Cardiovascular Topics

Proposed mechanisms for the anabolic steroid-induced increase in myocardial susceptibility to ischaemia/reperfusion injury

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Summary

Androgenic anabolic steroids (AAS) are often used by athletes to enhance athletic performance but are strongly associated with detrimental cardiovascular effects including sudden cardiac death.

Hypothesis: **AAS use increases myocardial susceptibility to ischaemia/reperfusion injury.**

Methods: **Rats were trained (swimming) with or without intramuscular injection of nandrolone laurate (0.375 mg/kg). Untrained rats with or without nandrolone served as controls. Hearts were mounted on the Langendorff perfusion apparatus and mechanical function was measured before and after 20-min normothermic global ischaemia. Myocardial tissue samples were collected for determination of tissue cyclic nucleotide and TNFα concentrations.**

Results: **Anabolic steroids decreased the rate pressure product (RPP) of the exercise-trained rat heart [34 582 ± 1 778 mmHg/min vs 28 868 ± 2 446 mmHg/min for exercise-trained steroid-treated hearts ($p < 0.05$)]. Reperfusion RPP was lower in both the sedentary, and the exercise-trained, steroid-treated hearts than in their concurrent vehicle-treated controls (18 276 ± 2 026 mmHg/min vs 12 018 ± 1 725 mmHg/min for sedentary steroid-treated hearts and, 21 892 ± 2 912 mmHg vs 12 838 ± 1 536 mmHg/min for exercise-trained steroid-treated hearts). Myocardial TNFα [267.75 ± 44.25 pg/g vs 190.00 ± 15.75 pg/g ($p < 0.05$)] and cAMP concentrations [406.04 ± 18.41 pmol/g vs 235.6 ± 43.26 pmol/g ($p < 0.05$)] were elevated in the steroid-treated hearts when compared with their untreated counterparts.**

Conclusions: **Supraphysiological doses of anabolic steroids, whether taken during exercise training or under sedentary conditions increase myocardial susceptibility to ischaemia/reperfusion injury in our model. This increased susceptibility may be related to steroid-induced increases in the pre-ischaemic myocardial cAMP concentrations and/or increases in both pre-ischaemic and reperfusion TNFα concentrations.**

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Non-therapeutic use of androgenic anabolic steroids is prevalent among young, competitive male and female high school athletes who hope to improve their exercise performance with these compounds.[1] They are administered in supraphysiological doses to enhance the development of muscle mass and strength and to reduce the recovery time after strenuous training bouts.[2,3] These doses are however associated with pathologic changes in numerous physiological systems. Studies using rats have shown that supraphysiological doses of anabolic steroids cause pathophysiological myocardial hypertrophy in this model.[3] In the mouse it has been associated with inadequate vascularisation of the hypertrophied myocardium,[4] and in isolated rat ventricular myocytes it has been linked to increased apoptosis.[5] When combined with exercise, anabolic steroid use has been shown to change exercise-induced physiological cardiac hypertrophy to pathophysiological cardiac hypertrophy.[3] In this study using the exercising rat, the anabolic steroid-induced changes in myocardial hypertrophy were associated with changes in the ratio of the left ventricular wall thickness to internal radius. These changes are thought to lead to detrimental increases in LV wall stress and to act as one of the stimuli for abnormal heart growth and development.

The exact mediators of myocardial hypertrophy are diverse and vary from mechanical stimuli to circulating humoral factors released by the heart and peripheral organs. Exercise-induced cardiac hypertrophy is thought to be due

to increases in the pre-load (diastolic filling) on the heart,[6] while the exact mechanisms for anabolic steroid-induced myocardial hypertrophy are at present unknown. Recent studies have shown that circulating cytokines such as TNFα may play a role in cardiac remodeling and that anabolic steroids strongly stimulate leukocyte TNFα production.[7,8] The question of whether a link exists between anabolic steroid use, serum and myocardial TNFα concentrations and myocardial hypertrophy remains to be established.

Exercise training in rats has been shown to improve myocardial resistance to ischaemia/reperfusion injury.[9-11] In addition, exercise-induced physiological cardiac hypertrophy alters the heart's susceptibility to ischaemia and reperfusion and renders it more resistant to ischaemia/reperfusion injury in *in vivo* rat hearts.[12] These changes were associated with an increased energy charge and decreased lipid peroxidation during ischaemia and reperfusion in the hearts from these animals.[12] What, however, remains unclear is whether anabolic steroid-induced hypertrophy alters the susceptibility of the heart to ischaemia/reperfusion injury. In addition, should these hearts be more susceptible to ischaemia/reperfusion injury, the mechanism/s that contribute to this phenomenon need to be elucidated.

