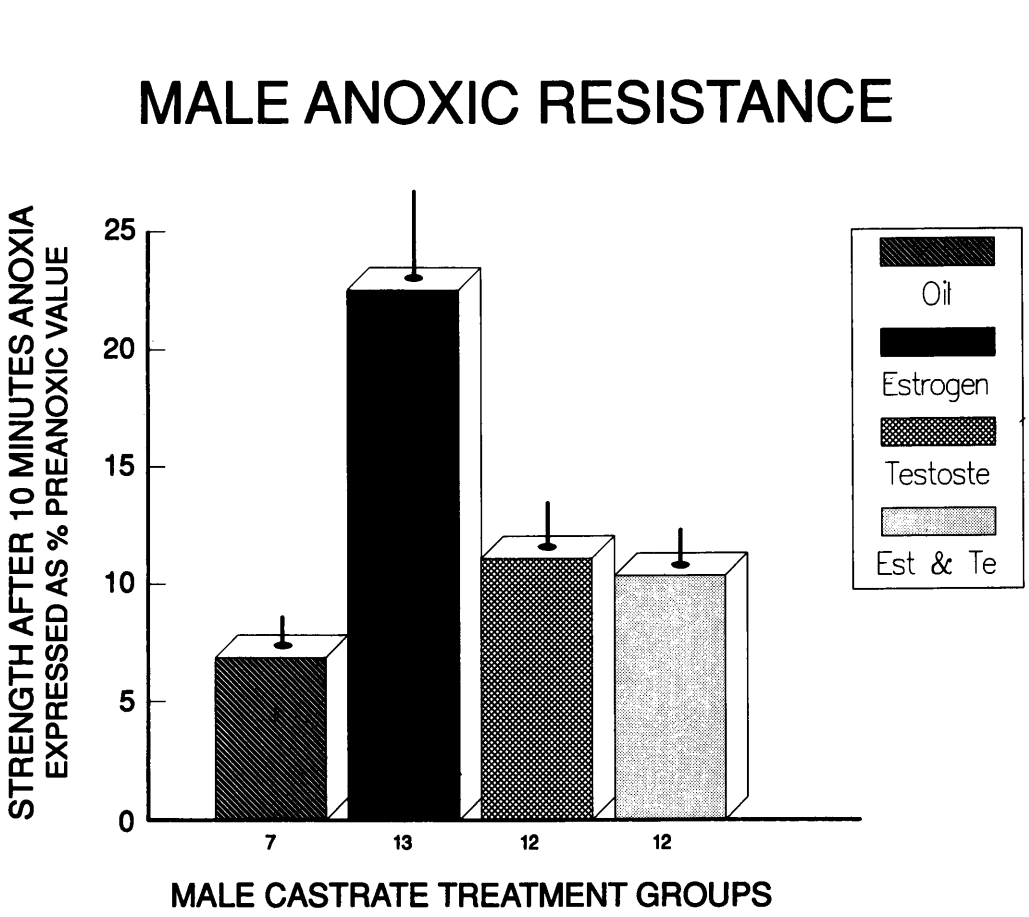

Figure 3. Sex steroid influence upon right ventricular anoxic resistance in the male castrate treatment groups. Bars represent group means ± 1 SE. Numbers below bars represent group *n* values.

further demonstrated that whereas males were protected from this type of necrotic cardiac lesion by large acute doses of exogenous estrogen, females were made more susceptible after acute testosterone administration.

Hormonal replacement levels employed in the present study were chosen to more closely approximate the hormonal environment to which the heart might actually be exposed *in vivo*. The estradiol benzoate replacement dosage used in the present study was derived from previous studies in our own laboratory and is consistent with values reported in the literature. Whereas the immature rat is much more sensitive to estrogen (13) and can be physiologically replaced with only 0.02–0.03 μg estradiol benzoate/100 g body wt/day (14), replacement values for adult female rats range from 2 to 7 μg/100 g body wt/day (15, 16). In a previous pilot study of estradiol benzoate replacement at 0.0, 1.5, and 15.0 μg/100 g body wt/day for 16 weeks, linear regression analysis yielded a requirement of 7.0–8.0 μg/100 g body wt/day for the 16-week period if coronary blood flow and myocardial anoxic resistance values were to be restored to intact female control values (17–19). The bioassay data reported in the present study confirm that this dosage provides a similar near-physiologic level of uterine stimulation. The replacement dosage for testosterone propionate was derived from published values (20, 21), and the bioassay data reported here again confirm that this dosage provides a similar near-physiologic level of seminal vesicle stimulation.

