CARDIAC EFFECTS OF HIGH DOSE ANDROGENIC STEROID ADMINISTRATION

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ABSTRACT

Androgenic steroids are commonly used at supraphysiological doses by athletes and body builders. There are an increasing number of reports of sudden cardiac death and myocardial infarcts associated with androgenic steroid abuse. In this thesis the effect of an androgenic steroid on cardiac mechanical performance, cellular function and interstitial properties was examined in rats.

A biweekly intramuscular injection of nandrolone decanoate (5 mg/kg) (steroid) for three months to sedentary rats supressed testosterone production. Chronic steroid administration increased left ventricular end diastolic (LVED) chamber stiffness (k), as determined both *in vivo* in anaesthetised, open-chest animals, using piezoelectric ultrasonic transducers to measure LV dimensions, as well as *ex vivo*, using an isolated, perfused, constant flow, isovolumic LV preparation. The increased LVED chamber k was attributed to changes in myocardial properties, as myocardial ED k was also increased. The altered L ED chamber performance mediated by chronic steroid administration subsequently resulted in a decreased slope of the LV stroke work-LVEDP relation (determined *in vivo*), a preload-independent index of LV systolic performance. However, load-insensitive indices of LV systolic performance, assessed both *in vivo* and *ex vivo* (slopes of LV end systolic or peak systolic pressure-dimension, pressure-volume and stress-strain relations) were either modestly increased or unchanged following steroid administration. Alternatively, in

the presence of a β-adrenoceptor agonist- isoproterenol ($10^{-8.5}$M), steroid treated rats had a decreased myocardial systolic performance as determined *ex vivo*.

The androgenic steroid attenuated an exercise (voluntary running on exercise wheels)-induced decrease in LVED myocardial and chamber *k*. However, the steroid also produced an increased LV internal diameter as determined over a low-normal range of LVEDPs in exercised rats. Hence, despite the steroid-mediated alteration in LVED chamber *k*, ventricular remodelling (dilatation) offset the potential detrimental actions of the androgenic steroid on the relationship between diastolic pressures and dimensions in exercised rats. Nevertheless, the presence of ventricular dilatation in nandrolone decanoate treated exercised rats might indicate a deleterious effect of androgenic steroids on LV remodelling processes in exercise.

Neither the myocardial hydroxyproline concentration, perivascular collagen volume fraction, the ratio of type I to type III phenotypes of myocardial collagen, the percentage of myocardial total, type I or type III collagen extracted after cyanogen bromide digestion (an index of collagen cross-linking), nor the activity of the myocardial sarcoplasmic reticulum Ca^{2+} ATPase (SERCA) pump were altered by nandrolone decanoate administration. However, acute administration of verapamil (10^{-3} mmol/kg)(a cardiovascular Ca^{2+} channel blocker) to rats *in vivo*, which resulted in decreases in LV loads, systolic performance and heart rate, was able to attenuate the increased LVED chamber and myocardial *k* produced by the steroid. In contrast, verapamil infusion failed to alter increments in LVED stiffness produced in rats with

streptozotocin-induced diabetes mellitus and in spontaneously hypertensive rats. The increased myocardial k in both of the latter animal models was ascribed to changes in myocardial interstitial properties.

In conclusion, the results described in this thesis indicate that supraphysiological doses of androgenic steroids to sedentary rats produce functional abnormalities of LV mechanical performance determined in diastole, as well as in systole in the presence of a controlled adrenergic stimulus. The alterations in diastolic performance are ascribed to changes in myocardial properties and are relatively unique in that they are not attributed to remodelling of the cardiac interstitium or to modifications of the activity of the SERCA pump, but are sensitive to alterations in either heart rate, systolic performance, or load. The steroid-mediated increase in myocardial stiffness and decrease in adrenergic-inotropic responsiveness may be of clinical importance in that these changes could contribute toward cardiac pathology in athletes who abuse androgenic steroids.

DECLARATION

I declare that this thesis is my own work, with guidance and assistance as indicated in the acknowledgements. It is being submitted for the Degree of Doctor of Philosophy in the Faculty of Medicine at the University of the Witwatersrand, Johannesburg, South Africa. This work has not been previously submitted for any degree or examination at this or any other University.

.....24th.....day of.....July.....1998.

Boris Trifunovic

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I certify that the studies in this thesis have been approved by the Animal Ethics Screening Committee of the University of the Witwatersrand, Johannesburg. The animal ethics clearance certificate numbers are: 93/24/3, 93/20/2B, 93/19/2B, 93/105/4, 94/24/4 and 96/08/5.

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PREFACE

Much of the work in support of this thesis has been published and presented at various conferences:

1. Trifunovic B, Norton GR, Duffield MJ, Avraam P, Woodiwiss AJ. An androgenic steroid decreases left ventricular compliance in rats. *American Journal of Physiology* 1995: 268: H1096-H1105.
2. Trifunovic B, Woodiwiss AJ, Duffield MJ, Norton GR. Novel attributes of an androgenic steroid-mediated increase in end diastolic myocardial stiffness in rats. *Canadian Journal of Physiology and Pharmacology* 1998 (in press).
3. Trifunovic B, Woodiwiss AJ, Norton GR. Androgenic steroid-induced myocardial stiffness is attributed to alterations in active rather than passive myocardial properties in rats. Poster presented at *33rd International Congress of Physiological Sciences, St. Petersburg, Russia, 1997.*
4. Trifunovic B, Woodiwiss AJ, Norton GJ. Total myocardial collagen content and collagen subtypes are not altered in the stiff myocardium of steroid-treated rats. Paper presented at *24th Annual Congress of the Southern African Physiological Society, Cape Town, 1996.*

5. **Trifunovic B**, Norton GR, Woodiwiss AJ. An androgenic steroid decreases left ventricular compliance in rats. Paper presented at *Zeneca 16th Annual Clinical Research Day, Johannesburg, 1995.*

6. **Trifunovic B**, Norton GR, Duffield M, Woodiwiss AJ. Acute alterations in myocardial non-material properties improve the stiff myocardium produced by an androgenic steroid in rats. Paper presented at *23rd Annual Congress of the Southern African Physiological Society, Johannesburg, 1995.*

7. **Trifunovic B**, Norton GR, Woodiwiss AJ, Duffield M. An androgenic steroid decreases cardiac compliance without influencing blood pressure in rats. Paper presented at *Southern African Hypertension Society's IXth Conference, Midrand, 1995.*

8. **Trifunovic B**, Duffield M, Woodiwiss A, Norton G. An androgenic steroid decreases left ventricular compliance in rats. Poster presented at *2nd International Conference of Pathophysiology, Kyoto, Japan, 1994.*

9. **Trifunovic B**, Duffield M, Woodiwiss A, Norton G. An androgenic steroid decreases left ventricular compliance in rats. Paper presented at *22nd Annual Congress of the Southern African Physiological Society, Stellenbosch, 1994.*

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LIST OF ABBREVIATIONS

α	alpha
ANOVA	analysis of variance
ATP and ATPase	adenosine trisphosphate and trisphosphatase
β	beta
b	intercept
BP	blood pressure
BW	body weight
Ca^{2+}	calcium
CVF	collagen volume fraction
CON	control
CNBr	cyanogen bromide
DBP	diastolic blood pressure
DM	diabetes mellitus
dP\dt	differential of left ventricular pressure change over time
E_{max}	slope of the systolic P-V relationship
E^n_{max} or E^n	slope of the systolic stress-strain relationship
ECM	extracellular matrix
ED and EDP	end diastolic and end diastolic pressure
EDV	end diastolic volume

