The effects of 17α-methyltestosterone on myocardial function in vitro

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ABSTRACT


Testosterone analogs have been used as performance enhancers by athletes for more than 40 yr. We asked whether the anabolic steroid 17α-methyl-4-androstene-17-ol-3-one (17α-MT) would affect intrinsic contractile function of the heart. Male Sprague-Dawley rats, 125–150 g, were treated with 17α-MT either parenterally or orally for up to 8 wk. Intrinsic contractile function of the hearts was assessed utilizing both the isolated working heart and isovolumic perfused heart preparations. Isolated working hearts from 17α-MT-treated rats had a 45% decrease in heart work attributable largely to a similarly decreased stroke volume. Isovolumic perfused hearts from treated animals had elevated left ventricular systolic and diastolic pressures at similar interventricular volumes compared to controls. Rates of ventricular pressure development (+dP/dT) or relaxation (−dP/dT) were unchanged as a result of the treatment. However, static elastance was reduced in potassium-arrested hearts from the 17α-MT treatment (63% increase in interventricular pressure), consistent with a limitation being imposed on stroke volume by a decreased myocardial compliance. Hydroxyproline content of the hearts was not altered by 17α-MT treatment suggesting that increased stiffness was not a consequence of collagen proliferation. Treatment of the steroid rats with β-aminopropionitrile, a compound that inhibits lysyl oxidase, restored the left ventricular volume-pressure relationship (elastance curve) to that of control hearts. Thus, chronic treatment with anabolic steroids appears to reduce left ventricular compliance, possibly related to an enhanced activity of lysyl oxidase, and results in increased crosslink formation between collagen strands in the extracellular matrix. Key Words: ANABOLIC STEROIDS, ISOLATED PERFUSED HEART, LEFT VENTRICULAR FUNCTION, LYSYL OXIDASE

Throughout history athletes have sought to enhance performance through the use of a variety of chemical substances. Over the last 4 decades the most widely used substances utilized for this purpose have been anabolic-androgenic steroids; derivatives or analogs of testosterone. The ability of these compounds to increase muscle mass and decrease body fat is well established (2). The abuse of these compounds now reaches to every level of competition from high school to international sports to professional athletes. Current legal statues classify anabolic steroids as controlled substances similar to opiates and barbiturates (13). Recent data also implicates a spreading of the usage of testosterone analogs to female high school athletes.

Anabolic steroids are known to induce a variety of alterations in hemodynamics and myocardial function. These include hypertension, hypo-α-lipoproteinemia, ultrastructural morphological changes, and alterations in left ventricular diastolic dimensions (1,6,8,9,20,21,25). Investigations into anabolic steroid induced alterations in cardiac function have produced inconsistent results. These appear to be due to differences in the specific steroid used, the dose, and the route by which it was administered. Ramo (21) reported that administration of methandienone (1.5 mg·kg⁻¹·d⁻¹ for 6 wk) decreased inotropic and chronotropic responses of the canine left ventricle in situ. Extending these studies to endurance trained animals, Ramo (21) demonstrated that steroid administration attenuated the exercise induced increase in left ventricular stroke work and the decrease in resting systemic vascular resistance. By contrast, Liang et al. (12) could find no significant differences in cardiac function between exercised rats and rats treated with nandrolone (3 mg·kg⁻¹·bw⁻¹ for 10 wk).

We designed experiments to determine the effects of chronic administration of 17α-methyl-4-androstene-17β-ol-3-one (17αMT) on intrinsic myocardial performance using isolated perfused hearts. This steroid was chosen because of the high incidence of its illicit use (4). The laboratory rat was chosen as an experimental animal because in the absence of cholesterol feeding atherogenesis does not occur in this animal model (5). This effectively eliminated influences of 17αMT on atherogenesis-mediated myocardial effects,
thus allowing the more direct effects of the drug on myocardial function to be determined. When the initial experiments demonstrated significant changes in myocardial pumping capacity, several additional experiments were performed. Finally, experiments were designed to determine whether the inhibition of lysyl oxidase, the enzyme responsible for the construction of the cross links between adjacent strands of collagen, might attenuate the 17αMT induced changes observed in myocardial volume pressure relationships. We report that the steroid-induced changes in the heart are independent of the route of administration of the drug and the elevated blood pressure often associated with anabolic steroid usage (26).

