Testosterone-propionate impairs the response of the cardiac capillary bed to exercise

CHRISTOS V. M. TAGARAKIS, WILHELM BLOCH, GEORG HARTMANN, WILDOR HOLLMANN, and KLAUS ADDICKS

Institute I of Anatomy, University of Cologne; Institute of Cardiology and Sports Medicine, German Sport University, Cologne; and Bayer Vital GmbH & Co. KG, Geschäftsbereich Pharma, Leverkusen, GERMANY

ABSTRACT

TAGARAKIS, C. V. M., W. BLOCH, G, HARTMANN, W, HOLLMANN, and K, ADDICKS. Testosterone-propionate impairs the response of the cardiac capillary bed to exercise. *Med. Sci. Sports Exerc.,* Vol. 32, No. 5, pp. 946–953, 2000. **Objective:** Experimental application of anabolic-androgenic steroids and exercise training induce cardiac hypertrophy. This study quantifies for the first time, on microscopical level, the adaptation of the cardiac capillaries and myocytes to the concomitant application of testosterone-propionate and exercise training. **Methods:** Female SPF-NMRI mice were studied over 3 and 6 wk. Experimental groups: (i) sedentary control (C); (ii) exercise (treadmill running, E); (iii) testosterone-propionate (TP); and (iv) testosterone-propionate+exercise (TPE). Morphometric parameters: 1) papillary muscles: capillary density, intercapillary distance, number of capillaries around a myocyte, and minimal myocyte diameter; and 2) left ventricular wall: capillary density and intercapillary distance. **Results:** Papillary muscle: A striking suppression of the exercise-induced improvement in capillary supply occurs in the testosterone-propionate+exercise groups over 3 and 6 wk. Exercise without drugs increases significantly ($P < 0.05$) the capillary density, shortens significantly ($P < 0.05$) the intercapillary distance, whereas it increases the number of capillaries around a myocyte. These alterations are not observed in the testosterone-propionate treated sedentary animals; e.g., capillary density after 6 wk (mean values ± standard deviation, capillaries·mm^{-2}): C: 4272 ± 287, E: 5411 ± 758, TP: 4221 ± 364, and TPE: 3997 ± 397. Moreover, only in the testosterone-propionate+exercise groups occurs a mild myocyte hypertrophy after both time periods: there is a trend toward hypertrophy ($P < 0.1$) in comparison with the C groups and a significant hypertrophy ($P < 0.05$) in comparison with the E groups. **Conclusions:** Testosterone-propionate profoundly inhibits the exercise-induced augmented capillarization, whereas (under training conditions) it leads to a mild myocyte hypertrophy. The microvascular impairment could trigger an imbalance between the myocardial oxygen supply and demand, especially during physical exercise. **Key Words:** ANDROGENS, ANABOLIC STEROIDS, PHYSICAL EXERCISE, MICE, HEART, LEFT VENTRICLE, PAPILLARY MUSCLES, HYPERTROPHY, MYOCYTE, ANGIOGENESIS, MICROCIRCULATION

Androgens are utilized in the clinical practice (7), e.g., in the cases of aging (35), osteoporosis (45), HIV infection (61), chronic obstructive pulmonary disease (20), and induction of male contraception (63), often in combination with a physical rehabilitation regimen (20,61). Recently, androgens have been experimentally applied in the field of cardiomyoplasty (26). In addition to the legal application to exercising individuals for research purposes (11), anabolic-androgenic steroids are abused in sports (7,44,65) for the improvement of physical performance and the increase of skeletal muscle mass.

Numerous side effects have been reported in connection with anabolic steroid treatment. Some of them are: cardiac sympathetic overstimulation (30), increased blood pressure (37) and atherosclerosis (3,41), impaired coronary flow and perfusion (46), inhibition of nitric oxide-mediated vasodilation (21), abnormal blood coagulation (19), thrombosis (41,46), apoptosis of skeletal myofibers (1), and toxic action upon the cardiac muscle cells (40) as well as pathological ultrastructural alterations of the cardiomyocytes (6,10,38,46). Furthermore, anabolic steroid abusers suffered various degrees of left ventricular dysfunction (16,41,46,60), ventricular fibrillation (38,46), hypertrophic cardiomyopathy (33), myocardial infarction (33,46) and myocarditis (33). Even cases of cardiac transplantation (41) and cardiovascular fatalities have been reported among these individuals (17,33,38).

Animal experiments document that anabolic steroids combined with muscular exercise increase the heart weight (9,32). Until now, the response of the cardiomyocytes to the concomitant application of these drugs and exercise training has not been quantified.

An adequately developed capillary network is of vital importance for the sufficient perfusion of the normal as well as the hypertrophied heart, especially during physical activity. Similar to the case of myocytes, the response of the

0195-9131/00/3205-0946/0
MEDICINE & SCIENCE IN SPORTS & EXERCISE®
Copyright © 2000 by the American College of Sports Medicine

Submitted for publication April 1998.
Accepted for publication July 1999.

coronary capillaries to anabolic steroids under training conditions has never been studied.

Thus, the aim of the present experiments was to quantify for the first time the short-term (3 wk) as well as the long-term (6 wk) effect of the combined stimuli of testosterone-propionate and muscular exercise on: (i) the structural response of the cardiac capillary bed and (ii) myocyte hypertrophy.

METHODS

Experimental Design

The following groups of 10-wk-old female SPF-NMRI mice were studied: (i) control (C), (ii) exercise (E), (iii) testosterone-propionate (TP), and (iv) testosterone-propionate+exercise (TPE).

The exercise regimen was treadmill running for five $d \cdot wk^{-1}$, 30 $min \cdot d^{-1}$, with 3% slope and progressively increasing speed until a maximum of 33 $m \cdot min^{-1}$.

The steroid treated groups received intramuscular injections of testosterone-propionate (Eifelfango, Bad Neuenahr), which was dissolved in sesame oil. The dose was 3 mg·kg body weight/wk.

Ethics

The mice were handled according to the policy statement of the American College of Sports Medicine on research with experimental animals (4), the German law for the care and use of laboratory animals, as well as the experimental Approval Procedures of the German Federal State Northrhine Westphalia.