ES and ESP	end systolic and end systolic pressure
h	wall thickness
HDL	high density lipoproteins
HPRO	hydroxyproline
h/R	wall thickness to radius ratio
HR	heart rate
HW and HW/BW	heart weight and heart weight to body weight ratio
EC_{50}	concentration which produces 50% of the maximum excitatory response
ISO	isoproterenol
i.v.c.	inferi · vena cava
k	stiffness constant
kg	kilogram
km	kilometre
ln	natural log
log	log
LV	left ventricle
LVED	left ventricular end diastolic
LVEDD	left ventricular end diastolic diameter
$LVEDD_0$	left ventricular end diastolic diameter at LVEDP=0 mm Hg
LVEDL	left ventricular end diastolic length

$LVEDL_0$	left ventricular end diastolic length at LVEDP=0 mm Hg
LVEDP	left ventricular end diastolic pressure
LVEDV	left ventricular end diastolic volume
LV Ees	left ventricular end systolic elastance
LVES	left ventricular end systole
LVESL	left ventricular end systolic length
LVESP	left ventricular end systolic pressure
LVH	left ventricular hypertrophy
LVP	left ventricular pressure
LVW or LVW/HW	LV weight or LV to body weight ratio
m	slope
M	molar
MAP	mean arterial pressure
mg	milligram
Mg^{2+}	magnesium
mM	millimolar
mRNA	messenger ribonucleic acid
NEFA	non-esterified fatty acids
NS	non-significant
PaO_2	arterial partial pressure of oxygen
P_{max}	peak systolic pressure

$p<$	probability value less than
PBS	phosphate bufferred saline
P-D	pressure-dimension
P-L AREA	pressure-length area
PSS	physiological saline solution
P-V	pressure-volume
R	radius
r	correlation coefficient
R/h	radius to wall thickness ratio
RV	right ventricle
SBP	systolic blood pressure
SDS	sodium dodecyl sulphate
SEM	standard error of mean
SERCA	SR Ca^{2+} ATPase pump
SHR or shr	spontaneously hypertensive rats
SR	sarcoplasmic reticulum
SW	stroke work
V	volume
V_0	volume intercept of the systolic or diastolic P-V relationship
V_m	left ventricular wall volume
w	wet
WKY or wky	Wistar Kyoto control rats

Prologue

Androgenic-anabolic steroid abuse has been implicated in the pathogenesis of cardiovascular disease (Am. College of Sports Med., 1984). In particular, high doses of anabolic steroids are reported to produce atherogenic lipid profiles as well as increase blood pressure (Molteni et al., 1969, Skelton, 1953, Salgado and Selye, 1954, and Wexler, 1971). Despite these reports of the detrimental effects of steroids on the systemic circulation, the consequence of prolonged steroid use on cardiac performance is largely unknown. Anabolic steroid administration is reported to decrease cardiac systolic performance when given to animals on exercise training programmes (Karhunen et al., 1988, Liang et al., 1993, Ramo et al., 1987b). In contrast, anabolic steroid treatment does not influence systolic performance in sedentary animals (Liang et al., 1993). Nevertheless, the effects of steroids on either 1) load-independent measures of cardiac systolic performance as determined *in vivo*, 2) load-independent measures of cardiac systolic performance as determined in the presence of an adrenergic stimulus *ex vivo*, or 3) myocardial as opposed to chamber systolic performance, have not been investigated. Furthermore, the influence of androgenic steroids on cardiac diastolic function has only been investigated using load and heart rate-dependent echocardiographic indices (Urhausen et al., 1989).

In this thesis, I describe the effects of chronic androgenic steroid administration on left ventricular (LV) chamber and myocardial diastolic stiffness and

load-independent indices of systolic performance, as determined both *in vivo* and *ex vivo* in the presence and absence of a controlled adrenergic stimulus in rat hearts. Nandrolone decanoate, an androgenic steroid commonly used by athletes at high doses, was administered biweekly for 3 months to rats. As discussed in chapter 2, as a consequence of an enhanced myocardial stiffness, LV chamber stiffness was increased following chronic steroid administration to sedentary rats. However, LV systolic chamber and myocardial performance were either unchanged or modestly enhanced as assessed in intact rats or *ex vivo* in the absence of an adrenergic stimulus (chapter 2). Alternatively, chronic steroid administration reduced myocardial adrenergic-inotropic responses. As the androgenic steroid produced consistent alterations in diastolic performance, I subsequently examined the effect of these changes on exercised animals. In chapter 3 I describe results which indicate that the androgenic steroid prevents an exercise-induced improvement in diastolic performance. However, simultaneous chamber remodelling offset the potential detrimental actions of the steroids on diastolic pressure-dimension relations. Nevertheless, the steroid-mediated increase in end diastolic stiffness suggested either the presence of deleterious myocardial interstitial changes or a decrease in the activity of the sarcoplasmic reticulum (SR) Ca^{2+} ATPase pump, alterations that are frequently associated with diastolic abnormalities in cardiac disease. However, the ensuing chapter presents results which indicate that the androgenic steroid produces an increased diastolic myocardial stiffness which is attributed to neither alterations in the

cardiac interstitium (myocardial total collagen, cross-linked collagen properties and phenotypes remained unchanged) nor the activity of the SR Ca^{2+} ATPase pump. In addition, the steroid-induced increase in myocardial stiffness was shown to be uniquely sensitive to acute alterations in cardiac load, systolic performance and/or heart rate.

The first chapter of my thesis provides the reader with a brief background on androgenic-anabolic steroids and a review of the current scientific literature pertaining to cardiovascular effects of steroid abuse. I have also highlighted the importance of investigating cardiovascular performance using the techniques and approaches employed to obtain the data described in this thesis. The final chapter provides the reader with an expanded summary and the conclusions reached from the data obtained.

CHAPTER 1

Literature review

1.1 Introduction.

At the end of the 19th century studies performed on dogs and pigs resulted in claims that testosterone is able to enhance physical strength and intellectual capacity. Since then testosterone and its derivatives have been artificially synthesised (Hoberman and Yesalis, 1995, Kopera, 1985) and marketed for therapeutic use. However, the availability of artificial androgenic steroids has resulted in the abuse of these substances by individuals attempting to increase their muscle strength and bulk. The beneficial effects of anabolic steroids on athletic performance are controversial, although athletes who use them usually insist that their strength, muscle endurance and exercise performance are improved. In addition, a number of detrimental effects of anabolic steroids, when used at supra-physiological doses by athletes and body builders, are well described (rev- Kibble and Ross, 1987).

In the following sections, I will discuss the structure and biological effects of testosterone and it's derivatives (androgenic-anabolic steroids), and differentiate between androgenic and anabolic effects. The extent of the abuse of as well as the side effects produced by anabolic-androgenic steroids is also highlighted.

1.2 Androgenic-anabolic steroids

Both natural and synthetic hormones chemically similar to testosterone are classified as androgenic-anabolic steroids. The two most important and potent natural androgenic-anabolic steroid hormones (androgens) are testosterone and androstenedione (Astwood, 1970). These natural androgens are rapidly absorbed

and broken down by the body, which limits their therapeutic usefulness. To overcome this problem most of synthetic androgenic-anabolic steroids are modified by esterification of the 17β-hydroxyl group, alkylation at the 17α position and by changing the ring structure of natural androgens in order to retard absorption and degradation (Wilson and Griffin, 1980).

1.2.1 Biological action of androgens

Androgenic-anabolic steroids have been shown to influence the growth of almost every organ or tissue (Kochakian, 1976). Androgen receptors have been found in most organs including the prostate, kidney, thymus, spleen (Bergink et al., 1985), skeletal muscles (Krieg, 1976) and also in heart muscle (McGill et al., 1980). However, the sensitivity of target organs to anabolic-androgenic steroids is complex and depends not only on the presence of androgen receptor sites, but also upon the pubertal stage of the individual (Lucas, 1993) as well as gender, nutritional and hormonal status and temperature (Wright, 1980).