TNFα has been implicated in ischaemia/reperfusion injury. Mice devoid of myocardial TNFα (knockouts) have been shown to be more resistant to ischaemia/reperfusion injury than their wild-type counterparts and treatment of rat hearts with anti-murine TNFα antibody before ischaemia also improved reperfusion function of these hearts.[13,14]

Besides cytokines, elevations in cytosolic cAMP concentrations during ischaemia would be expected to increase cytosolic calcium concentrations and exacerbate ischaemia/reperfusion injury in this model of ischaemia and reperfusion.[15] Interestingly, the basal myocardial cAMP concentrations are elevated by anabolic androgenic steroids in isolated rat atrial muscle and contribute to the positive inotropic response observed with steroid treatment.[16] These recent findings suggest that anabolic steroids could also potentially promote calcium overload during ischaemia in the anabolic steroid-treated heart. The final question remains whether chronic anabolic steroid treatment alters ventricular myocardial cAMP concentrations, and if so, what the effects of this alteration are on susceptibility to ischaemia/reperfusion injury. Several studies have also suggested that there is a correlation between myocardial cAMP and cGMP concentrations[17] and that the ratio between these cyclic nucleotides during ischaemia may be important in determining the severity of ischaemia/reperfusion injury.[18] The effects of anabolic steroids on NO-cGMP pathway activity, as assessed by measuring myocardial cGMP concentrations, also remain unknown.

In this study we set out to determine how anabolic steroids in the absence of, or together with an exercise training programme, would influence: (1) heart size and mechanical function, (2) myocardial susceptibility to ischaemia/reperfusion injury, and (3) myocardial TNFα, cAMP and cGMP concentrations. To determine the effects of chronic androgenic anabolic steroid administration on the heart, we used the chronically treated rat and the isolated rat heart perfusion system.

Methods

Male Long–Evans rats weighing 80–100 grams were weaned and used in this study. Rats had free access to standard laboratory chow and water and were housed at 22°C and 40% humidity with a 12-hour day–night cycle. The project was approved by the ethics committee of the University of Stellenbosch and conforms with the 'Guide for the care and use of laboratory animals' of the National Academy of Sciences (NIH publication no 80-23, revised 1978).

Forty 12-week-old rats were randomly assigned to four experimental groups; (1) a sedentary control group, (2) a sedentary steroid-treated group, (3) an exercising control group, and, (4) an exercising steroid-treated group. These rats were used to document the effects of anabolic steroids on heart size and function. An additional group of 50 rats was randomly divided into two groups, for the collection of myocardial tissue samples. One group served as the control, and the other as an anabolic steroid-treated group. Myocardial tissue samples were collected at the end of the pre-ischaemic perfusion period, 10 and 20 min into ischaemia, and at the end of reperfusion. These samples were used to determine myocardial TNFα and cyclic nucleotide concentrations.

Training programme used during study

Rats were subjected to swimming training in baths kept at 30–32°C. The training programme consisted of six swimming sessions per week for six weeks and would be considered endurance-type exercise. Rats were started off swimming 5 min/day for the first week, then the duration of swimming sessions was increased by 10-min increments each week. By the sixth week of the training programme, rats swam 55 min/day, for six days per week.

Anabolic steroid administration

Nandrolone laurate (Laurabolin 25, Intervet, SA) was diluted in sterile peanut butter oil and injected intramuscularly at a dose of 0.375 mg/kg body weight into the hind limb as previously described.[19] Intramuscular injections were repeated once weekly for the six weeks of training and the dosage was chosen as it had been shown to induce cardiac growth in rats in a previous study.[19] The volume of peanut butter oil and steroid injected varied between 10 and 50 µl depending on rat weight. Control animals received the same volume of peanut butter oil intramuscularly.

Isolation of hearts and perfusion on the Langendorff perfusion apparatus

Rats were anaesthetised by intraperitoneal injection of pentobarbital (30 mg/rat). The hearts were excised and placed in ice-cold Krebs-Henseleit buffer before being mounted on a Langendorff perfusion apparatus for perfusion at 37°C with Krebs-Henseleit buffer at constant pressure (100 cmH_2O). The buffer was equilibrated with 95% CO_2/5% O_2 and had the following composition (mM) NaCl 118.0, KCl 4.7, $MgSO_4$ 1.2, $CaCl_2$ 1.25, $NaHCO_3$ 25, KH_2PO_4 1.2, and glucose 10.0. Retrograde perfusion was resumed within 45 s of excision of the heart. A 'cling film' balloon

was inserted into the left ventricle and attached to a pressure transducer and PowerLab data recording system (AD Instruments, Australia).[20] Cardiac parameters were recorded continuously and included heart rate, left ventricular developed pressure and coronary flow rates. Heart temperature was monitored throughout the experiment using an electronic temperature probe.