**MATERIALS AND METHODS**

Conventionally bred male Sprague-Dawley rats weighing 125–150 g were purchased from Hilltop Farms (Scottsdale, PA) and were used in all experiments. They were housed in groups of three to four per cage and kept in animal quarters on a 12-h light-dark cycle at a constant temperature with *ad libitum* access to food and water. Animals were allowed to acclimatize for a week before the initiation of an experiment. The animal facility is American Association of Animal Laboratory Committee approved. All animal protocols were approved by the LSU Medical Center Animal Care and Use Committee and were in accordance with the guidelines set forth by the American College of Sports Medicine.

**Drug Administration Routes**

In the first set of experiments, silastic capsules containing 17αMT were implanted subcutaneously in the nape of the neck after the method of Moon and Bunge (16). Under sterile conditions, four capsules fabricated from polydimethylsilastic tubing (Dow Corning silastic medical grade tubing) with an inner diameter of 0.157 cm and an outer diameter of 0.318 cm each 3.0 cm long filled with 33.5 ± 2.4 mg 17αMT dissolved in 0.5 mL of sesame seed oil were implanted in the nape of the neck. These generated a release of 1 mg d−1. Two groups were defined: sham controls in which empty capsules were implanted and experimental rats whose capsules contained the steroid (*N* = 8 per group). After the implantation, the rats were returned to the animal quarters and individually housed for a period of 8 wk.

Because many abusers of anabolic steroids use orally administered analogs of testosterone, we wished to determine whether changing the route of administration of the 17αMT changed the effect on the heart. In these experiments, the 17αMT was administered in the food. Two groups were defined: a group that received powdered chow and tap water to drink *ad libitum*, and an experimental group fed 17αMT (0.3%) mixed with the powdered chow and tap water to drink (*N* = 8 per group). We determined that approximately 55 ± 5 mg of 17αMT were ingested per day. Anabolic steroid use is also associated with the development of hypertension; rats implanted with the steroid containing capsules were marginally hypertensive at the time of sacrifice. Changing the route of administration attenuated the increase in blood pressure observed when the steroid was delivered via the silastic capsules.

**In Vitro Estimation of Myocardial Performance**

**Isolated working perfused heart.** At the end of the treatment period and after an overnight fast, the rats were anesthetized with ketamine and xylazine, a thoracotomy was performed, and the hearts were removed. After excision, the hearts were placed in cold (4°C) saline until contractions ceased. The hearts were then mounted by the stump of the aorta on a Langendorf type perfusion column, and retrograde aortic flow was initiated. Additional cannulae were placed in the left atrium and the pulmonary artery. The isolated working heart preparation used was similar to that described by Neely et al. (17). The perfusate was Krebs-Henseleit bicarbonate buffer containing 1.25 mM calcium chloride and 5 mM glucose as substrate. After a 5-min equilibration period, Starling-type ventricular function curves were generated by sequentially elevating left atrial filling pressure from 7.5 to 10, 12.5, 15, and 20 cm of water. At each preload, cardiac output (defined as the sum of coronary and aortic flows), heart rate, and peak systolic and diastolic pressures were determined.

**Isovolumic perfused heart.** In the studies utilizing the isovolumic contracting heart preparation, hearts were removed from the animal and mounted on a modified retrograde aortic (Langendorff) apparatus. A small piece of PE-90 polyethylene tubing was inserted into the apex of the left ventricle to allow for drainage of the thebesian vessels. A latex balloon attached to a 5 French Swan-Ganz double lumen catheter was introduced into the left ventricle via the left atrium. The intraventricular balloons used in these studies were fabricated from the reservoir tip of a latex condom cut and standardized for a receptacle tip to catheter tip length of 1.25 cm. The dual lumen catheters which permitted measurement of intraventricular pressures as well as the introduction of known volumes of fluid into the balloon, were connected to a Narco P-1000B pressure transducer attached to a Narco Biosystems (Austin, TX) Physiograph DMP-4A. This permitted the determination of left ventricular pressures and heart rate. In some experiments, the left ventricular pressure signal was coupled to a Narco Biosystems 7301 differentiator for estimation of the maximum rate of change of pressure development with time (+dP/dT) and maximum rate of pressure relaxation with time (−dP/dT).