Histological Procedure—Morphometry

After the defined time intervals (3 and 6 wk), the animals were anesthetized with Nembutal (Abbott, Ingelheim, Germany) and fixed by perfusion, using a 0.1 M cacodylate buffer containing 2.5% glutaraldehyde at pH 7.4. Left ventricular wall samples and the papillary muscles were removed, postfixed, and embedded in plastic. The perfusion and fixation methods are described in detail elsewhere (30). Semithin cross-sections were stained with methylene blue and were studied by using light microscopic morphometry. Ultrathin sections of the tissue specimens (microtome Ultracut, Fa. Reichert Jung, Vienna, Austria) were subjected to ultrastructural morphologic study (electron microscope Zeiss EM 902A).

The morphometry of the left ventricular papillary muscles included the parameters (the number of animals per group (N) used for each one of the studied variables is given in parenthesis): 1) capillary density and 2) intercapillary distance (3 wk: C: $N = 6$, E: $N = 5$, TP: $N = 3$, TPE: $N = 9$, 6 wk: C: $N = 7$, E: $N = 8$, TP: $N = 6$, TPE: $N = 13$), 3) number of capillaries around a myocyte and 4) minimal myocyte diameter (3 wk: C: $N = 4$, E: $N = 4$, TP: $N = 3$, TPE: $N = 5$, 6 wk: C: $N = 6$, E: $N = 7$, TP: $N = 5$, TPE: $N = 9$).

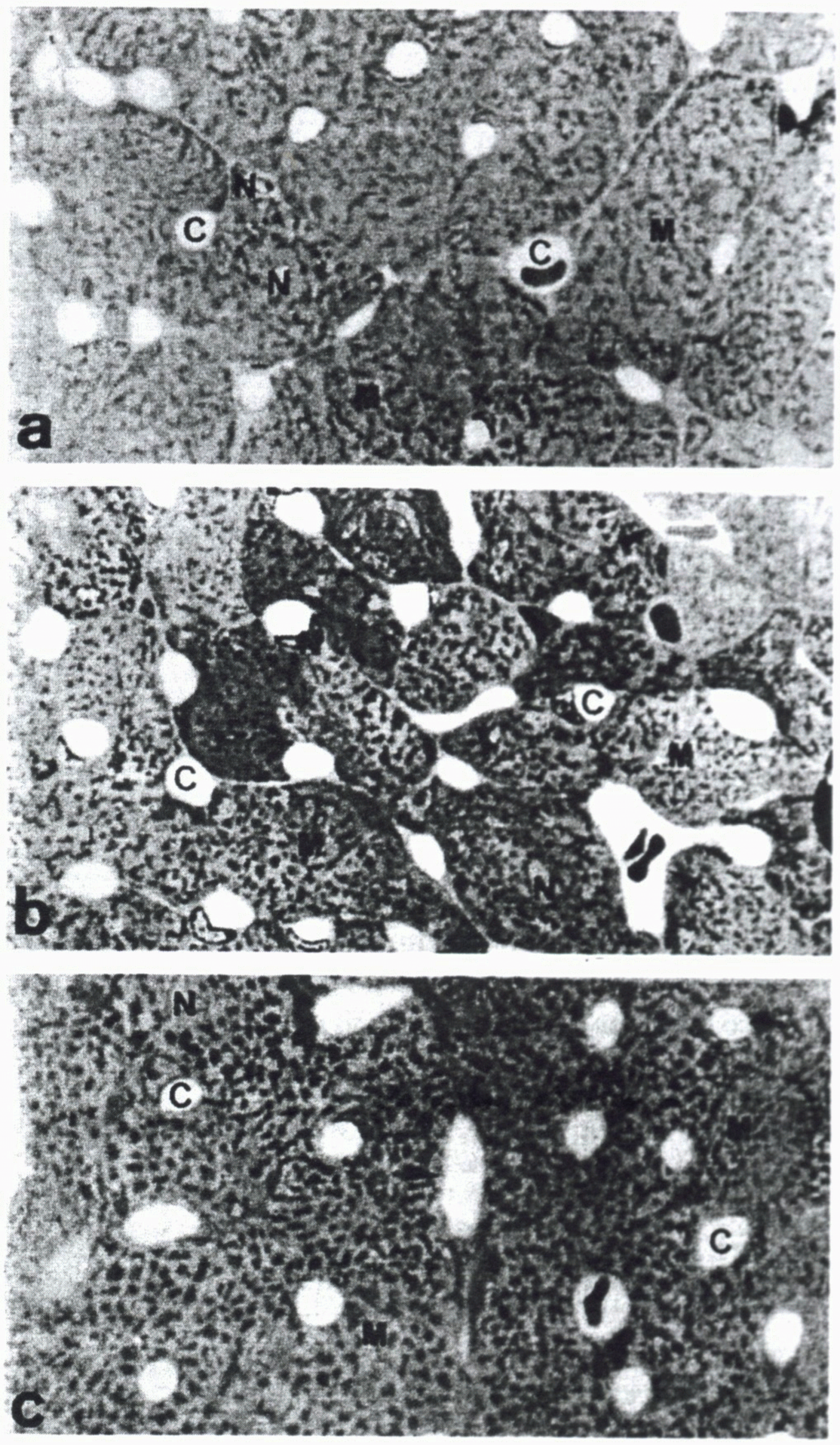

Figure 1—Semithin cross-sections of the left ventricular papillary muscles, after the 6-wk experiment: M: myocyte, N: nucleus, C: capillary. a) Control: normal distribution of the capillaries; most of them are cross-sectioned. b) Exercise without drug treatment: increased capillarization; many capillaries are oblique sectioned (*arrow* →), due to increased capillary brunching. The myocytes are not hypertrophied. c) Testosterone-propionate+exercise: the capillarization is similar to that of the control group; no capillary brunching is observable. Magnification ×1000.

The morphometry of the left ventricular *wall* included: 1) the capillary density and 2) the intercapillary distance (3 wk: C: $N = 5$, E: $N = 3$, TP: $N = 3$, TPE: $N = 3$, 6 wk: C: $N = 4$, E: $N = 4$, TP: $N = 4$, TPE: $N = 6$).