Perfusion protocol

Hearts were equilibrated and baseline haemodynamic parameters [heart rate (HR), left ventricular developed pressure (LVDevP) and coronary flow rates (CF)] were determined (for 30 min). Hearts were subjected to 20 min total global ischaemia at 37°C. Hearts were reperfused with 1% lignocaine hydrochloride monohydrate for the first two min to reduce the incidence of reperfusion ventricular fibrillation. For the remaining 28 min of reperfusion a standard Krebs-Henseleit buffer was used. Heart function was again determined during reperfusion.

Biochemical analysis

For biochemical determinations, two groups of hearts (control and anabolic steroid-treated hearts) were freeze-clamped: (1) immediately before ischaemia, (2) 10 min after the onset of ischaemia, (3) at the end of ischaemia and, (4) at 30-min reperfusion. Samples were stored in liquid nitrogen until assays were performed.

Cyclic nucleotide determinations

The cAMP and cGMP concentrations were determined using radio-immunoassay kits obtained from Amersham, UK. For cGMP assays, freeze-clamped hearts were freeze-dried and 10–15 mg of dry tissue was extracted in 5% TCA. The extracted sample was ether-washed three times for 5-min wash cycles. These samples were acetylated and then diluted 1:8 for the ^{125}I cGMP assay. The IC_{50} for the cGMP assay was 20–25 fmol/tube.[21] For the cAMP assays, 10–15 mg freeze-dried samples were extracted with PCA, neutralised with KOH and assayed with the appropriate RIA assay. The IC_{50} for this assay was 1.91 pmol/tube.[21]

Myocardial TNFa determinations

Myocardial TNFα content was determined using the enzyme-linked immunosorbent assay (OptEIA™ rat TNFα ELISA kit, PharMingen, USA). Lysates were prepared according to Meldrum and co-workers and 100 µl of sample (containing equal amounts of protein) was used in each well of a 96-well plate.[22] The lower detection limit for the assay is 13 pg/gram ww.

Calculations and statistical methods

Results were expressed as mean and SEM. Recovery of function: rate pressure product (RPP) during reperfusion is expressed as a percentage of its pre-ischaemic value.

RPP = left ventricular developed pressure (LVDevP) × heart rate (HR).

$$\% \text{ RPP recovery} = \frac{\text{Reperfusion RPP (10, 20, 30 min)}}{\text{Pre-ischaemic RPP (30 min)}} \times 100$$

The unpaired Student's *t*-test was used to determine statistical significance in the biochemical and biological data where only two groups of data were compared. For multiple comparisons, where four study groups were compared with each other, the ANOVA followed by the Bonferroni correction for multiple comparisons were applied. A value of $p < 0.05$ was considered significant.

Results

Effects of exercise training and/or anabolic steroids on body and heart weight

There were no differences in body weight when comparing rats from the four groups before and after the training programme and/or anabolic steroid administration for six weeks. Rat weights after the training programme were 310.3 ± 10.8 g for sedentary control rats, 298.3 ± 8.6 g for sedentary steroid-treated rats, 300.3 ± 13.2 g for exercising control rats and 294.1 ± 16.3 g for exercising steroid-treated rats. Heart weight-to-body weight ratios were different when comparing the sedentary steroid- and the exercising steroid-treated groups [4.00 ± 0.19 mg/g and 4.68 ± 0.13 mg/g, respectively ($p < 0.05$)] (Fig. 1).

Myocardial haemodynamic function

Pre-ischaemic coronary flow rate, heart rate and left ventricular developed pressure

Exercise training decreased resting heart rate compared with sedentary controls (241 ± 12 bpm and 282 ± 9 bpm respectively, $p < 0.05$). This exercise-induced bradycardia was lost when the rats were on chronic anabolic steroid treatment. Pre-ischaemic coronary flow rates and left ventricular developed pressures were unchanged by exercise and/or the administration of anabolic steroids in these animals (Table I).