Androgens pass through cellular membranes, then bind to steroid receptors in the cytoplasm of target tissues. The hormone-receptor complex subsequently moves to the nucleus. Within the nucleus, the complex is bound to chromatin thus inducing transcription and production of specific messenger ribonucleic acid (mRNA). The new mRNA is translated into proteins which mediate cellular changes related to androgen actions (Lucas, 1993). However, the androgenic-anabolic steroid receptor has not been cloned. In addition, the gene(s) activated by androgens have not been elucidated (Mooradian et al., 1987).

The effects of anabolic-androgenic steroids on target tissues are divided into androgenic and anabolic effects. This division is fairly artificial, because all steroids exert their effects in the same basic manner described above, i.e. "androgenicity" differs from "anabolism" only in location and not in essence.

1.2.1 Androgenic effects

Numerous studies have attempted to draw a clear distinction between anabolic and androgenic effects of steroids. Generally, androgenic effects of anabolic-androgenic steroids involve masculinisation, while anabolic effects involve hypertrophy of tissue and organ systems. Hershberger et al. (1953) described the classical pharmacological test to discriminate between anabolic and androgenic characteristics. According to this test, the ability of the compound to increase the weight of the *musculus levator ani* in male castrated rats reflects its anabolic activity, while an increase in the weight of the seminal vesicle or the ventral prostate reflects androgenic activity.

Androgenic effects of anabolic-androgenic steroids include the control of the development of the urogenital tract during embryonic life in males. During puberty, anabolic-androgenic steroids induce growth of sexual organs and mediate the development of secondary sexual characteristics. Reproductive tissues also require androgens for their maintenance. Castration produces not only regression and atrophy of all sexual organs, but also changes in sexual behaviour and libido (Mooradian et al., 1987).

1.2.1.2 Anabolic effects

Anabolic effects of natural androgenic-anabolic steroids are exerted during puberty. An androgen-mediated acceleration of the growth rate of bones and muscles results in broadening of the thorax and shoulders, epiphyseal closure, an increase in bone density and an enhanced size of muscle cells during puberty (Cheek et al., 1968) The growth of internal organs (e.g kidneys and heart) as well as an increase in red blood cell numbers is also due to an androgen surge in puberty.

Manipulation of the chemical structure of natural anabolic-androgenic steroid molecules improves not only pharmacokinetic parameters, but also enhances anabolic as opposed to androgenic effects. However, it is not possible to produce steroids with only anabolic actions. Therefore Kochakian (1976) suggested that the synthetic steroids would be more accurately termed "androgenic-anabolic" rather than "anabolic" steroids.

The first synthetic steroid with distinct anabolic properties, shown to be several times more potent that testosterone in stimulating growth of the rat *levator ani* was 19-nortestosterone (Hershberger et al., 1953). Synthetic changes to steroid molecules decreases the polarity of the molecules thereby increasing their solubility, which enhances and prolongs their activity (Junkmann, 1957). Decanoate, heptanoate and cypionate esters of steroids are all useful modifications of naturally occurring steroids. Esterification of 19-nortestosterone with decanoate forms 19-nortestosterone (or nandrolone) decanoate (Figure 1), the steroid used in the studies described in this thesis.

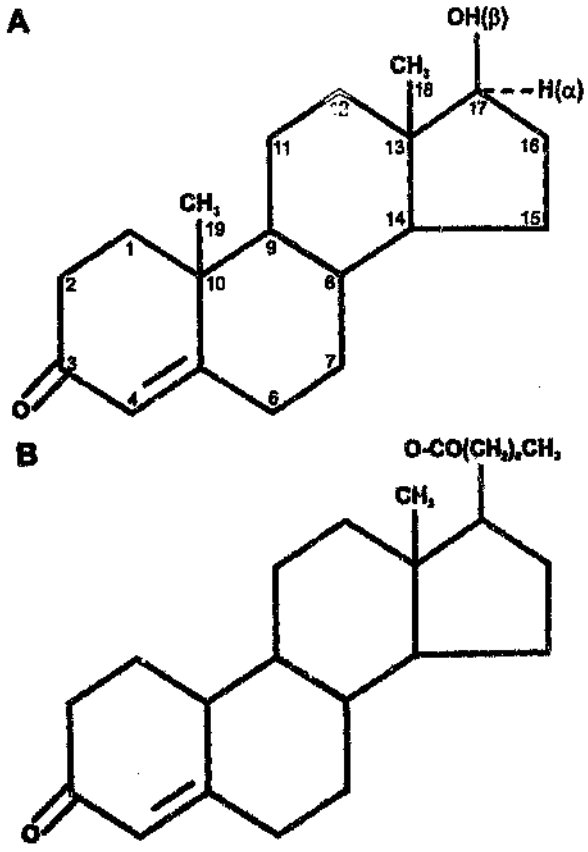

Figure 1. A: The molecular structure of testosterone. The carbon atoms are marked with numbers. α and β refer to the stereochemistry of the hydroxyl and hydrogen groups at carbon 17. B: The nandrolone (19-nortestosterone) decanoate molecule. The decanoate ester is attached at the 17β position, while the 19 methyl group is removed. (Wilson and Griffin, 1980)

1.2.2 Abuse of androgenic-anabolic steroids in sport

Steroids have become popular and widely used by athletes, mostly in power sports to improve their physical performance. These agents are considered capable of enhancing muscle mass and strength, although the scientific evidence for these claims is controversial (Bower and Reardan, 1972, Casner et al., 1971, Crist et al., 1983, Fahey and Brown, 1973, Golding et al., 1974, Johnson and O'Shea, 1969). The International Olympic Committee's Medical Commission banned the use of steroids in sport in 1976, not only for ethical reasons related to fair competition, but also because of the well-documented deleterious side effects of these substances. Steroid abuse in sport is the main reason why androgenic-anabolic steroids have never been legitimised as a method of birth control for men, although they are an efficient male contraceptive mediated via endocrine feedback loops. The use of anabolic-androgenic steroids for contraception would devalue the testing technique that is currently being used to trace steroids in urine in athletes (Hoberman and Yesalis, 1995).

Nandrolone decanoate, the steroid used in the studies described in this thesis is one of the most potent injectable androgenic-anabolic steroids used by athletes seeking improved skeletal muscle performance (Titlestad et al., 1994). The relative anabolic potency of nandrolone decanoate compared to the other steroids as computed by Potts et al. (1976) is as follows: nandrolone decanoate = 11.3, stanozolol = 5, methyltestosterone and mesterolone = 1.15, and methandrostenolone = 1.7. Athletes have been reported to use up to 10-100 times

higher doses of nandrolone decanoate than the accepted therapeutic dose range normally used in hypogonadal syndromes (Brower et al., 1990).

1.2.3 General side effects of androgenic-anabolic steroids

While controversy and conflicting experimental data exist regarding the beneficial effects of androgenic-anabolic steroids in sport, there is apparent unanimity pertaining to their detrimental actions. Side effects subsequent to androgenic-anabolic steroid administration are mostly due to their androgenic actions.

Although androgen supplementation in conjunction with regular monitoring is an accepted approach to hypogonadal patients (Odell and Swerdloff, 1976), Heller et al. (1958) and Jackson and Jones (1972) described a decrease in plasma gonadotrophin levels, sperm production and motility after androgenic-anabolic steroid administration to non-hypogonadal male subjects. Masculinising effects, such as beard growth and a deepened voice, as well as lowered fertility rates are likely to occur in female athletes subsequent to androgenic-anabolic steroid use.