The minimal diameter of 60 myocytes per animal (Fig. 1) was measured at the level of the nucleus at a magnification of ×630, by using an image analysis system (CBA 8000, Leica). At the same time, the number of capillaries around each one of the evaluated myocytes was counted. In some semithin cross-sections, it was not possible to discriminate the borders of the neighboring myocytes. This difficulty reduced the number of animals that were used for the parameters: (i) myocyte diameter and (ii) number of capillaries around a myocyte.

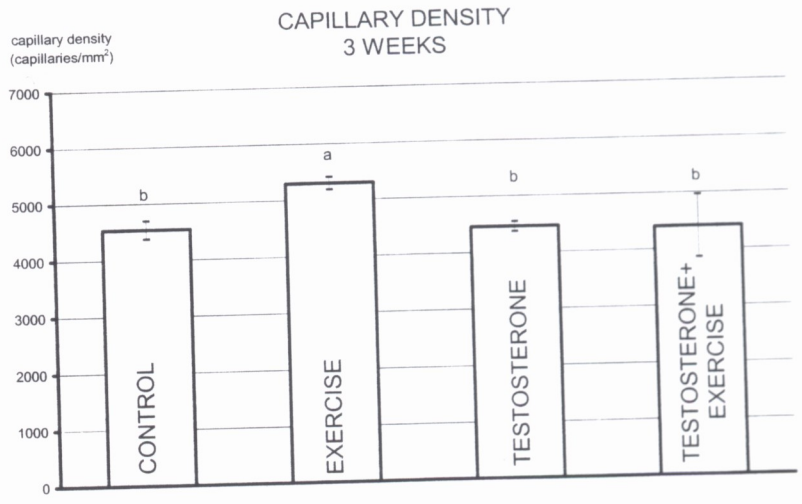

Figure 2—Papillary muscle capillary density after the 3-wk experiment; statistically significant differences in comparison with the control (a) and the exercise (b) group.

The number of capillary profiles per field was counted according to the method of Gundersen (28). The counting was conducted only in regions where the myocytes were cross-sectioned (magnification ×1000, microscope Axioskop, Zeiss). Subsequently, (i) the capillary density (definition: number of capillaries·mm^{-2}) and (ii) the maximal intercapillary distance (definition: distance between two adjacent capillaries, according to the hexagonal model of tissue capillarization) were calculated (50).

Statistical Analysis

The statistical evaluation was conducted by using the statistical program system SPSS 6.0 for Windows. Based upon the results of the Levene's test for homogeneity of the variables, one of the following procedures was performed: a) the one-way ANOVA, with multiple range test and the Scheffe's test, or b) the Kruskal-Wallis test and the Mann-Whitney U test. Statistical significance was accepted, if $P < 0.05$. Cases of $P < 0.1$ were interpreted as showing a trend toward a difference.

RESULTS

Papillary Muscles

Capillary density. After 3 (Fig. 2) and 6 wk (Fig. 3), exercise training, unlike all other regimens, leads to a significant increase in capillary density. The striking absence of this increase in the testosterone-propionate+exercise groups is of special interest. Application of testosterone-propionate on sedentary animals does not induce any alterations in comparison with the control values over both time periods.

Due to the exercise-induced increased capillary branching, many oblique sectioned capillaries can be observed in the exercise groups (Fig. 1). This alteration is not present in the testosterone-propionate+exercise and testosterone-propionate groups.

Intercapillary distance. Even after 3 wk of exercise training the intercapillary distance is shortened significantly in comparison with the control and testosterone-propionate group, whereas there is a trend toward shorter intercapillary distance in comparison with the testosterone-propionate+exercise group (Fig. 4). The most interesting finding is that

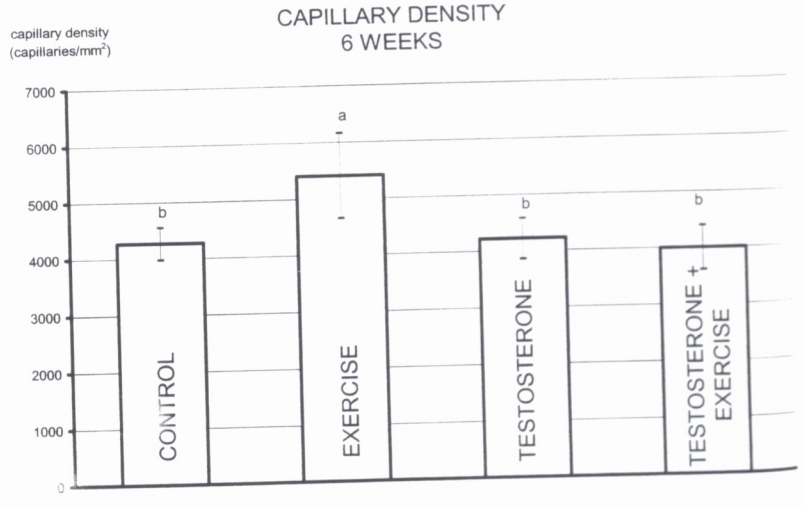

Figure 3—Papillary muscle capillary density after the 6-wk experiment; statistically significant differences in comparison with the control (a) and the exercise (b) group.

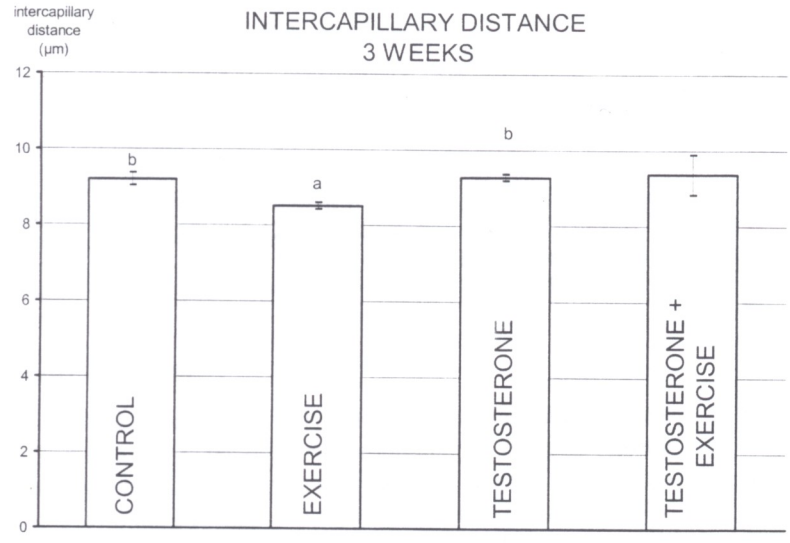

Figure 4—Papillary muscle intercapillary distance after the 3-wk experiment; statistically significant differences in comparison with the control (a) and the exercise (b) group.

this adaptation does not occur in the testosterone-propionate+exercise group. No alteration can be observed in the testosterone-propionate sedentary mice.