Reperfusion coronary flow rates, heart rate and left ventricular developed pressure

Exercise improved reperfusion left ventricular developed pressure at 20- and 30-min reperfusion when compared with

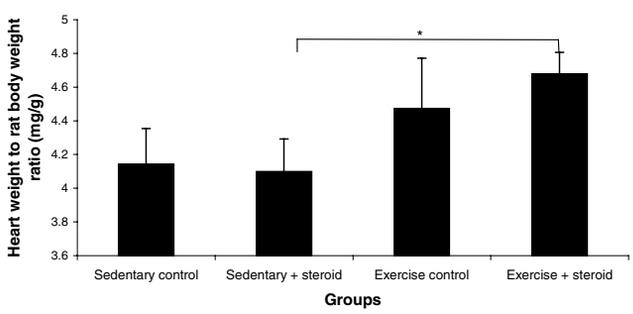

Fig. 1. Heart weight-to-body weight ratio for the sedentary control rats, the sedentary steroid-treated rats, the exercising control rats and the exercise-trained steroid-treated rats.
Sedentary control = sedentary vehicle-treated hearts; sedentary + steroid = sedentary steroid-treated hearts; exercise control = exercise-trained vehicle-treated hearts; exercise + steroid = exercise-trained steroid-treated hearts.
***$p < 0.05$; n = 8–10.**

TABLE I. CORONARY FLOW (CF), HEART RATE (HR) AND LEFT VENTRICULAR DEVELOPED PRESSURE (LVDevP) FOR SEDENTARY VEHICLE-TREATED HEARTS, SEDENTARY STEROID-TREATED HEARTS, EXERCISE-TRAINED, VEHICLE-TREATED HEARTS AND EXERCISE-TRAINED STEROID-TREATED HEARTS.

Groups	*Functional parameters*	*Pre-ischaemia*	*Reperfusion*	
		30 min	*20 min*	*30 min*
Sedentary control (n = 8)	CF (ml/min)	16.5 ± 0.5	15.7 ± 0.8	14.8 ± 1.1
	HR (beats/min)	282 ± 10	255 ± 17	259 ± 20
	LVDevP (mmHg)	113.7 ± 6.1	62.8 ± 4.3	70.4 ± 5.2
Sedentary + steroid (n = 9)	CF (ml/min)	14.2 ± 0.7	12.9 ± 0.8	11.6 ± 0.6
	HR (beats/min)	278 ± 14	240 ± 25	216 ± 18
	LVDevP (mmHg)	111.1 ± 4.2	52.2 ± 8.8	55.9 ± 6.4
Exercise control (n = 10)	CF (ml/min)	17.9 ± 0.6	16.8 ± 1.5	16.6 ± 1.8
	HR (beats/min)	241 ± 12#	239 ± 16	233 ± 20
	LVDevP (mmHg)	128.8 ± 5.9	89.6 ± 9.3#	91.3 ± 8.9**
Exercise + steroid (n = 9)	CF (ml/min)	13.9 ± 0.7	13.4 ± 1.2	12.0 ± 1.4
	HR (beats/min)	247 ± 21	215 ± 26	220 ± 19
	LVDevP (mmHg)	115.1 ± 4.1	57.7 ± 9.4*	58.9 ± 5.0*

Sedentary control = sedentary vehicle-treated hearts
Sedentary + steroid = sedentary steroid-treated hearts
Exercise control = exercise-trained vehicle-treated hearts
Exercise + steroids = exercise-trained steroid-treated hearts
#$p < 0.05$ vs sedentary control; *$p < 0.05$ vs exercise control; **$p < 0.05$ vs sedentary control.

the hearts from sedentary animals (89.57 ± 9.28 mmHg vs 62.75 ± 4.27 mmHg and 91.29 ± 8.94 mmHg vs 70.37 ± 5.21 mmHg respectively, $p < 0.05$). The use of anabolic steroids during the exercise training programme decreased left ventricular developed pressure at 20- and 30-min reperfusion when compared with the exercise-trained control animals (57.67 ± 9.36 mmHg vs 89.57 ± 9.28 mmHg, and 58.89 ± 5.00 mmHg vs 91.29 ± 8.94 mmHg, $p < 0.05$).

Rate pressure product

Pre-ischaemic rate pressure product values were similar for sedentary animals and their anabolic steroid-treated controls. The reperfusion rate pressure product values for these two groups of hearts were, however, different (18 276 ± 2 026 mmHg/min vs 12 018 ± 1 725 mmHg/min after 30-min reperfusion, $p < 0.05$)

Rate pressure product values were higher in the exercise-trained hearts than in the exercise-trained, steroid-treated hearts both before ischaemia and during reperfusion. Pre-ischaemic rate pressure product values were 34 582 ± 1 778 mmHg/min vs 28 868 ± 2 446 mmHg/min ($p < 0.05$) at 20-min perfusion and 34 582 ± 1 778 mmHg/min vs 28 868 ± 2 446 mmHg/min ($p < 0.05$) at 30-min perfusion. This difference persisted at 10-min (22 196 ± 2 904 mmHg/min vs 10 128 ± 1 447 mmHg/min), 20-min (21 753 ± 2 917 mmHg/min vs 11 275 ± 1 941 mmHg/min) and 30-min [21 892 ± 2 912 mmHg/min vs 12 838 ± 1 536 mmHg/min ($p < 0.05$)] reperfusion (Fig. 2).