Toxic side effects of androgenic-anabolic steroid administration are mainly hepatic. Elevation of plasma alkaline phospatase as well as raised conjugated bilirubin concentrations, indicating hepatocellular damage, have been described by many authors following androgenic steroid administration (Foss and Simpson, 1959; Arias, 1962; de Lorimier et al., 1965). In addition, Johnson et al. (1972), found an association between androgenic-anabolic steroid therapy and the development of hepatocellular carcinoma. Furthermore, Bagheri and Boyer (1974)

reported on the development of *peliosis hepatis*, a severe form of hepatic injury, subsequent to androgenic-anabolic steroid therapy.

The unfavourable psychological effects of steroid administration have only recently been recognized. The most commonly described psychological effect of androgenic steroids is an increased level of aggressiveness (Scaramella and Brown, 1978). Euphoria with diminished fatigue, changes in libido and mood swings have been also reported in athletes who abuse androgenic steroids (Wilson and Griffin, 1980, Lamb, 1984, Haupt and Rovere, 1984).

1.3 Cardiovascular effects of androgenic-anabolic steroids.

Androgenic-anabolic steroid administration has been implicated as a risk factor for cardiovascular disease (Skelton, 1953, Salgado and Selye, 1954). There are an increasing number of reports of sudden cardiac death and acute myocardial infarction amongst athletes and body builders who use anabolic-androgenic steroids (Mc Nutt et al., 1988, Luke et al., 1990). In addition, anabolic-androgenic steroid users who have undergone weight training have been reported to suffer an increased frequency of cardiac arrhythmias during cardiovascular stress (Wright, 1980). However, the mechanisms by which androgenic-anabolic steroids may induce deleterious cardiac effects remains unclear. Mechanisms which may play a role include changes in blood lipid profiles and blood pressure, as well as direct effects on myocardial tissue.

In body builders and weight lifters the use of anabolic-androgenic steroids reduces high density lipoprotein (HDL) and elevates low density lipoprotein

plasma concentrations, hence producing an atherogenic lipid profile (Strauss et al., 1985, Haffner et al., 1983). The unfavourable changes in lipid profiles in athletes who use anabolic-androgenic steroids is in marked contrast to the increased plasma HDL concentrations that occur in athletes who do not (Webb et al., 1984).

Anabolic steroid administration may lead to an elevation in blood pressure, a further risk factor for cardiovascular disease, (Am College of Sports Med., 1984, Kuipers et al., 1991, Lenders et al., 1988). Molteni et al. (1969) produced hypertensive vascular disease in rats following chronic androgenic-anabolic steroid administration. A number of other authors have also implicated androgens in hypertension, atherosclerosis and even nephrosclerosis in experimental animals (Skelton, 1953, Salgado and Selye, 1954, and Wexler, 1971). Greenberg et al. (1974) provided evidence that indicates that steroids may lead to hypertension in part, by increasing vascular responses to vasoactive stimuli. Wolinsky (1972) showed that testosterone enhanced the quantity of fibrous proteins, elastin and collagen in the rat aortic wall which might be, in part, responsible for increases in blood pressure. Messerli and Frohlich (1979) suggested that a direct inhibition of 11β-hydroxylation, leading to an overproduction of 11-deoxycorticosterone by the adrenal cortex, may be responsible for an increase in blood pressure in athletes taking steroids.

Despite the evidence in favour of an effect of androgenic steroids on cardiovascular risk factors, whether long-term steroid administration induces deleterious cardiac effects through changes in lipid profiles and increments in

blood pressure, or through a direct effect on the myocardium has not been established.

1.3.1 Anabolic-androgenic steroid effects on cardiac morphology and structure.

The differences in morbidity and mortality rates from cardiovascular disease in men versus women led to a search for sex hormone receptors in cardiovascular tissue. Estrogen receptors in atrial myocytes (Stumpf et al., 1977) and androgen receptors in heart muscle (Krieg et al., 1978) have been identified in rats. In addition, androgen receptors have been found in atrial and ventricular myocytes of baboons and rhesus monkeys (Mc Gill et al., 1980). The discovery of androgen receptors in cardiac myocytes has prompted further research on the effect of androgens on the heart.

Although a number of studies have been performed to establish a possible link between androgenic steroids and cardiac hypertrophy, controversy still exists with regards to the conditions under which this occurs. Koenig et al. (1982) induced myocardial hypertrophy in rats following repeated testosterone injections over a prolonged period. In earlier studies, Scow and Hagan (1965) showed that androgens markedly potentiate growth hormone-induced cardiac hypertrophy. Conversely, Kinson et al. (1991) reported that ventricular enlargement caused by aortic constriction in castrated infant rats was reduced following androgen administration. Furthermore, we have demonstrated that androgenic steroid administration to sedentary rats reduces both cardiac and somatic growth

(Trifunovic et al., 1995) but has no effect on physiological cardiac hypertrophy (chapters 2 and 3).

The mechanisms by which androgenic steroids mediate cardiac growth are also more complex than what was originally thought. Iversen and Salt (1970) showed that steroids inhibit presynaptic reuptake of catecholamines in the rat heart, which could increase the actions of catecholamines on cardiac tissue. Hence. androgenic steroid-induced actions on cardiac growth might occur as a consequence of either direct effects on intracellular protein synthesis, or indirect actions mediated by catecholamines.

Echocardiographic studies in humans examining the influence of anabolic steroids on the heart have also produced conflicting results. De Piccoli et al. (1991) found enlargement and thickening of the left ventricle (LV) in bodybuilders who abuse anabolic-androgenic steroids. Urhausen et al. (1989) showed that the effects of anabolic-androgenic steroids on cardiac size is only apparent when combined with intense bodybuilding. Alternatively, Salke et al. (1985) found no differences in LV morphology between groups of bodybuilders either with or without a history of previous or current anabolic-androgenic steroid abuse. Echocardiographic studies in humans are however often difficult to interpret because of the overlapping influence of strenuous isometric exercise on heart size as well as the difficulties associated with attempting to assess the degree of steroid abuse amongst athletes.

A wide range of ultrastructural and morphological changes, other than simply measures of heart size, are associated with androgenic-anabolic steroid

administration. Appell et al. (1983) described an increase in myocardial mitochondrial volume subsequent to chronic steroid administration. When exercise and the use of anabolic-androgenic steroids were combined, destruction of myocardial mitochondria and myofibrils, similar to those changes described after severe hypoxia or after an overdose of catecholamines, was found (Appell et al., 1983). Behrendt and Boffin (1977) found the following effects of steroid administration on heart cells in rats. Myofilaments had an irregular arrangement with intermingling clusters of swollen and elongated mitochondria. A sparse mitochodrial matrix and fewer than normal cristae were noted in the steroid treated group. Myofibrils were irregularly shaped, and the thick and thin myofilaments and Z-bands were noted to have disintegrated following steroid administration. The extracellular matrix (ECM) was occupied by increased numbers of bundles of collagen fibrils indicating the possible presence of fibrosis subsequent to chronic androgenic-anabolic steroid administration. These changes mediated by anabolic-androgenic steroid administration resemble those described in the early stages of heart failure. Although myocyte ultrastructural changes apparently decrease in severity subsequent to ongoing anabolic-androgenic steroid administration (Behrendt and Boffin, 1977), the results imply that chronic steroid treatment can induce significant myocardial cellular and interstitial pathology and, thus, perhaps could affect cardiac performance.

1.3.2 Andro[illegible]nic steroid-induced effects on cardiac performance

Although a number of potentially deleterious histological and morphological effects of anabolic steroids on cardiac tissue have been demonstrated, the action of androgenic-anabolic steroids on cardiac performance has been investigated by a limited number of groups. A decrease in LV systolic mechanical performance follows steroid administration to sedentary dogs (Ramo, 1987a) and to rats and dogs on exercise-training programmes (Karhunen et al., 1988, Liang et al., 1993, Ramo et al., 1987b).