After 6 wk (Fig. 5), the exercise-induced shortening of the intercapillary distance becomes significant in comparison with all other regimens. It should be stressed that the difference between the exercise group and the testosterone-propionate+exercise one is progressively enhanced (compare Figs. 4 and 5) and becomes more pronounced after 6 wk. During the same time period, the intercapillary distance of the testosterone-propionate group does not differ from the control values.

Number of capillaries around a single myocyte. Short-term exercise (Table 1) induces a trend toward an increased number of capillaries around a single myocyte in comparison with all other regimens. After 6 wk there are significantly more capillaries around a myocyte in the exercise group than in the control, the testosterone-propionate as well as in the testosterone-propionate+exercise group.

The progressive suppression of the exercise-induced increase in the number of capillaries around a single myocyte in the testosterone-propionate+exercise groups is a noteworthy finding: it is coupled with concomitant alterations occurring in the other indices of capillarization, namely the capillary density and the intercapillary distance (Figs. 2–5). No alterations in the number of capillaries around a single myocyte are observed in the testosterone-propionate groups in the course of both 3 and 6 wk.

Myocyte diameter. After the short-term period (Table 2) the myocytes of the testosterone-propionate+exercise group show a trend toward hypertrophy, when compared with the exercise group. This difference becomes significant after 6 wk. The testosterone-propionate+exercise group shows a trend toward myocyte hypertrophy in comparison with the control group after 3 and 6 wk.

Over both time intervals, the myocyte diameters of the exercise, testosterone-propionate and control groups are comparable.

Electron microscopic evaluation documents that the myocyte hypertrophy of the testosterone-propionate+exercise groups is due to increased myofibrillar components and not to cellular swelling.

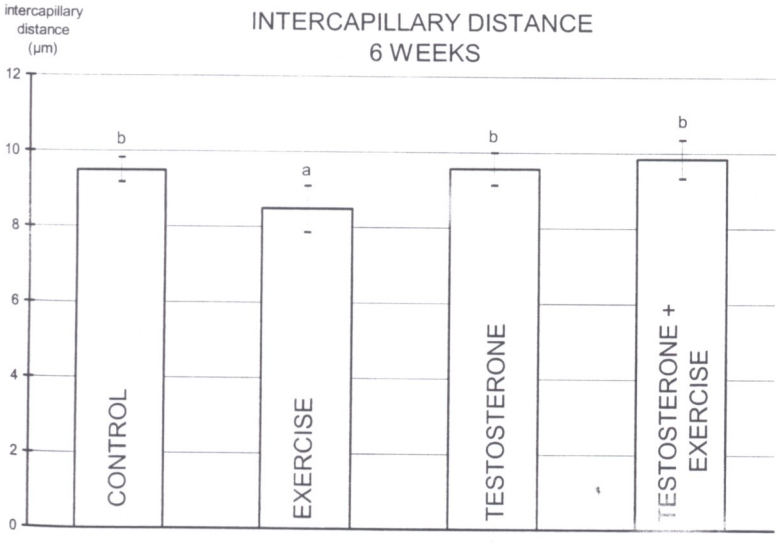

Figure 5—Papillary muscle intercapillary distance after the 6-wk experiment; statistically significant differences in comparison with the control (a) and the exercise (b) group.

TABLE 1. Papillary muscle: number of capillaries around a single myocyte (*N*).

	C	E	TP	TPE
3 W	4.20	4.76	4.14	4.51
SD	0.11	0.65	0.20	0.44
	b	a	b	b
6 W	4.35	4.85	4.23	4.46
SD	0.40	0.20	0.35	0.26

Control (C), exercise (E), testosterone-propionate (TP), testosterone-propionate + exercise (TPE). 3 W: 3 wk, 6 W: 6 wk. Mean values ± SD; significant differences in comparison with the control (a) and the exercise (b) group of the same period.

TABLE 3. Left ventricular wall: capillary density (capillaries · mm^{-2}).

	C	E	TP	TPE
			a,b	
3 W	4594	4648	4135	3849
SD	72	181	330	144
			b	b
6 W	4024	4960	3362	3599
SD	616	476	172	592

Control (C), exercise (E), testosterone-propionate (TP), testosterone-propionate + exercise (TPE). 3 W: 3 wk, 6 W: 6 wk. Mean values ± SD; significant differences in comparison with the control (a) and the exercise (b) group of the same period.

Left Ventricle

Capillary density. It is noteworthy that even after 3 wk (Table 3) the testosterone-propionate+ exercise group shows a trend toward reduced capillary density in comparison with the exercise as well as the control group. Over the same period testosterone-propionate treated sedentary animals show a significantly lower capillary density than the exercise and the control groups.

The difference between the exercise and the testosterone-propionate treated groups increases progressively and becomes more striking over 6 wk ($P < 0.05$). Moreover, after the long-term period the testosterone-propionate and the testosterone-propionate+exercise groups exhibit lower capillary density than the respective control values. However, this difference cannot be statistically documented.

Three weeks of exercise alone do not alter the capillary density in the ventricular wall as compared with the control group. An increased capillary density in comparison with the control values is to be observed after the 6-wk experiment without, however, reaching statistical significance.

Intercapillary distance. Even after 3 wk (Table 4) the testosterone-propionate+exercise group exhibits a significantly longer intercapillary distance in comparison with the control values and a trend toward longer intercapillary distance than the exercise group. Noticeably, this increase becomes more evident over 6 wk and reaches statistical significance compared with the exercise group. The 6-wk values of the testosterone-propionate+exercise and the control group do not differ significantly.