**Static elastance measurements.** For these experiments hearts were removed and perfused free of blood as described above. After a 5-min stabilization period, the perfusate was changed to KRH containing 20 mM KCl and perfusion continued for another 5 min to permit complete arrest to occur. At the end of this time a small incision was made in the left atrium and a PE-190 catheter was guided into the left ventricle toward the apex of the heart. The catheter was fashioned with a sharp and a flanged end. The sharp end was pushed through the apex until the flanged end rested against the left ventricle wall. A ligature was tied
around the atria to isolate the ventricle and excess fluid was expressed out of the ventricles. Sequential 50-μL volumes of warm saline were then introduced into the arrested ventricle and intraventricular pressure monitored. Resting ventricular elastance (ΔP/ΔV) was determined. After perfusion, hearts were utilized for determination of hydroxyproline after the method of Woessner (27).

**β-aminopropionitrile (βAPN) experiments.** The possible role of the enzyme lysyl oxidase in elastance changes was assessed using the inhibitor β-aminopropionitrile (3). Four groups were defined: one group given βAPN in tap water (1 mg·mL⁻¹), a group given 17α-MT (0.3%) in ground chow, a group given βAPN and 17α-MT at the concentrations indicated above, and finally an untreated control group that was allowed powdered chow and water *ad libitum*. Food and water intakes as well as body weights were monitored for all groups. The daily intake of βAPN was calculated as 30 mg·d⁻¹.

**In vivo blood pressure determinations.** Rats were anesthetized and under sterile conditions a PE-50 polyethylene catheter placed in the left common carotid artery. The catheter was externalized to the dorsal region of the neck filled with heparinized saline sealed and taped in place; 24 h later, the catheter was opened flushed and connected to a pressure transducer. Heart rate and systolic and diastolic pressures were recorded, and mean systolic blood pressure was calculated.

**Statistical reduction of the data.** All data were reduced using the analysis of variance with students *t*-test used to compare groups (22) Significance was set at the *P* < 0.05 level.

**RESULTS**

**Drug administration routes.** The effect of 8 wk of 17αMT administration via the silastic capsule route on body weight is illustrated in Figure 1 (top panel). The 17αMT-treated rats gained significantly less weight than their sham-treated counterparts beginning day 25 and continuing for the rest of the exposure period. Wet weights of the hearts and the testes are illustrated in Figure 1 (lower panel). The treatment consistently caused a decrease in the size of the testes. Wet to dry weight ratios of either the heart or the testis did not differ as a consequence of the treatment. Similar effects on body weight, heart and testis size were observed when the drug was administered at the level of 0.3% mixed with powdered food.

**Isolated working perfused heart.** The effect of chronic 17αMT treatment on *in vitro* myocardial performance assessed using the isolated working perfused heart is shown in Figure 2A. Hearts from sham-treated controls responded to increases in left atrial filling pressure with consistent increases in cardiac work (both cardiac output and peak systolic pressure) through the entire range of preloads employed. By contrast, the hearts from the 17αMT-treated group did not respond as efficiently to preload increases with the CO × PSP product becoming significantly depressed compared with control at the two high-

**Body weights**

![Image](https://example.com/image.png)

Figure 1—(Top) Body weights of rats (mean ± standard error) receiving 17-α methyl testosterone (17αMT) via silastic catheter versus sham implanted controls over 8 wk of treatment. *N* = 15 for the control group and *N* = 16 for the 17αMT-treated group. * indicates significantly different from controls *P* < 0.05. (Bottom) Weights (mean ± SE) of selected organs of 17α MT-treated vs sham-treated controls. *N* = 53 for controls and 50 for 17αMT treated. * indicates significantly different from controls *P* ≤ 0.05.
volumes. To achieve this parity of pressures, control hearts required 110.6 ± 4.8 μL of saline, whereas hearts from the 17αMT-treated groups only needed 67.7 ± 4.4 μL of fluid. The difference was highly significant \((P < 0.05)\). All fluid was then removed from the balloon and the intraventricular balloon catheter connected to a syringe contained in an infusion pump and saline pumped into the ventricle at a rate of 34 μL/min. Systolic and diastolic pressure levels were determined at 45-s intervals until a final volume of 175 μL was reached. Developed pressure was also calculated. At all measured intraventricular volumes both diastolic and systolic pressures of the 17αMT-treated group were higher than at similar volumes for sham-treated controls (Fig. 3, top panel). Neither \(+dP/dT\) nor \(-dP/dT\) were different at any volume tested as a consequence of the treatment. Similarly,
developed pressure did not differ over this range of left ventricular volumes (data not shown).