Effects of androgenic steroids on cardiac performance in sedentary animals

Liang et al. (1993) using an isolated, perfused heart preparation (i.e. an *ex vivo* heart preparation) found that steroid administration produced no alterations in the slope of the systolic pressure-volume (P-V) relationship, a preload and heart rate-independent measure of systolic cardiac performance that is also insensitive to inc[illegible]nents in afterload (Kass et al., 1993, Sagawa et al., 1988) in sedentary rats. However, despite the strengths of this study with regards to the choice of systolic performance measurements, there were a number of deficiencies in the study. Although these authors (Liang et al. ,1993) measured diastolic P-V relations, these relations were not adequately evaluated by comparing the slopes of the relations between the groups. Indeed, from the data presented, the steroid treated group had an apparent increase in diastolic stiffness (Liang et al., 1993). Furthermore, Liang et al. (1993) also failed to account for the variability in LV

geometry when assessing LV performance by converting both diastolic and systolic P-V relations into stress-strain relations.

Using thermal washout curves to measure ejection fraction, stroke volume, cardiac output, and the maximum velocity of LV contraction (V_{max}) as well as $+dP/dt_{max}$ to assess cardiac performance, Ramo (1987a) reported a decreased inotropic response to isoproterenol infusion in steroid treated sedentary dogs. However, a potential weakness of this study is that all of the indices of systolic performance used by Ramo (1987a) were either load or heart rate-dependent. Indeed, Ramo (1987a) also showed a decreased heart rate subsequent to isoproterenol infusion after 6 weeks of androgenic steroid treatment, which suggests that differences in systolic performance could be attributed to changes in heart rate. In addition, these authors (Ramo, 1987a) failed to identify whether the differences noted between the steroid-treated and control groups occurred subsequent to altered adrenergic responsiveness (i.e. steroid induced changes in β-adrenoceptor kinetics, G_s protein activity or concentrations or second messenger system concentrations) or to changes in myofilament mechanical performance. Furthermore, although single cycle LV P-V loops were constructed by Ramo et al. (1987a), diastolic P-V relations were not assessed.

Effects of androgenic steroids on cardiac performance in exercise trained animals

Most investigators agree on the effects of androgenic steroids on systolic cardiac performance in exercise trained animals. Both Wang et al. (1993) and Ramo et al. (1987b), using the same measurement approaches discussed above,

have shown that steroid administration attenuates systolic performance in exercised animals. Again, despite certain strengths of these studies, both studies have a number of inadequacies (see above discussion). In addition, Karhunen et al. (1988), also using load and heart rate-dependent measures of systolic LV performance, showed that the enhanced isoproterenol mediated increase in LV pump performance induced by exercise training was attenuated following chronic androgenic steroid administration. The weakness of the study by Karhunen et al. (1988) includes the use of load and heart rate-dependent measures of systolic LV performance and a notable lack of diastolic performance measurements.

Effects of androgenic steroids on cardiac performance in human studies

Human echocardiographic studies have also focussed on assessing LV load and heart rate-dependent measures of cardiac systolic and diastolic performance in body builders and weight lifters using steroids (Pearson et al., 1986, Urhausen et al., 1989). Pearson et al. (1986) using two-dimensional echocardiography showed concentric hypertrophy in weight lifters, but no differences in LV filling rate or in ejection fraction. Urhausen et al., (1989) found isovolumetric relaxation time to be modestly prolonged in steroid users. The latter result indicates that androgenic steroid abuse might lead to alterations in diastolic performance.

Apparent deficiencies in the scientific literature promoted the studies described in this thesis

Prompted by the distinct lack of comprehensive data reported on in the scientific literature describing the actions of androgenic steroids on cardiac systolic and diastolic mechanical performance, as well as the fact that most of the studies performed to date have focussed on either load and/or heart rate-dependent measures of systolic LV performance, I set out to assess the effects of androgenic-anabolic steroids on preload and heart rate-independent measures of both LV chamber and myocardial systolic and diastolic performance which are insensitive to increases in afterload (Krass et al., 1993, Sagawa et al., 1988). In this thesis I have shown that supraphysiological doses of an androgenic steroid induce marked alterations in both myocardial and LV chamber diastolic function both in sedentary and exercised rats (Trifunovic et al., 1995, chapters 2, 3 and 4). Moreover, these steroid-induced changes in diastolic performance were associated with a preserved systolic LV chamber function, as assessed *in vivo* (Trifunovic et al., 1995, chapter 2), a normal systolic myocardial performance, as determined *ex vivo* in the absence of an adrenergic-inotropic stimulus (chapter 2), but a decreased LV chamber and myocardial adrenergic-inotropic responsiveness as determined *ex vivo* (chapter 2). As the consistent LV mechanical alteration noted subsequent to chronic steroid administration was in the diastolic period of the cardiac cycle, all subsequent work described in this thesis pertains to steroid-induced changes in diastolic mechanical performance. As such, it is pertinent to provide a summary of the potential implications of alterations in LV diastolic

performance, as well as an overview of those factors which determine LV diastolic function.

1.4 Left ventricular diastolic performance

Diastole is defined as the period from aortic valve closure to mitral valve closure during which LV relaxation and filling occurs. It is essential to generate prompt and complete relaxation of the LV in order to create conditions which are ideal for promoting low-pressure LV filling. An increase in LV filling pressures might result in an enhanced myocardial extra-capillary pressure during diastole, when most myocardial blood flow occurs. An increase in myocardial extra-capillary pressure will result in a reduction in myocardial capillary radius, and the consequence might be an attenuated myocardial blood supply. In addition, a further potential deleterious spin-off of increases in LV filling pressures during diastole is enhanced left atrial and hence pulmonary venous pressures and a subsequent increase in pulmonary capillary hydrostatic pressure. The consequence of increased pulmonary capillary hydrostatic pressures is pulmonary oedema.

Although the mechanisms by which changes in diastolic function may induce detrimental effects on the heart have not been established, it is well known that diastolic performance abnormalities precede systolic abnormalities in many forms of heart disease (Brilla et al., 1991, Norton et al., 1996, Norton et al., 1997). In addition, heart failure may occur in the presence of a preserved systolic performance, where the major abnormality is a reduced diastolic performance

(Dougherty et al., 1984). Hence, currently it is accepted that cardiac diastolic abnormalities either produce or are associated with deleterious cardiac effects.

1.4.1 Diastolic pressure-volume relationships

Left ventricular diastolic pressure-volume (P-V) or pressure-dimension (P-D) relationships and the slope or linearised slope of these relations (LV chamber stiffness) are used to determine LV diastolic performance. Left ventricular diastolic P-V or P-D relationships may be assessed from P, V and/or D values obtained over a single cardiac cycle (usually from the period starting with end isovolumic relaxation and ending with end diastole) or from P, V and D values obtained at end diastole over a range of LV preloads. It is unlikely that the factors that determine these relationships contribute to the same extent toward P-V or P-D relations determined over a single beat or at end diastole over a range of preloads.

The relationship between LV diastolic pressure and volume is determined by the balance between the forces due to pressures within the ventricular cavity that expand the LV and those forces generated through myocardial elasticity that resist LV expansion (rev- Gilbert and Glantz, 1989). A typical diastolic P-V or P-D relationship is curvilinear. At low pressures the relationship is a linear function, but at higher pressures it resembles an exponential function (Diamond et al., 1971).