Short-term treatment of sedentary animals with testosterone-propionate induces a significantly longer intercapillary distance in comparison with the exercise and the control group. Over 6 wk the testosterone-propionate group exhibits a significantly longer intercapillary distance than the exercise group as well as a similar trend in comparison with the control values.

TABLE 2. Papillary muscle: minimal myocyte diameter (μm).

	C	E	TP	TPE
3 W	17.13	17.05	17.95	18.66
SD	2.32	2.16	1.38	1.93
				b
6 W	17.48	16.85	17.87	19.33
SD	2.58	1.96	1.68	1.42

Control (C), exercise (E), testosterone-propionate (TP), testosterone-propionate + exercise (TPE). 3 W: 3 wk, 6 W: 6 wk. Mean values ± SD; significant differences in comparison with the control (a) and the exercise (b) group of the same period.

Three weeks of exercise without drugs do not induce any alterations in comparison with the control values, but after 6 wk a trend toward shorter intercapillary distance in comparison with the control group can be observed.

DISCUSSION

The main outcomes of the present study are (Fig. 6): (i) testosterone-propionate inhibits the exercise-induced capillary growth, (ii) testosterone-propionate combined with exercise induces mild myocyte hypertrophy, and (iii) testosterone-propionate leads to a partial deterioration of the capillary supply in the sedentary animals. Under exactly the same experimental conditions, our group demonstrated that the combined stimuli of testosterone-propionate and exercise training result in a more pronounced cardiac sympathetic overstimulation than testosterone-propionate alone as well as exercise alone (30).

Anabolic-Androgenic Steroids Impair the Capillary Network

As far as the capillary impairment is concerned, which is documented in the testosterone-propionate+exercise groups, the following points should be mentioned:

a) The microvascular impairment occurs after only 3 wk and becomes more pronounced over the long-term period.

b) Although the papillary muscles and the left ventricular wall have different functions, they undergo a similar impairment. In both cardiac regions, all indices of capillarization are deteriorated (to various degrees) under concomitant testosterone-propionate+exercise treatment, which is in striking contrast to the respective exercise drug-free regimen.

c) Although the number of capillaries around a single myocyte increases in the testosterone-propionate+exercise

TABLE 4. Left ventricular wall: intercapillary distance (μm).

	C	E	TP	TPE
			a,b	a
3 W	9.15	9.10	9.66	10.00
SD	0.07	0.17	0.37	0.19
			b	b
6 W	9.84	8.82	10.71	10.95
SD	0.78	0.43	0.27	1.47

Control (C), exercise (E), testosterone-propionate (TP), testosterone-propionate + exercise (TPE). 3 W: 3 wk, 6 W: 6 wk. Mean values ± SD; significant differences in comparison with the control (a) and the exercise (b) group of the same period.

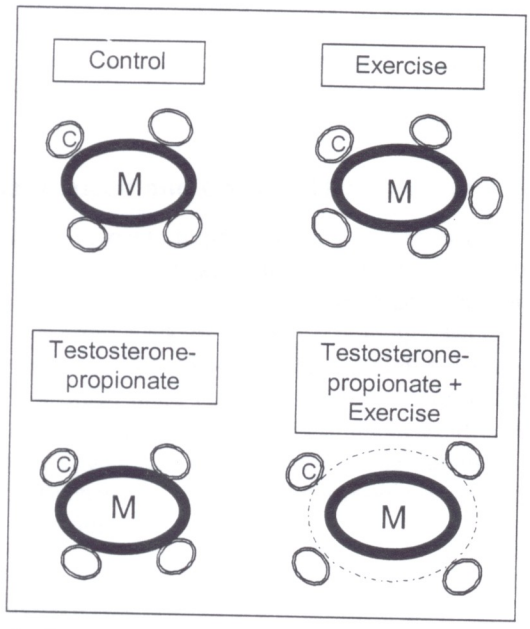

Figure 6—Summary of the main experimental findings; M: myocyte, C: capillary, *dashed line*: mild myocyte hypertrophy. Note the increased number of capillaries and the shorter distance between them in the exercised group; adaptations that are not present in the other regimens, especially in the testosterone-propionate + exercise one.

groups (Table 1), this increase is insufficient: it cannot counterbalance the mild myocyte hypertrophy (Table 2). The hypertrophic myocytes broaden the space between the capillaries and lead to a reduced capillary density (Figs. 2 and 3) as well as to a longer intercapillary distance (Figs. 4 and 5) in comparison with the exercise groups. This is one mechanism that could underlie the impaired capillarization.

The inhibition of angiogenesis (inhibition of new capillary formation) is a second mechanism that could have led to the inadequate adaptation of the capillaries in the testosterone-propionate+exercise groups. Androgens exert various degrees of angiogenesis inhibition under a lot of experimental conditions, e.g., in the chick embryo (testosterone) (15), rabbit cornea (5a-dihydrotestosterone) (64), breast tumor in rats (testosterone) (27), and rat mesentery (testosterone propionate) (47) as well as in cultured human microvascular endothelial cells (testosterone inhibits the formation of capillary-like tubes) (36). Due to the inhibition of neovascularization, which is a general characteristic of the steroids (corticosteroids have more pronounced angiostatic activity), these substances have been called "angiostatic steroids" (15).

Angiostatic steroids have a striking specificity against new capillaries, whereas mature microvessels remain unaffected (23,24). This specificity could underlie the reduced number of capillaries around a single myocyte in the testosterone propionate+exercise groups (in comparison with the exercise drug-free groups) of the present experiments. This could be the case in the rat skeletal muscles too: application of anabolic steroids+exercise training reduced the number of capillaries per number of fibers (56) as well as the number of capillaries around each fiber (18) as compared with the respective exercise groups. The trend toward decreased capillarization in the skeletal muscles of athletes (54) is attributed (56) to the anabolic steroids abuse as well.

Another type of muscle conditioning, namely electric stimulation, increased the capillary density in rabbit skeletal muscles (52). Application of nandrolone decanoate did not alter this response (52), whereas a reduction of the myocyte cross-sectional area was observed both in the electrically stimulated and in the electrically stimulated-steroid treated muscles (53). In this case, a direct counting of the number of capillaries around each myocyte can reveal whether the increased capillary density is due to capillary growth or (very probably) to the reduced myocyte area.