**Static elastance measurements.** To determine whether an increased stiffness of the left ventricle might be limiting stroke volume, static pressure development of potassium arrested hearts were made. Data in Figure 4 indicate that at intraventricular volumes above 100 μL, the pressures within the ventricles of the hearts from the treated animals were higher than those observed in the hearts of sham-treated controls. Such increased stiffness has been reported to occur in response to the development of hypertension. Increased collagen content within the myocardium can also contribute significantly to compliance loss. To test whether this might be an explanation for our findings in vivo blood pressure determinations and myocardial collagen content determinations were made on animals receiving the steroid.

**In vivo blood pressure determinations.** Systolic, diastolic and mean arterial pressure (MAP) were all elevated in the steroid group, whereas there was no difference between pulse pressure and heart rate between steroid-treated and control (Fig. 5). In contrast to the situation in which the steroid was administered by latex capsule, there was no significant difference in blood pressure between controls and animals receiving orally administered 17αMT. Myocardial collagen content did not differ as a result of the steroid treatment in either administration modality.

**β-aminopropionitrile (BAPN) experiments.** A final set of experiments was performed utilizing the lysyl oxidase inhibitor β-aminopropionitrile (BAPN) to determine whether inhibition of this enzyme, which catalyzes collagen cross-link formation, would attenuate the observed myocardial elastance changes. After 6 wk of administration of the two compounds the rats were sacrificed, their hearts removed and static elastance determinations performed. Data in Figure 5 indicate that administration of BAPN caused a downward shift in the elastance curve of the treated hearts. The curve was virtually superimposable upon that obtained from hearts of untreated controls.

**DISCUSSION**

These studies confirm an androgen-induced cardiomyopathy arising as a consequence of the chronic administration of the anabolic steroid 17αMT. The hearts from the 17αMT-treated animals showed a decreased intrinsic myocardial performance secondary to a stroke volume reduction. Consistent with this are data showing a decreased myocardial elastance. This stiffening of the ventricular chambers could limit left ventricular filling at physiological left ventricular end diastolic pressures. This in turn would limit stroke volume.

These effects were seen independent of the route of administration of the drug. This is particularly relevant to the question regarding the use of testosterone analogs as performance enhancers. Both oil-soluble (injected) and water-soluble (orally ingested) analogs are abused as androgenic aids by athletes. The effect on the myocardium observed in these studies is independent of the method of drug delivery.

No differences in wet or dry weights of the hearts were observed between control and the methyltestosterone-treated groups. This indicates that the chronic treatment with methyltestosterone did not induce cardiac hypertrophy in these studies. This is particularly important since there are significant differences in myocardial contractile and metabolic properties such as oxygen uptake (MVO₂) of hearts of differing sizes (17).

The slope of the left ventricular pressure curve (+dP/dT) was used in these studies as an estimation of contractility. Similarly slope of the left ventricular pressure curve during relaxation (−dP/dT) was used as the rate of relaxation. Contractility is defined as an increased force of contraction

![Figure 4](https://example.com/figure4.png)  
**Figure 4—Volume pressure relationship of potassium arrested hearts from 17α methyl testosterone-treated (MT) and control rats. Each point represents the means ± SE. N = 10 for control and N = 9 for 17αMT-treated rat hearts. * indicates significantly different from sham-treated controls P < 0.05.