Diastolic stress-strain relationships (determined from P-V or P-D values and wall thickness), which account for differences in ventricular geometry

between individuals or groups of individuals (rev- Gilbert and Glantz, 1989) are often used as a substitute for diastolic P-V relationships. The slope of the linearised stress-strain relationship is one assessment of myocardial elastic stiffness. Stress is a measure of the intensity of forces in the ventricular wall and strain is a dimensionless quantity that represents the percent change from the ventricular unstressed dimension (Mirsky and Parmley, 1973). In order to assess LV stress a spherical shell model of the LV may be assumed, and calculated using Laplace's Law (Stillwell, 1973, Gilbert and Glantz, 1989). According to Laplace's Law, stress is directly proportional to the internal radius and pressure and inversely proportional to twice the wall thickness.

The use of spherical shell models when assessing stress-strain relations, with a knowledge that the LV has in most circumstances an elliptical shape has been of concern to many investigators. However, Janz et al. (1980) showed that the use of spherical geometrical models of the LV for estimating the myocardial elastic stiffness constant is as accurate as are ellipsoidal geometric models. Moreover, Fester and Samet (1974) showed a close relationship between myocardial elastic stiffness constants calculated from an assumption of either a spherical or an ellipsoid geometry of the LV.

Many factors affect diastolic P-V relationships and hence ventricular chamber stiffness. These factors will be considered in four broad groups: (1) myocardial passive material properties and chamber geometry, 2) myocardial active properties, 3) viscoelasticity, diastolic suction and coronary vascular

engorgement as well as 4) external factors such as ventricular interaction, pericardial and pleural forces and lung constraints.

1.4.1.1 Myocardial passive properties and LV geometry; Effects on diastolic pressure-volume relationships.

Alterations in LV wall composition, thickness or geometry are in most instances caused by chronic processes and are unlikely to change LV diastolic P-V relations over short periods of time. These factors are often termed myocardial material or passive properties. The development of ventricular hypertrophy that occurs as a consequence of either pressure or volume overload will change the P-V relationship. Concentric hypertrophy may shift the relationship to the left and increase the slope, whereas eccentric hypertrophy may shift the relationship to the right without necessarily altering the slope. However, Mirsky and Parmley (1973) were able to show that although chamber stiffness (assessed from P-V relations) of the hypertrophied ventricle may increase, myocardial stiffness (assessed from stress-strain relations) is not necessarily affected.

An intrinsic myocardial material property with a substantial impact on myocardial stiffness is the composition of the ventricular wall (Mirsky, 1976). Processes altering ventricular wall matrix substances, such as fibrosis, will increase myocardial stiffness (Smith and Zile, 1991). The myocardial extracellular matrix (ECM), a fibrous framework of collagen, is described by many authors as a key factor determining myocardial stiffness. Weber and Brilla (1991) have attributed the increased myocardial stiffness constants that occur in pathological

hypertrophy to myocardial fibrosis due to overproduction of collagen by cardiac fibroblasts. Furthermore, Hood et al. (1970) noted that fibrosis, following myocardial infarction, was also associated with an increased ventricular stiffness. Moreover, substances which reduce the degree of myocardial fibrosis in cardiac disease, such as angiotensin-converting enzyme inhibitors (Brilla et al., 1991), or β-aminoproprionitrile (Bing et al., 1978), a substance which reduces myocardial collagen cross-linked properties, are able to attenuate the enhanced myocardial diastolic stiffness constants produced by cardiac disease. Recent data obtained from our laboratory shows that alterations in the cardiac interstitium which mediate increases in myocardial diastolic stiffness in cardiac disease cannot simply be attributed to changes in the total collagen content, but rather to alterations in the quality of the myocardial collagen (Norton et al., 1996, Norton et al., 1997).

1.4.1.2 Myocardial active properties; Effects on diastolic pressure-volume relationships.

The view that only myocardial passive properties determine ventricular end diastolic P-V relations has been changed since Bristow et al. (1970) and Dwyer (1970) showed that in patients with coronary artery disease the diastolic P-V curve is temporarily shifted upward following cardiac pacing-induced angina. In addition, ventricular relaxation abnormalities and alterations in the diastolic P-V relationship have been reported following the induction of myocardial ischaemia in dogs (Serizawa et al., 1980, and Paulus et al., 1982). A possible explanation for the altered diastolic P-V relationship noted during pacing-induced myocardial

ischaemia is that ventricular relaxation is reduced by ischaemia and at high heart rates the myocardium has an inadequate time period between beats to fully relax (Grossman and McLaurin, 1976). However, in animal studies the reduction in the rate of relaxation secondary to ischaemia is not sufficiently large enough to account for the increments in myocardial stiffness noted at the end of diastole (Palacios et al., 1976). As such it has been postulated that the extent, as opposed to the rate of ventricular relaxation may influence end diastolic function during pacing-induced ischaemia. The extent of relaxation depends directly on the number of active actin-myosin filament cross bridges formed in the cytosol. If an excess of cross bridges remain after contraction has ceased the equilibrium length of the muscle will be shortened which is manifested as a smaller equilibrium volume (V_0). The smaller equilibrium volume will result in larger strains and higher pressures and stresses at any given volume at the end of diastole (rev- Gilbert and Glantz, 1989).

A number of cellular theories have been proposed to explain why a decrease in the extent of ventricular relaxation during pacing-induced ischaemia induces an increased diastolic stiffness. Ischaemia and a consequent adenosine trisphosphate (ATP) shortage would limit Ca^{2+} reuptake by the sarcoplasmic reticulum (SR) and mediate Ca^{2+}-overload (Kihara et al., 1989). In addition, failure of the Na^{+}-Ca^{2+} exchanger or Ca^{2+}-pump in the sarcolemma during ischaemia would also contribute to Ca^{2+}-overload (rev- Gilbert and Glantz, 1989, Makino et al., 1987). The consequence of ischaemia might then be a Ca^{2+}-overload-mediated increase in myofilament cross-bridge formation during the

diastolic period of the cardiac cycle, an effect that would be unmasked at high heart rates when efficient relaxation processes are required. Data obtained by Grossman and colleagues suggests that the principal cellular change responsible for the increased LVED stiffness following pacing-induced ischaemia is a decreased activity of the SR Ca^{2+} ATPase pump (Kihara et al., 1989). However, recent data suggests that the cellular change responsible for the increased diastolic myocardial stiffness constant that occurs following pacing-induced ischaemia is a decrease in stretch-activated cationic channel activity (Takano and Glantz, 1995). A decreased activity of stretch-activated cationic channels is also likely to result in an accumulation of cytoplasmic Ca^{2+} during the diastolic period of the cardiac cycle and hence an increased myofilament cross-bridge formation.

1.4.1.3 Viscoelasticity, diastolic suction and coronary vascular engorgement; Effects on diastolic pressure-volume relationships.

Biological materials undergo stress during stretching which depends not only on the degree of stretch but also on how fast the material is stretched (Little and Wead, 1971). Viscoelasticity or viscoelastic forces which are present during ventricular stretching (filling) may play a role in characterising the P-V relationship, especially in early diastole when filling rates are high (rev- Gilbert and Glantz, 1989). However, the degree to which diastolic viscous forces contribute toward P-V relations and also the extent to which they are altered in pathology has not been determined.

Diastolic suction is a dynamic property of the ventricle that induces negative pressures in early diastole (Suga et al., 1968). Diastolic suction is caused by the elastic recoil of the ventricle to its unstressed volume after contraction has ceased. The force for elastic recoil is provided for by the release of potential energy at the end of systole. This potential energy is stored between the layers of myocardial fibres during contraction and is released at the end of systole (Rushmer et al., 1953). As diastolic suction occurs mainly in early diastole, it is unlikely to significantly affect events at the end of diastole except at very high heart rates when filling periods are short.