These chronically administered near-physiologic doses of estradiol and testosterone could have modified cardiovascular function at many levels in the intact animal. The present study, employing an *in vitro* isolated right ventricular preparation, allows us to examine the ability of isolated myocardial cells, divorced from their usual coronary supply system, to maintain contractile function in response to electrical stimulation during the anoxic state. Thus, any differences seen between various treatment groups will primarily represent differences in the ability of the isolated myocardial cells to utilize anaerobic metabolic processes for ATP production during the 10-min anoxic period. In order for this mechanism to be physiologically relevant, sex steroid receptors must exist that are capable of influencing the cardiovascular system. Such target sites have indeed been demonstrated in the walls of coronary blood vessels (4), in fresh human aortic tissue (7), and within the myocardial muscle cells themselves in the rat and primate (5, 6, 22). The presence of vascular estrogen receptors, the observed decrease in uterine,

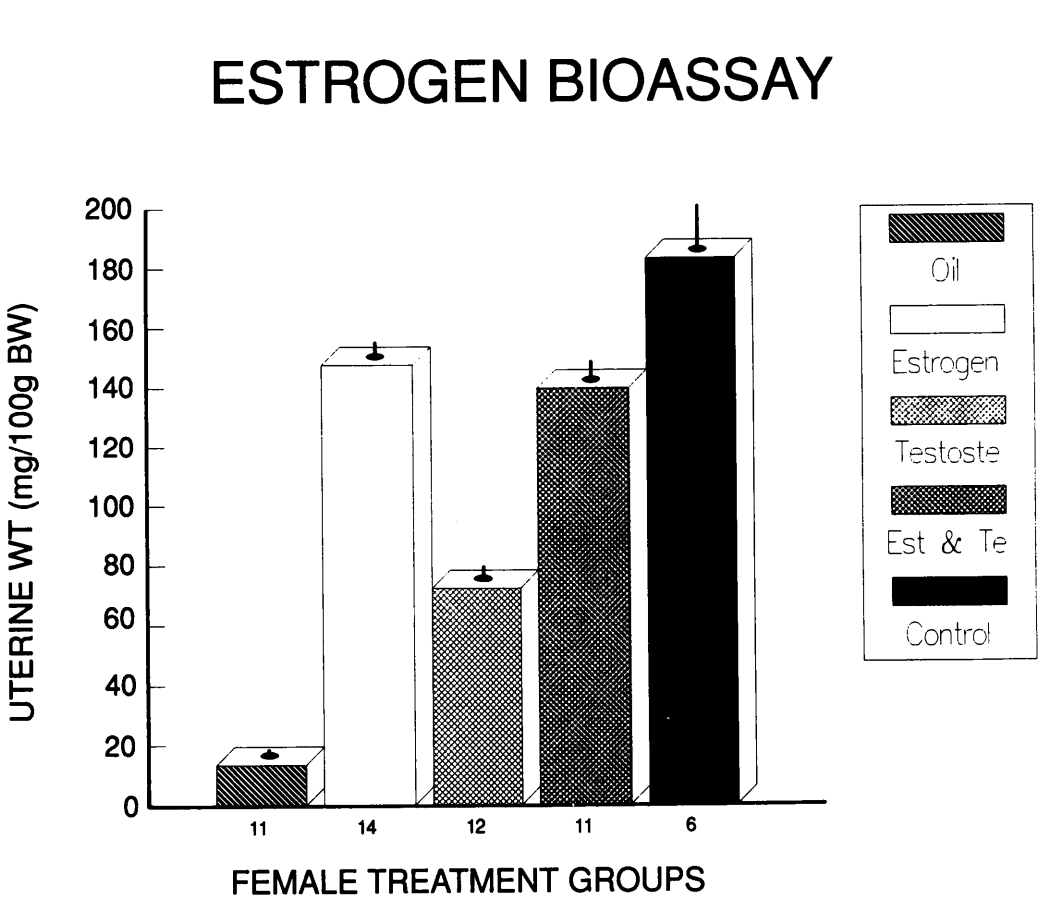

Figure 4. Sex steroid influence upon uterine weights in the female castrate and intact control groups. Bars represent group means ± 1 SE. Numbers below bars represent group *n* values.

renal, and total peripheral resistances that accompanies pregnancy, the emulation of these resistance changes by estrogen administration (23, 24), and the fact that uterine blood flow peaks concomitantly with the cyclic estrogen surges during the estrous cycle (25) would indicate that estrogen may modify vascular resistance and blood flow to various tissue beds. However, in contrast to the isolated whole-heart preparation, the *in vitro* right ventricular preparation is divorced from its usual coronary vascular supply system, and estrogen-induced differences in coronary perfusion would not be apparent in these experiments. The effects of estrogen demonstrated in these studies could result either from receptor activation within the cardiovascular system or secondarily from the *in vivo* activation of other agents capable of activity at the level of the isolated myocardial cell.