![Figure 5](https://example.com/figure5.png)  
**Figure 5—Volume pressure relationship of potassium arrested hearts from rats receiving 17α methyl testosterone (17αMT) 0.03% in chow or 17α MT and β-aminopropionitrile BAPN (1 mg·mL⁻¹ in drinking water). N = 7 for 17αMT, N = 7 for 17αMT plus BAPN and N = 8 for control. * indicates significantly different from either hearts from controls or BAPN + MT-treated rats.**

**POTENTIAL ARRESTED VOLUME—PRESSURE RELATIONSHIP**

- **SHAM IMPLANTS**
- **MT IMPLANTS**

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**STEROIDS AND THE HEART**

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independent of fiber length (left-ventricular end-diastolic volume). The rate of relaxation is related to the rate of calcium sequestration into the sarcoplasmic reticulum. The use of the isovolumic contracting heart preparation is advantageous in these measurements because it permits the standardization of preload and heart rate conditions that must be comparable when attempting to quantitate contractility.

It is generally accepted that prolonged administration of testosterone analogs causes hypertension and that hypertension alters myocardial compliance through hypertrophy and increased collagen accumulation. Trifunovic et al. (24) reported that nandrolone decanoate decreased ventricular compliance in rats. Utilizing an external strain gauge appliance, they showed a change in the left-ventricular elastic stiffness constant (left-ventricular end-diastolic stress vs left-ventricular end-diastolic strain). They concluded that this was a result of a direct effect on the heart since systolic blood pressure was not different between control and treated groups. They did not, however, evaluate diastolic blood pressure so systemic hypertension cannot be ruled out. Similarly, no chemical determinations of myocardial interstitial parameters (specifically collagen content or concentration) were made.

Our data suggest that the changes in myocardial elastance is not a consequence of hypertension. The ventricular changes were present irrespective whether or not significant elevations in blood pressure occurred as when the steroid was administered via the silastic capsules or when blood pressure was not elevated as occurred in the oral administration experiments.

Neither treatment caused any increases in myocardial collagen content. This would suggest that the effect on myocardial elastance was not a consequence of the presence of additional collagen in the myocardium. This is significant at two different levels. First, clinical literature regarding structural changes in the heart resulting from anabolic steroid abuse has documented extensive fibrosis probably resulting from atherosclerotic degeneration of the coronary vasculature (14). The rat model used here does not develop atherosclerosis, and as expected there was no evidence of increased collagen in the heart. Second, the intrinsic contractility of the hearts from the treated animals did not differ from that of control animals. This, too, is consistent with a lack of significant structural damage to the myocardium.

A common feature of anabolic steroid abuse are tendon injuries. These have usually been ascribed to either decreases in collagen synthesis as proposed by Karpakka et al. (11), who found that prolyl-4-hydrolyase activity fell as a consequence of anabolic steroid administration, or, alternatively, that the steroid may accelerate collagen degradation as proposed by Michna and Stang-Voss (15), who found increased lysosome activity in fibroblasts isolated from the tendons of anabolic steroid-treated mice.

Lysyl oxidase is the enzyme responsible for initiating the formation of irreducible crosslinks between adjacent collagen molecules. These crosslinks are a necessary component of the collagen matrix. One possible mechanism for increasing the stiffness of the heart could be increasing numbers of crosslinks, possibly by increasing lysyl oxidase activity. Increased activity of this enzyme has been implicated in the increased stiffness in dystrophic skeletal muscle in chickens (7). Inhibiting the activity of this enzyme by βAPN caused a shift in the myocardial elastance curve toward normal. The ability of βAPN to reverse the 17α-MT effect suggests that the steroid treatment increases the number of crosslinks in myocardial collagen. Other steroids have been shown to increase the activity of lysyl oxidase, specifically estrogen and dehydroepiandrosterone (18,19).

Whether activation of the enzyme can be accomplished by anabolic steroids is unknown. Very little is known about the regulation of the enzyme (10).

In conclusion, our data and previous reports in the literature underscore two consequences of anabolic steroid abuse. First, testosterone and its analogs exert a profound effect on collagen metabolism. The extent of this and its impact on tissue repair after injury, susceptibility of collagen rich structures, such as tendons, to injury or interactions with various training regimes have been only slightly examined. Data in the literature combined with anecdotal reports implicating anabolic steroids in connective tissue injury suggests that this would be a fertile field for further investigation.

Second, as to the myocardium, our data show that even a relatively short exposure to anabolic steroids can negatively impact cardiac function. This effect appears to be independent of changes in blood pressure and similarly does not require the development of atherosclerosis. Whether these changes are reversible following discontinuation of the drug remains to be studied.

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REFERENCES