It can be concluded that androgens impair the capillary network. The microvascular impairment results in insufficient oxygen supply, e.g., to the cerebral cortex (42) and to the heart (49,57), whereas it can lead to tumor regression (8,22). Moreover, the decreased capillary density impairs the blood flow and increases the vulnerability of the heart to ischemia (12). The inadequate adaptation of the coronary capillaries could be one of the causes underlying the cardiovascular complications from which anabolic steroid abusers suffer (16,33,38,41,46,60). The mechanisms leading to a possible interaction between the myocyte hypertrophy, the microvascular impairment, and other factors, e.g., the adrenergic overstimulation (30) and the modulation of the macrophage activity (43), which have been reported after anabolic steroid treatment, remain to be elucidated.

Exercise Training Augments the Capillary Supply

In the exercised drug-free groups of the present experiments, an increased capillarization can be observed. This well-known adaptation facilitates the adequate oxygen transport to the tissue. It occurs both in the heart (48) and in the skeletal muscle (48,55). The improved capillary supply is due to: (a) neovascularization and/or (b) elongation or increased branching of the existing capillaries. The mechanisms underlying these microvascular responses are discussed in detail elsewhere (2,31,39,59). An interesting finding of this study is that exercise training induces a more pronounced improvement in the papillary muscle capillarization than in the ventricular wall. This could result from regional variations in the ventricular wall myocyte hypertrophy (5,13,62). Due to methodological difficulties (see Methods/Morphometry), a possible hypertrophy of the ventricular wall myocytes could not be quantified.

Anabolic Steroids, Exercise, and Cardiac Hypertrophy

The present experiments reveal a mild myocyte hypertrophy in the testosterone-propionate+exercise groups. In accordance with an earlier investigation (6), our ultrastructural study documents that this hypertrophy results from the increase of the myofibrillar components, but we could not confirm the cellular swelling that other authors observed (6,10). The present findings (mild myocyte hypertrophy) support other animal experiments: they showed increased heart weight after concomitant application of anabolic

steroids and exercise training (9,32). Some human studies report the incidence of cardiac hypertrophy in connection with anabolic steroid treatment (16,51,60) while other investigations did not reveal this adaptation (14,29,58).

Exercise-Induced and Cardiac Hypertrophy

The lack of myocyte hypertrophy in the exercised drug-free groups of the current experiments supports the results of other studies. Neither did they demonstrate an alteration of heart weight after 7 wk of intensive running (34). In the same experiments, 12 wk of exercise training led to an increase in cardiac mass (34). A different exercise mode, e.g., swimming, induced hypertrophy of the heart after 9 wk (25). Among other factors the exercise mode, intensity and duration influence the cardiac hypertrophic response to physical conditioning.

CONCLUSIONS

Testosterone-propionate impairs the adaptation of the coronary capillaries to exercise training (Fig. 6). This impairment could contribute to the cardiovascular complications reported among anabolic steroid abusers (16,33,38,41,46,60). Moreover, the compatibility of androgen treatment, for therapeutic (7,20,45,61,63) as well as experimental purposes (7,11), with muscular exercise remains to be elucidated: the cardiovascular consequences (inadequate capillary supply) of their concomitant application to human subjects have been never evaluated. The anabolic steroid-induced microvascular impairment is the fundamental aim, e.g., in the case of the tumor treatment (8,22), but it could lead to an imbalance between the myocardial oxygen supply and demand, especially during physical activity.

The authors would like to express their gratitude to Mr. C. Hofmann and the technical personnel of the Institute I of Anatomy, University of Cologne for their technical support, Mr. G. Marxen and Dr. H. Lüpsen from the Regional Computer Center of the University of Cologne for the statistical advice, and Mr. M. Spicka and Mr. J. Schiffer for the correction of the manuscript.

Address for correspondence: Christos V. M. Tagarakis, Institute I of Anatomy, University of Cologne, Joseph-Stelzmann-Str. 9, 50931 Cologne, Germany.