An increase in coronary perfusion pressure may stiffen the myocardium (Salisbury et al., 1960), an effect which is likely to impact mainly at low LV pressures, such as occur during diastole. This is most evident when the coronary arteries are maximally perfused in late diastole. Gaasch et al. (1977) substantiated this hypothesis by demonstrating a reduced diastolic stiffness subsequent to coronary artery ligation. However, Huyghe (1986) and Olsen et al. (1981) showed that increments in coronary vascular engorgement produce only modest effects on chamber stiffness, while Spadaro et al. (1982) failed to show alterations in diastolic P-V relations subsequent to alterations in coronary perfusion pressure.

1.4.2 External factors affecting diastolic pressure-volume relationships; Ventricular interaction, pericardial and pleural forces and lung constraints.

A manifestation of the mechanical forces transmitted through the septum between the two cardiac ventricles is termed direct ventricular interaction, as

opposed to series ventricular interaction which is the effect of right ventricular output on left ventricular input. Series ventricular interaction is delayed, while direct ventricular interaction is immediate. Slinker and Glantz (1986) used this "time-lag" approach and determined that series ventricular interaction was twice as important in determining ventricular end-diastolic size than direct ventricular interaction. Direct ventricular interaction is unlikely to impact to any significant extent over a range of normal filling volumes on diastolic P-V relations once the pericardium is removed.

As the pericardium wraps the heart, any increase in end-diastolic volume will stretch the pericardium. This will impact on the epicardium and contribute substantially to ventricular diastolic pressure (rev- Gilbert and Glantz, 1989). However, a normal pericardium, through external constraints, is thought to have little or no influence on the pressures in the heart over a normal operating range of cardiac preloads (Tyson et al., 1984). Nevertheless, following an increased preload, pericardial forces will influence LV diastolic P-V relations.

The lungs and pleura also interact mechanically with the heart by exerting contact pressures on the pericardium and through the pericardium further on the heart. This interaction significantly impacts on diastolic forces during artificial positive end [illegible]y pressure ventilation. The decrease in cardiac output that occurs following positive end expiratory pressure ventilation is due, in part, to a mechanical interaction between the lungs and the heart, which reduces LV filling volumes and subsequently attenuates Frank Starling effects (rev- Gilbert and Glantz, 1989).

1.5 Choice of cardiac performance measurement techniques employed in the studies described in this thesis.

In the studies described in this thesis a number of approaches were employed to attempt to characterise the changes in cardiac mechanical performance that occur in rats subsequent to chronic administration of the androgenic steroid, nandrolone decanoate. These measurement techniques were chosen in order to eliminate confounding variables that would have made the results described in this thesis difficult to interpret. The reasons for the choice of techniques employed in the light of those factors known to influence cardiac systolic performance as well as those factors discussed above that are known to modulate diastolic performance are underscored in subsequent discussion.

In order to evaluate the influence of chronic androgenic steroid administration on systolic mechanical performance, I chose to assess systolic performance by using a number of preload and heart rate-independent approaches which are insensitive to increases in afterload (Kass et al., 1993, Sagawa et al., 1988). Systolic mechanical performance was determined using both auxobaric and isovolumic LV preparations (Sagawa et al., 1988). Systolic function was assessed from the slope of end systolic (ES) or peak systolic P-V, P-D, and stress-strain relations constructed from data obtained both *in vivo*, in anaesthetised, open-chest, ventilated rats, as well as *ex vivo*, using an isolated, constant flow, paced and perfused heart preparation in both the presence and absence of an adrenergic stimulus. The slopes of the LV ES P-D and stress-strain relations represent LV FS chamber (LV Ees) and myocardial (E^m) elastance respectively. The slopes of

the LV peak systolic P-V and stress-strain relations represent LV peak systolic chamber (E_{max}) and myocardial (E^{n}_{max}) elastance respectively. Using these approaches I could account for differences in load and heart rate, LV geometry, the degree of adrenergic stimulation or sympathetic versus parasympathetic activation, problems associated with isovolumic/isometric cardiac preparations, as well as anaesthetic effects on systolic mechanical performance.

In order to assess the influence of chronic androgenic steroid administration on diastolic mechanical performance, I chose to examine end diastolic P-V, P-D and stress-strain relations (the slopes of which, when linearised, represent either LV chamber or myocardial diastolic stiffness [*k*]) constructed from data obtained both *in vivo*, in anaesthetised, open-chest, ventilated rats, as well as *ex vivo*, using an isolated, constant flow, paced and perfused heart preparation. Using these approaches, again I could account for differences in load and heart rate, LV geometry, the degree of adrenergic stimulation or sympathetic versus parasympathetic activation, problems associated with isovolumic/isometric cardiac preparations, as well as anaesthetic effects on diastolic performance. In addition, by using an open-chest, pericardectomised preparation *in vivo*, and an isolated perfused, constant flow preparation *ex vivo* to assess diastolic performance, I could exclude the potential contribution of pericardial, pleural, right ventricular and lung forces as well as the contribution of coronary perfusion pressures when explaining differences in diastolic P-V and P-D relations between steroid treated and control rats.

As changes in diastolic performance subsequent to chronic androgenic steroid administration were noted in the presence of an intact systolic performance *in vivo* in the studies described in this thesis, I also used indices of preload-recruitable stroke work (Glower et al., 1985, Sunagawa et al., 1985), (assessed *in vivo)*, to examine the effect of alterations in diastolic performance on systolic function. Stroke work was expressed as a function of incremental LV filling pressures as well as a function of an index of LV filling volumes. The assumption is that an increased LV diastolic stiffness (an impaired diastolic performance) will result in a reduced systolic function when employing filling pressures but not indices of filling volume as a measure of preload (Norton et al., 1993).

CHAPTER 2

High dose androgenic steroid administration alters ventricular mechanical performance in rats.

2.1 Introduction

Androgenic-anabolic steroids are synthesised for use in modern medicine for therapeutic reasons. However, athletes use androgenic-anabolic steroids at supraphysiological doses in order to improve physical performance through an effect on muscle mass and strength (1.2.2 of chapter 1, page 28). However, a number of hepatic, psychological and endocrine adverse effects as well as changes in lipid profiles which are considered atherogenic have been described to occur subsequent to androgenic-anabolic steroid use (1.2.3 and 1.3 of chapter 1, pages 29-31).

Although histological effects of anabolic steroids on cardiac tissue have been demonstrated (Appell et al., 1983, Behrendt and Boffin (1977), our understanding of the actions of androgens on cardiac mechanical performance is incomp ·ie evidence that exists describing the influence of chronic high dose anabolic-androgenic steroids on cardiac mechanical performance in sedentary animals or humans is not comprehensive. Cardiac systolic performance has been shown to be either unchanged (Liang at al., 1993), or decreased when assessed in the presence of an inotrope (Ramo, 1987a) in untrained animals following androgenic steroid adminstration, and is reduced in exercise trained animals (Karhunen et al., 1988, Liang at al., 1993, Ramo et al., 1987b). Only minor alterations in indices of diastolic function have been found in humans (Urhausen at al., 1989). Nevertheless, the interpretation of the data obtained in these studies is obscured by a number of inadequacies. These include the use of load and heart rate-dependent measures of cardiac mechanical performance employed by these

authors (Karhunen et al., 1988, Ramo et al., 1987a,b), the inability of these authors to account for variations in LV geometry between animals or individuals, or between groups of animals or humans (Karhunen et al., 1988, Liang at al., 1993, Ramo et al., 1987a,b, Urhausen at al., 1989), and lastly the distinct lack of, or inadequacies in, measurements used to evaluate diastolic mechanical performance (Karhunen et al., 1988, Liang at al., 1993, Ramo et al., 1987a,b).