Percentage rate pressure product recoveries after 20-min global ischaemia (at 10-, 20- and 30-min reperfusion) were

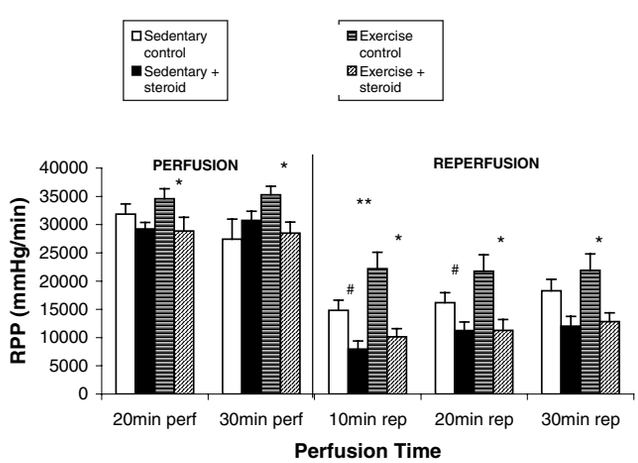

Fig. 2. Rate pressure product before and after ischaemia for the sedentary control hearts, the sedentary steroid-treated hearts, the exercise-trained control hearts and the exercise-trained steroid-treated hearts.
rep = reperfusion; n = 8–10;
***$p < 0.05$ exercise control vs exercise + steroid**
#$p < 0.05$ sedentary control vs sedentary steroid
****$p < 0.05$ sedentary control vs exercise control.**

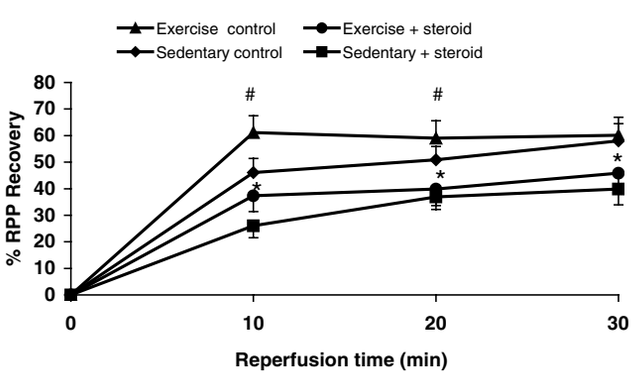

Fig. 3. Percentage rate pressure product recovery for sedentary control hearts, sedentary steroid-treated hearts, exercise-trained vehicle-treated hearts and exercise-trained steroid-treated hearts.
***n* = 8–10;**
***$p < 0.05$ sedentary control vs sedentary + steroid**
#$p < 0.05$ exercise control vs exercise + steroid.

TABLE II. MYOCARDIAL cAMP AND cGMP CONCENTRATIONS (PMOL/G WW) BEFORE, DURING AND AFTER ISCHAEMIA FOR HEARTS FROM SEDENTARY CONTROL RATS AND SEDENTARY ANABOLIC STEROID-TREATED RATS.

		Pre-ischaemia	*10-min ischaemia*	*20-min ischaemia*	*30-min reperfusion*
Sedentary control	cAMP	235.60 ± 43.26	343.12 ± 17.04	339.32 ± 50.61	235.83 ± 36.03
	cGMP	7.38 ± 0.28	9.98 ± 2.47	20.55 ± 3.03	4.49 ± 0.33
Sedentary + steroid	cAMP	406.04 ± 18.41*	289.53 ± 44.18	300.36 ± 17.66	193.66 ± 29.38
	cGMP	9.16 ± 0.81*	20.81 ± 3.46*	11.63 ± 2.81*	8.14 ± 2.24

*$p < 0.05$ vs sedentary + steroid group; $n = 4–6$.

higher for the hearts from sedentary animals than those for sedentary animals on the anabolic steroids (46.01 ± 5.37% vs 26.09 ± 4.56%, 50.90 ± 5.04% vs 36.93 ± 4.79% and 58.01 ± 6.47% vs 39.89 ± 5.94%, $p < 0.05$). Similarly, rate pressure product recoveries at 10-, 20- and 30-min reperfusion were higher in exercise-trained rats than in the exercise-trained, steroid-treated hearts (61.12 ± 6.39% vs 37.32 ± 5.96%, 59.04 ± 6.56% vs 39.87 ± 6.22% and respectively, $p < 0.05$) (Fig. 3).