REFERENCES

1. Abu Shakra, S., M. S. Alhalabi, F. C. Nachtman, R. A. Schemidt, and W. S. Brusilow. Anabolic steroids induce injury and apoptosis of differentiated skeletal muscle. *J. Neurosci. Res.* 47:186–197, 1997.
2. Adair, T. H., W. J. Gay, and J. P. Montani. Growth regulation of the vascular system: evidence for a metabolic hypothesis. *Am. J. Physiol.* 259:R393–R404, 1990.
3. Adams, M. R., J. K. Williams, and J. R. Kaplan. Effects of androgens on coronary artery atherosclerosis and atherosclerosis-related impairment of vascular responsiveness. *Arterioscler. Thromb. Vasc. Biol.* 15:562–570, 1995.
4. American College of Sports Medicine. Policy statement of the American College of Sports Medicine on research with experimental animals. *Med. Sci. Sports Exerc.* 29:vi, 1997.
5. Anversa, P., R. Ricci, and G. Olivetti. Effects of exercise on the capillary vasculature of the rat heart. *Circulation* 75:I12–8, 1987.
6. Appell, H. J., B. Heller Umpfenbach, M. Feraudi, and H. Weicker. Ultrastructural and morphometric investigations on the effects of training and administration of anabolic steroids on the myocardium of guinea pigs. *Int. J. Sports Med.* 4:268–274, 1983.
7. Bagatell, C. J., and W. J. Bremner. Androgens in men: uses and abuses. *N. Engl. J. Med.* 334:707–714, 1996.
8. Barinaga, M. Designing therapies that target tumor blood vessels. *Science* 275:482–484, 1997.
9. Bauman, D. H., J. T. Richerson, and A. L. Britt. A comparison of body and organ weights, physiologic parameters, and pathologic changes in target organs of rats given combinations of exercise, anabolic hormone, and protein supplementation. *Am. J. Sports Med.* 16:397–402, 1988.
10. Behrendt, H., and H. Boffin. Myocardial cell lesions caused by an anabolic hormone. *Cell Tissue Res.* 181:423–426, 1977.
11. Bhasin, S., T. W. Storer, N. Berman, et al. The effects of supraphysiologic doses of testosterone on muscle size and strength in normal men. *N. Engl. J. Med.* 335:1–7, 1996.
12. Breisch, E. A., F. C. White, L. E. Nimmo, and C. M. Bloor. Cardiac vasculature and flow during pressure-overload hypertrophy. *Am. J. Physiol.* 251:H1031–7, 1986.
13. Brown, M. D., M. K. Davies, and O. Hudlicka. The effect of long-term bradycardia on heart microvascular supply and performance. *Cell Mol. Biol. Res.* 40:137–142, 1994.
14. Cheriex, E., F. Hartgens, T. Gordijn, K. Depuydt, H. A. Keizer, and H. Kuipers. Cardiac dimensions and function are not affected by eight weeks androgenic-anabolic steroid use. *Med. Sci. Sports Exerc.* 28:S35, 1996.
15. Crum, R., S. Szabo, and J. Folkman. A new class of steroids inhibits angiogenesis in the presence of heparin or a heparin fragment. *Science* 230:1375–1378, 1985.
16. De Piccoli, B., F. Giada, A. Benettin, F. Sartori, and E. Piccolo. Anabolic steroid use in body builders: an echocardiographic study of left ventricle morphology and function. *Int. J. Sports Med.* 12:408–412, 1991.
17. Dickerman, R. D., F. Schaller, I. Prather, and W. J. McConathy. Sudden cardiac death in a 20-year-old bodybuilder using anabolic steroids. *Cardiology* 86:172–173, 1995.
18. Dimauro, J., R. J. Balnave, and C. D. Shorey. Effects of anabolic steroids and high intensity exercise on rat skeletal muscle fibres and capillarization: a morphometric study. *Eur. J. Appl. Physiol.* 64:204–212, 1992.
19. Ferenchick, G. S., S. Hirokawa, E. F. Mammen, and K. A. Schwartz. Anabolic-androgenic steroid abuse in weight lifters: evidence for activation of the hemostatic system. *Am. J. Hematol.* 49:282–288, 1995.
20. Ferreira, I. M., I. T. Verreschi, L. E. Nery, et al. The influence of 6 months of oral anabolic steroids on body mass and respiratory muscles in undernourished COPD patients. *Chest* 114:19–28, 1998.
21. Ferrer, M., A. Encabo, J. Marin, and G. Balfagon. Chronic treatment with the anabolic steroid, nandrolone, inhibits vasodilator responses in rabbit aorta. *Eur. J. Pharmacol.* 252:233–241, 1994.
22. Folkman, J. Seminars in Medicine of the Beth Israel Hospital, Boston: clinical applications of research on angiogenesis. *N. Engl. J. Med.* 333:1757–1763, 1995.
23. Folkman, J., and D. E. Ingber. Angiostatic steroids: method of discovery and mechanism of action. *Ann. Surg.* 206:374–383, 1987.
24. Folkman, J., R. Langer, R. J. Linhardt, C. Haudenschild, and S. Taylor. Angiogenesis inhibition and tumor regression caused by heparin or a heparin fragment in the presence of cortisone. *Science* 221:719–725, 1983.
25. Frenzel, H., B. Schwartzkopff, W. Holtermann, H. G. Schnurch, A. Novi, and W. Hort. Regression of cardiac hypertrophy: morphometric and biochemical studies in rat heart after swimming training. *J. Mol. Cell. Cardiol.* 20:737–751, 1988.