The purposes of the present study were to (1) determine whether the presence of estrogen alone might protect the myocardium during anoxic episodes, (2) determine whether the presence of testosterone alone might decrease the ability of the heart to maintain function during anoxia, (3) examine interactions of these two sex steroids upon myocardial anoxic resistance, and (4) determine whether or not genetic differences in the heart might preclude the ability of the myocardium of one gender from responding to the major sex steroid produced by the other gender.

Our results indicate that estradiol augments the ability of the myocardium to generate tension during a 10-min anoxic bout in both sexes when estradiol-replaced castrates are compared with sham (oil)-injected controls. Thus, it would appear that the genetically determined sex of the animal is less important than the hormonal milieu in determining the susceptibility of the heart to ischemic damage. The fact that the anoxic resistance of the male castrate is augmented by estradiol replacement to a much greater extent (225%), however, than is the same parameter of the estradiol-treated female castrate (125%) does not rule out a genetic difference in cardiac responsiveness to the same steroid environment. Treatment of castrate rats of either gender with testosterone alone did not significantly alter myocardial anoxic resistance from that demonstrated by the sham (oil)-injected control. Thus, it would appear that the absence of estrogen, rather than the presence of testosterone, accounts for the decreased ability of the intact male myocardium to maintain contractile function during anoxia. On the other hand, the simultaneous administration of both estradiol and testosterone reduces the anoxic resistance of the heart to that seen in castrate animals receiving no hormone. It thus

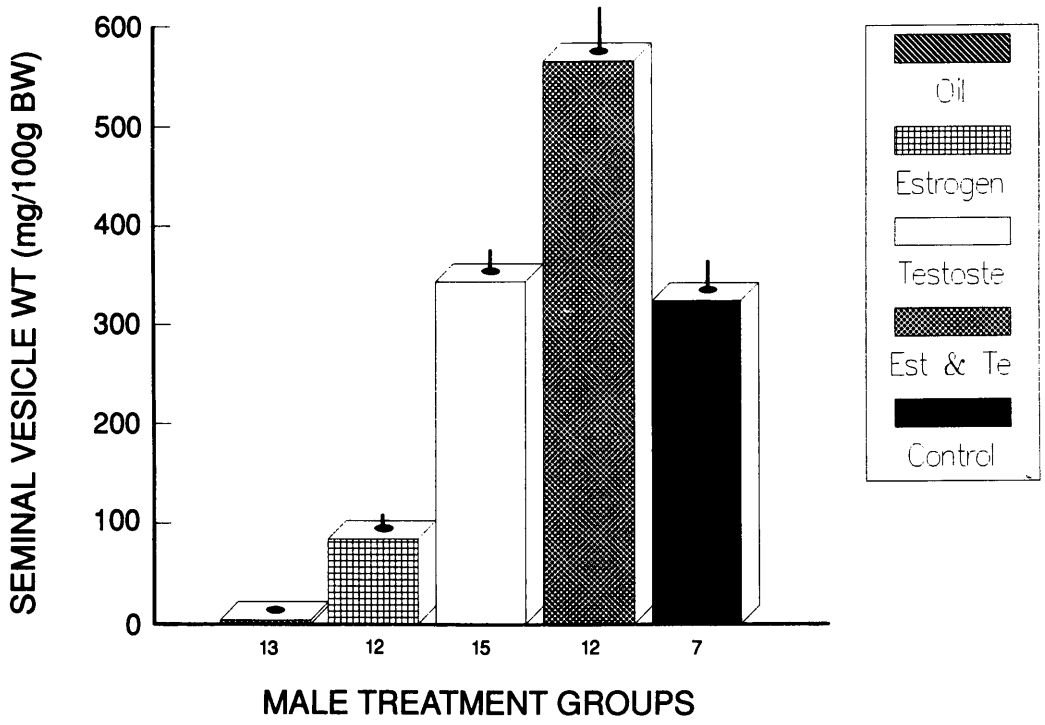

Figure 5. Sex steroid influence upon seminal vesicle weights in the male castrate and intact control groups. Bars represent group means ± 1 SE. Numbers below bars represent group *n* values.

appears that while estrogen may increase the ability of the myocardium to continue to generate tension during periods of reduced oxygen availability, the simultaneous presence of testosterone abolishes this protection.

In summary, these data would suggest that the presence of testosterone is not directly responsible for reduced anaerobic competency in the male myocardium, but testosterone might negate any beneficial effect of estrogen in the intact male. It would further appear that the protection afforded to the premenopausal female, with respect to coronary heart disease and cardiovascular mortality, is not determined strictly genetically, but rather is phenotypically modified through the presence of estrogens in the circulatory system.

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