Myocardial cyclic nucleotide and cytokine concentrations

Tissue cGMP concentrations: Myocardial cGMP concentrations were elevated in the normoxic steroid-treated sedentary hearts when compared with their untreated counterparts (9.16 ± 0.82 pmol/g vs 7.38 ± 0.28 pmol/g, $p < 0.05$). Myocardial cGMP concentrations were also elevated in these steroid-treated hearts by 10-min ischaemia but returned to basal concentrations by 20-min ischaemia (20.81 ± 3.46 pmol/g and 11.63 ± 2.81 pmol/g respectively). There was a delay in the elevation in cGMP concentrations in the vehicle-treated hearts. After 10-min ischaemia, cGMP concentrations were 9.98 ± 2.48 pmol/g and after 20-min ischaemia they were 20.55 ± 3.03 pmol/g. During reperfusion, myocardial cGMP concentrations returned to basal concentrations by 30-min reperfusion in both groups (Table II).
Tissue cAMP concentrations: cAMP concentrations were significantly elevated under normoxic conditions (before ischaemia) in the steroid-treated animals when compared with the vehicle-treated ones (406.04 ± 18.41 pmol/g vs 235.60 ± 43.26 pmol/g, $p < 0.05$). cAMP concentrations were similar in both groups during ischaemia and reperfusion.
Myocardial TNFα concentrations: Myocardial TNFα concentrations were elevated in the normoxic hearts from steroid-treated sedentary animals, compared with their control (vehicle-treated) counterparts (267.75 ± 44.25 pg/g vs 190.00 ± 15.75 pg/g, $p < 0.05$). During ischaemia there were no differences in myocardial TNFα concentrations, but during reperfusion, the TNFα concentrations were again elevated in the hearts of the steroid-treated sedentary animals (511.13 ± 13.37 pg/g vs 287.63 ± 59.95 pg/g for controls, $p < 0.05$) (Fig. 4).

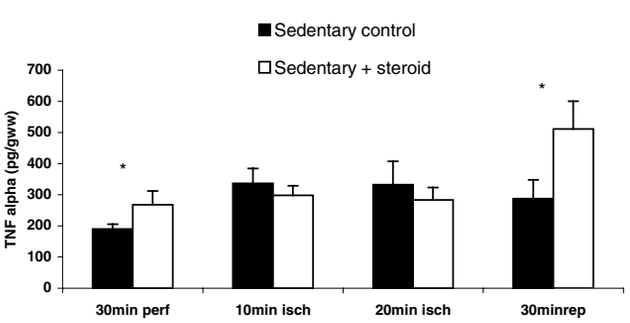

Fig. 4. Myocardial TNFα concentrations for the sedentary control rats and the sedentary steroid-treated rats.
***n* = 4–6**
***p < 0.05**

Discussion

The aim of the study was to determine whether anabolic steroid-induced pathophysiological cardiac hypertrophy (as opposed to exercise-induced physiological hypertrophy) is associated with increased myocardial susceptibility to ischaemia/reperfusion injury. We also wished to identify potential mechanisms for: (1) the anabolic steroid-induced cardiac hypertrophy and, (2) the differences in susceptibility of the steroid-treated hearts to ischaemia/reperfusion injury. We found that chronic use of supra-physiological doses of anabolic steroids: (1) decreases mechanical function of the heart when used in conjunction with an exercise training programme and, (2) decreases post-ischaemic mechanical function of the normal sedentary and exercise-trained heart. We also found that the chronic use of anabolic steroids increases myocardial cAMP and TNFα concentrations in the normoxic heart under basal conditions.

Although there is mounting evidence to implicate anabolic steroids in the development of cardiovascular abnormalities that could result in death, most evidence is indirect and is based on a limited number of clinical case studies.[23-25] Very little is known about the mechanisms for anabolic steroid-induced pathophysiological myocardial hypertrophy seen in athletes. Also, little is known about the effects of chronic anabolic steroid use on the susceptibility of the heart to ischaemia/reperfusion injury, or which changes in these hearts contribute to the changes in the susceptibility of the heart to ischaemia/reperfusion injury.

Exercise training and steroids: effects on function and morphology in the heart

The effects of chronic anabolic steroid use on the exercise-trained and the sedentary heart are not well understood. Although there is evidence to suggest that TNFα may con-

tribute to myocardial remodeling,[7] and we did see increases in myocardial TNFα concentrations in the normoxic steroid-treated hearts, we have no direct evidence to suggest that these elevations played a causative role in the myocardial hypertrophy in our study.