26. Fritzsche, D., R. Krakor, G. Asmussen, et al. Anabolic steroids (metenolone) improve muscle performance and hemodynamic characteristics in cardiomyoplasty. *Ann. Thorac. Surg.* 59:961–969, 1995.
27. Fukuda, M., J. Maekawa, Y. Hosokawa, et al. Hormone-dependent changes of blood vessels in DMBA-induced rat mammary carcinoma and its regression studied by ^{3}H-thymidine autoradiography. *Basic Appl. Histochem.* 29:21–43, 1985.
28. Gundersen, H. J. Notes on the estimation of the numerical density of arbitrary profiles. *J. Microsc.* 111:219–223, 1977.
29. Hartgens, F., G. Rietjens, E. Cheriex, B. H. R. Wolffenbuttel, H. A. Keizer, and H. Kuipers. Effects of an anabolic steroid on cardiovascular risk factors and the left ventricle in bodybuilders. *Med. Sci. Sports Exerc.* 29:S292, 1997.
30. Hartmann, G., K. Addicks, M. Donike, and W. Schanzer. Testosterone application influences sympathetic activity of intracardiac nerves in non-trained and trained mice. *J. Auton. Nerv. Syst.* 17:85–100, 1986.
31. Hudlicka, O., and M. D. Brown. Postnatal growth of the heart and its blood vessels. *J. Vasc. Res.* 33:266–287, 1996.
32. Karhunen, M. K., M. P. Ramo, and R. Kettunen. Anabolic steroids alter the haemodynamic effects of endurance training and deconditioning in rats. *Acta Physiol. Scand.* 133:297–306, 1988.
33. Kennedy, M. C., and C. Lawrence. Anabolic steroid abuse and cardiac death. *Med. J. Aust.* 158:346–348, 1993.
34. Kiiskinen, A., and E. Heikkinen. Physical training and connective tissues in young mice-heart. *Eur. J. Appl. Physiol.* 35:167–171, 1976.
35. Lamberts, S. W., A. W. van den Beld, and A. J. van der Lely. The endocrinology of aging. *Sci.* 278:419–424, 1997.
36. Lansink, M., P. Koolwijk, H. van, V, and T. Kooistra. Effect of steroid hormones and retinoids on the formation of capillary-like tubular structures of human microvascular endothelial cells in fibrin matrices is related to urokinase expression. *Blood* 92:927–938, 1998.
37. Lenders, J. W., P. N. Demacker, J. A. Vos, et al. Deleterious effects of anabolic steroids on serum lipoproteins, blood pressure, and liver function in amateur body builders. *Int. J. Sports Med.* 9:19–23, 1988.
38. Luke, J. L., A. Farb, R. Virmani, and R. H. Sample. Sudden cardiac death during exercise in a weight lifter using anabolic androgenic steroids: pathological and toxicological findings. *J. Forensic Sci.* 35:1441–1447, 1990.
39. Mall, G., G. Zimmer, S. Baden, and T. Mattfeldt. Capillary neoformation in the rat heart: stereological studies on papillary muscles in hypertrophy and physiologic growth. *Basic Res. Cardiol.* 85:531–540, 1990.
40. Melchert, R. B., T. J. Herron, and A. A. Welder. The effect of anabolic-androgenic steroids on primary myocardial cell cultures. *Med. Sci. Sports Exerc.* 24:206–212, 1992.
41. Menkis, A. H., J. K. Daniel, F. N. McKenzie, R. J. Novick, W. J. Kostuk, and P. W. Pflugfelder. Cardiac transplantation after myocardial infarction in a 24-year-old bodybuilder using anabolic steroids. *Clin. J. Sport. Med.* 1:138–140, 1991.
42. Metzger, H., S. Heuber Metzger, A. Steinacker, and J. Struber. Staining PO_2 measurement sites in the rat brain cortex and quantitative morphometry of the surrounding capillaries. *Pflugers Arch.* 388:21–27, 1980.
43. Michna, H. The human macrophage system: activity and functional morphology. *Bibl. Anat.* 1–84, 1988.
44. Millar, A. P. Licit steroid use: hope for the future. *Br. J. Sports Med.* 28:79–83, 1994.
45. Muchmore, J. S., D. K. Cooper, Y. Ye, V. Schlegel, A. Pribil, and N. Zuhdi. Prevention of loss of vertebral bone density in heart transplant patients. *J. Heart Lung Transplant.* 11:959–963, 1992.
46. Nieminen, M. S., M. P. Ramo, M. Viitasalo, et al Serious cardiovascular side effects of large doses of anabolic steroids in weight lifters. *Eur. Heart J.* 17:1576–1583, 1996.
47. Norrby, K., A. Jakobsson, and J. Sorbo. On mast-cell-mediated angiogenesis in the rat mesenteric-window assay. *Agents Actions* 30:231–233, 1990.
48. Petren, T. The effect of exercise on the density of capillaries in the heart and in the skeletal muscle (Der Einfluss des Trainings auf die Häufigkeit der Capillaren in Herz- und Skeletmuskulatur). *Arbeitsphysiologie* 9:376–386, 1936.
49. Rakusan, K., M. F. Flanagan, T. Geva, J. Southern, and R. Van Praagh. Morphometry of human coronary capillaries during normal growth and the effect of age in left ventricular pressure-overload hypertrophy. *Circulation* 86:38–46, 1992.
50. Rakusan, K., B. Korecky, K. Sarkar, and Z. Turek. Merits and pitfalls in morphological assessment of cardiac growth. *Fed. Proc.* 45:2580–2584, 1986.
51. Sachtleben, T. R., K. E. Berg, B. A. Elias, J. P. Cheatham, G. L. Felix, and P. J. Hofschire. The effects of anabolic steroids on myocardial structure and cardiovascular fitness. *Med. Sci. Sports Exerc.* 25:1240–1245, 1993.
52. Salmons, S. Myotrophic effects of anabolic steroids. *Vet. Res. Commun.* 7:19–26, 1983.
53. Salmons, S. Myotrophic action of an anabolic steroid (nandrolone decanoate) in normal, but not in chronically stimulated, rabbit limb muscles. *Basic Appl. Myol.* 1:241–251, 1991.
54. Schantz, P. Capillary supply in heavy-resistance trained non-postural human skeletal muscle. *Acta Physiol. Scand.* 117:153–155, 1983.
55. Schön, F. A. Light and electron microscopic findings in the vastus lateralis muscle and their relation to physiological measures in normal subjects, sport students and endurance athletes (Licht- und elektronenmikroskopische Befunde am Musculus vastus lateralis und ihr Bezug zu physiologischen Messgrössen bei Normalpersonen, Sportstudenten und Ausdauertrainierten). Doctoral Dissertation. German Sport University of Cologne (Deutsche Sporthochschule Köln), Germany, 1978.
56. Soares, J. M., and J. A. Duarte. Effects of training and an anabolic steroid on murine red skeletal muscle. a stereological analysis. *Acta Anat. Basel.* 142:183–187, 1991.
57. Suzuki, H., Y. Takeyama, S. Koba, Y. Suwa, and T. Katagiri. Small vessel pathology and coronary hemodynamics in patients with microvascular angina. *Int. J. Cardiol.* 43:139–150, 1994.
58. Thompson, P. D., A. Sadaniantz, E. M. Cullinane, et al. Left ventricular function is not impaired in weight-lifters who use anabolic steroids. *J. Am. Coll. Cardiol.* 19:278–282, 1992.
59. Tomanek, R. J. Exercise-induced coronary angiogenesis: a review. *Med. Sci. Sports Exerc.* 26:1245–1251, 1994.
60. Urhausen, A., R. Holpes, and W. Kindermann. One- and two-dimensional echocardiography in bodybuilders using anabolic steroids. *Eur. J. Appl. Physiol.* 58:633–640, 1989.
61. Wagner, G., J. Rabkin, and R. Rabkin. Exercise as a mediator of psychological and nutritional effects of testosterone therapy in HIV+ men. *Med. Sci. Sports Exerc.* 30:811–817, 1998.
62. White, F. C., G. Witzel, E. A. Breisch, C. M. Bloor, and L. E. Nimmo. Regional capillary and myocyte distribution in normal and exercise trained male and female rat hearts. *Am. J. Cardiovasc. Pathol.* 2:247–253, 1988.
63. World Health Organization. Task Force on methods for the regulation of male fertility: contraceptive efficacy of testosterone-induced azoospermia in normal men. *Lancet* 336:955–959, 1990.
64. Yamamoto, T., N. Terada, Y. Nishizawa, and V. Petrow. Angiostatic activities of medroxyprogesterone acetate and its analogues. *Int. J. Cancer* 56:393–399, 1994.
65. Yesalis, C. E., and M. S. Bahrke. Anabolic-androgenic steroids: current issues. *Sports Med.* 19:326–340, 1995.