Chronic anabolic steroid use suppresses basal mechanical function and contractile reserve capacity of the normal untrained heart.[20-22] In contrast with these adverse effects, exercise training is known to increase the contractile reserve capacity of the heart.[29,30] The unresolved question is what the combination of exercise and anabolic steroids does to the function of the heart. Some studies suggest that anabolic steroids compromise both the contractile reserve capacity and the basal work capacity of the exercise-trained heart.[27,31]

The exercise-trained rat hearts in our study had basal RPP values similar to those of their untrained counterparts (Fig. 2). The contractile reserve capacity of the hearts was, however, not tested in this study. In our study, we chose not to challenge the hearts with positive inotropes before ischaemia, as these interventions may also affect the severity of ischaemia/reperfusion injury in these hearts. We did not find any differences between the basal pre-ischaemic function of the hearts from sedentary animals and their sedentary steroid-treated counterparts. This lack of effect of anabolic steroids in sedentary animals may be related to the dose of the steroid we used. In the studies where mechanical function of the hearts was suppressed, the doses were 100-fold[26] and 50-fold[28] higher. We did, however, find that the hearts from animals that were on the training programme developed higher RPP than their anabolic steroid-treated counterparts. These data support the findings of Liang and co-workers who found that anabolic steroids only attenuated mechanical function of hearts subjected to an exercise training programme.[31]

LeGros and co-workers proposed that the anabolic steroid-induced decrease in myocardial function observed in their study on untrained rats may be attributed to structural changes, possibly in collagen cross-link formation.[28] They proposed that this could lead to a decreased left ventricular compliance and consequent decreased stroke volume in these animals.

The exact myocardial cellular changes that occur when combining exercise training with anabolic steroid use is not well defined. Besides possible changes in collagen cross-link formation,[28] anabolic steroids evidently change collagen synthesis and distribution in the left ventricle.[32] It has also been shown that anabolic steroids may prevent the exercise-induced increase in LV wall thickness to internal diameter ratio.[3] This change may cause an increase in the LV wall stress in the anabolic steroid-treated heart and contribute to the decreased cardiac performance seen in these hypertrophied hearts.[31] We found that in our model the use of anabolic steroids combined with exercise training decreased mechanical function of the normoxic heart (Fig. 2). These data support the work reported by Liang and co-workers that showed that hearts from rats trained on a treadmill and treated with nandrolone decanoate performed poorly when compared with their trained, vehicle-treated counterparts.[31]

Exercise training, anabolic steroids and myocardial susceptibility to ischaemia/reperfusion injury

The reduced susceptibility of the exercise-induced hypertrophied heart to ischaemia/reperfusion injury is well documented.[9,10,12] In our study, exercise-trained rats were also more resistant to ischaemia/reperfusion injury, as reflected by the better absolute RPP values (Fig. 2) and RPP percentage recoveries (Fig. 3) seen in these hearts.

The effects of anabolic steroid treatment combined with exercise training on the susceptibility of the heart to ischaemia and reperfusion injury is, however, unresolved. Our data suggest that the chronic use of supra-physiological doses of anabolic steroids, whether used in sedentary animals or in animals subjected to an exercise training programme increases the susceptibility of the heart to ischaemia/reperfusion injury (Fig. 3). These hearts had poorer reperfusion mechanical function when compared with hearts from untreated animals (Figs 2, 3). Clinical case studies abound that implicate anabolic steroid use in myocardial infarction and sudden cardiac death (SCD).[33-36] What remains unclear is whether these increases in the incidence of SCD are directly related to the effects of steroids on the myocardium, or are due to the vascular effects of these steroids.

Chronic anabolic steroid use has been shown in primates to alter serum lipid profiles by decreasing HDL-C.[37] These changes would increase the animal's risk for coronary heart disease. Although we did not investigate the effects of the anabolic steroids on vascular morphology in our rats, we found that the coronary flow rates of all hearts perfused on the perfusion apparatus were similar (Table II). These data would suggest that the function and morphology of the coronary vasculature in all our rats was similar and would therefore not contribute to the differences in function and post-ischaemic recovery observed in this study.

Effects of anabolic steroids on myocardial cyclic nucleotide and cytokine concentrations

We wished to determine whether anabolic steroid use altered myocardial cyclic nucleotide concentrations and whether there was a change in myocardial cytokine and stress-activated protein kinase activity associated with anabolic steroid use.

A recent study has shown that chronic anabolic steroid treatment attenuates beta-adrenoceptor-mediated contractile response in the rat heart.[38] Also, Velasco and co-workers found that the positive inotropic response induced by androgens in the isolated rat atrium was associated with elevated tissue cAMP concentrations.[16] We found that chronic anabolic steroid use caused an elevation in basal cAMP concentrations in the normoxic heart before ischaemia. This chronic increase in the cAMP concentrations was, however, not associated with an increase in mechanical function of these anabolic steroid-treated hearts (see Fig. 2). Contrary to expectations, the anabolic steroid-treated hearts performed poorly when compared with their concurrent untreated counterparts, despite the approximately 73% increase in cAMP concentrations seen in these hearts. Anabolic steroid-treated hearts are thought to have structural abnormali-

ties such as increased or abnormal collagen distribution. Indications are that these abnormalities adversely affect systolic and diastolic function of the heart. The possibility exists that the elevated beta-adrenoceptor–cAMP pathway activity seen in these steroid-treated hearts is simply not adequate to overcome the restrictions placed on the heart by the abnormal collagen distribution and structure.

Increases in the concentrations of myocardial cAMP would in turn be expected to increase cytosolic calcium concentrations in these hearts. We and others have shown that elevated cytosolic calcium concentrations during ischaemia and early reperfusion increase the severity of ischaemia/reperfusion injury in the rat heart.[15,39,40] The elevated cAMP concentrations in these anabolic steroid-treated hearts could potentially exacerbate myocardial calcium overload during early ischaemia and worsen ischaemia/reperfusion injury. Although myocardial cAMP concentrations were similar in the steroid-treated and untreated hearts during ischaemia, the elevated cAMP (and presumably calcium) concentrations in the steroid-treated hearts before ischaemia may set the stage for more severe calcium overload during ischaemia in these hearts.

Myocardial cGMP concentrations were moderately elevated in the normoxic steroid-treated hearts but rose sharply early in ischaemia when compared with untreated animals. These small differences in cGMP concentrations (relative to the cAMP changes) before and during ischaemia appear to have had little or no effect on the severity of ischaemia/reperfusion injury.

The anabolic steroid-induced elevation in myocardial cyclic nucleotide concentrations could be due to stimulatory effects on myocardial guanylyl and adenyl cyclase or to suppressor effects on the myocardial phosphodiesterases responsible for the breakdown of these cyclic nucleotides. The exact mechanism responsible for this effect of anabolic steroids on the cyclic nucleotide concentrations is currently unknown. Similarly, no information is available on the cross-talk between signaling pathways under these conditions.

Several studies have shown that elevated myocardial TNFα concentrations may increase the severity of ischaemia/reperfusion injury. Gurevitch and co-workers have shown that treating the rat heart with anti-TNFα antibodies before ischaemia, decreases the severity of ischaemic injury and improves functional recovery of the heart.[14] In similar studies, it has been shown that TNFα knockout mice are less susceptible to ischaemia/reperfusion injury than their wild-type counterparts.[13] These data suggest that the elevated cytokine concentrations during ischaemia may contribute to ischaemia/reperfusion injury. In our study, TNFα concentrations were elevated in the non-ischaemic steroid-treated hearts. There were, however, no differences in TNFα concentrations when comparing the treated and untreated groups during ischaemia. Our data would suggest that TNFα played no role in determining the severity of ischaemic injury in our model. TNFα concentrations were again elevated during reperfusion in the anabolic steroid-treated animals and it remains to be seen whether this elevation in TNFα during reperfusion contributed to the decreased reperfusion mechanical function seen in these steroid-treated hearts.

Limitations of study

Although we studied mechanical function before and after ischaemia in both the trained and untrained rat treated with vehicle or anabolic steroids, we only investigated the biochemical effects of anabolic steroids on the hearts of sedentary animals. However, the data clearly indicate that it would be worthwhile for future studies to be performed to determine the biochemical effects of anabolic steroids in exercise-trained hearts.

The exercise training routine possibly stressed the animals and could have lead to neurohumoral responses that may have influenced the data. We, however, believe that whether we had used treadmill running or swimming for the exercise training programme, the animals would have been subjected to some degree of stress and anxiety. This is a factor that may influence the physiological response of the animals to anabolic steroid administration but is unavoidable in this type of study.

Conclusions

- Chronic use of supraphysiological concentrations of anabolic steroids, whether taken during an exercise training programme or under sedentary conditions, increases myocardial susceptibility to ischaemia/reperfusion injury in our model.
- This increase in susceptibility of the steroid-treated heart to ischaemia/reperfusion injury may be related to anabolic steroid-induced increases in pre-ischaemic cAMP and/or pre-ischaemic and reperfusion TNFα concentrations in these hearts.

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