The aim of this study was therefore to examine the effect of chronic administration of supraphysiological doses of an androgenic steroid commonly abused by athletes on preload and heart rate-independent measures of LV diastolic and systolic mechanical performance that are insensitive to increments in afterload (Kass et al., 1993, Sagawa et al., 1988) and account for differences in geometry. These measures were assessed from data obtained both *in vivo* in anaesthetised open-chest ventilated rats, as well as *ex vivo* in an isolated, constant flow perfused LV preparation in both the presence and absence of an adrenergic stimulus. Both *in vivo* and *ex vivo* approaches were employed to account for the limitations that each approach has alone.

2.2 Methods

2.2.1 Experimental groups

Ninety five male Sprague Dawley rats (OLAC, UK) weighing 150-250 g were used for this study. Rats were randomly divided into two groups: steroid and control. The steroid group (n=49) received an intramuscular injection of the androgenic-anabolic steroid, nandrolone decanoate (ester-4-en-3-one, 17-[(1-

oxodecyl)oxy]-, (17β)-17β-hydroxyester-4-en-3-decanoate -Deca Durabolin, Organon), biweekly into the quadriceps femoris muscle at a dose of 5 mg/kg. The control group (n=46) received a biweekly injection of arachis oil with 10% vol/vol benzyl alcohol, the vehicle for the androgenic steroid. Steroid and vehicle administration continued for a 3 month period and rats received standard laboratory rat chow and water *ad libitum* throughout this study. All rats were housed in a room that was lighted between 0600 and 1800.

2.2.2 Body weight and composition and gastrointestinal energy absorption.

Body weights were measured weekly in a random sample of 25 steroid treated and 23 control rats. A random sample of 10 rats from the steroid group and 10 control rats were placed in metabolic chambers for 4 days before the end of the experiment. Food consumption and faecal weight were measured over a 3 day period after a 24 hour adaptation period in the chamber. Gastrointestinal energy absorption was calculated from the difference between energy consumed and faecal energy output. Food and faecal energy were measured using bomb calorimetry (DDS CP500 Calorific Value Processor, Digital Data Systems).

The percent lean body mass was measured in these same 10 rats in each group at the end of the metabolic study with the use of a whole body scanner (model SA2, EM Scan)(Walsberg, 1988). Rats were anaesthetised with xylazine (10 mg/kg) and ketamine-HCl (45 mg/kg) to immobilise them during the scanning procedure. Lean body mass and fat mass was calculated from the percentage of

the body weight measured as lean tissue (fat-free mass) and the percentage measured as fat tissue respectively.

2.2.3 Systolic blood pressure and heart rate

Systolic blood pressure and heart rate were measured fortnightly using a technique previously described (Bunag, 1973). Briefly, rats were trained in stocks prior to the first measurement of systolic blood pressure in order to allow them to adapt to the procedure. Using a tail cuff plethysmographic technique, measurements were taken on prewarmed conscious rats. Each read' , taken at midday to avoid diurnal variation and the mean of three measurements was recorded.

2.2.4 Measurements of LV mechanical performance

Left ventricular systolic and diastolic mechanical performance were assessed in steroid treated and control rats *in vivo*, in anaesthetised, open-chest, artificially ventilated animals, and *ex vivo* using an isolated, constant flow perfused, artificially paced, isovolumic heart preparation. The advantages of measurements made *in vivo* include the assessment of mechanical performance with relatively intact autonomic and other neurohumoral systems, the presence of a blood perfusate and measurements performed in a filling and ejecting preparation. The advantages of the *ex vivo* preparation include measurements performed at controlled heart rates and coronary flows, direct measures of LV

filling volumes, the absence of anaesthetic effects and the exclusion of neurohumoral influences.

2.2.4.1 Left ventricular performance as assessed *in vivo*

At the end of the three month steroid and vehicle treatment period, measurements of cardiac function were performed on a sample of rats from the 2 groups (steroid; n = 30, control; n = 30) *in vivo*. Rats were anaesthetised with fentanyl-droperidol (0.02 mg fentanyl and 1 mg droperidol intramuscularly, Janssen Pharmaceutica). A catheter was inserted into the right carotid artery for the measurement of systolic, diastolic and mean blood pressure as well as heart rate. A tracheostomy was performed and the animals were positive pressure ventilated (Harvard Apparatus, South Natick, Mass., USA) with room air. The animal's core temperature was monitored with a rectal thermometer and maintained within a physiological range with a table heater.

A second dose of anaesthetic (0.01 mg fentanyl and 0.5 mg droperidol) was administered intravenously. A midsternal thoracotomy was performed and the parietal pericardium dissected free from the heart. Room air was supplemented with oxygen to maintain arterial partial pressure of oxygen (PaO_2). PaO_2 was measured using a Corning model 168 (Corning, MA, USA) blood gas analyzer from carotid artery blood samples collected into 100 μl heparin coated capillary tubes. A dextran 70 solution in 0.9% NaCl (Baxter) with KCl, 4.7 mmol/ℓ^{-1}, $MgSO_4$, 1.19 mmol/ℓ^{-1}; and $CaCl_2$, 2.5 mmol/ℓ^{-1}, warmed to 35-37°C and buffered with $NaHCO_3$ to a physiological pH as previously described (Norton et

al., 1993) was infused to maintain mean blood pressure, which decreases with thoracotomy. The tip of a 23 gauge needle attached to a saline filled PP50 polyethylene catheter, coupled to a Statham (P23AA) pressure transducer, was inserted into the apex of the LV, directed toward the base of the heart, secured and used to record LV pressure (LVP), including LVEDP. Prior to performing these experiments, the amplitude-frequency response of the fluid-filled catheter system, including the pressure transducer and its dome was assessed as previously described (Norton et al., 1997). Catheter and needle combinations were only used if amplitude-frequency responses were shown to be flat to 10 Hz.

In order to measure LV dimensions *in vivo*, the technique of sonomicrometry as first described by Rushmer and Franklin, 1956 and later refined by this group (Franklin et al., 1962) was employed in this study. This approach which was originally developed for large animal studies has subsequently been modified by our group in order to avoid having to suture ultrasonic transducers to the LV wall, which in our hands results in decreases in systolic performance, or to glue the transducers to the LV wall which has proved to be of limited success in our hands because of the difficulties in positioning two piezoelectric transducers that are sufficiently aligned to obtain signals throughout the cardiac cycle. The two devices manufactured in our laboratory by Dr. G. Norton for measuring LV long axis segmental length changes and LV short axis diameter changes throughout the cardiac cycle in the rat LV allow for a practically "user friendly" and reproducible approach to sonomicrometry in

rodents (Trifunovic et al., 1995, Woodiwiss and Norton, 1995). These approaches, as applied to the study described in this thesis are as follows.

In a random sample of rats from each group (steroid; n = 18, control; n = 16), two 30 gauge needles, each attached to an arm of a metal hinge (Figure 2A), were inserted to a similar wall depth in the long axis of the anterolateral LV wall in order to measure LV segmental length changes throughout the cardiac cycle. Left ventricular segmental length changes were measured with piezoelectric transducers attached to the opposite arms of the metal hinge. The metal hinge was designed so that changes in the distance between the two 30 gauge needles was reflected in changes in the distance between the transducers attached to the opposite arms of the metal hinge. The distance between the piezoelectric transducers was precalibrated to accurately measure the distance between the needles. A medium for ultrasonic signals to transmit between the transducers was provided by suspending ultrasonic jelly (Aquasonic Parker Laboratories Inc., N.J., USA) between them. The ability of ultrasonic signals to be transmitted through this medium was checked during the experiment by monitoring signals on an oscilloscope (SS-5710 oscilloscope, Iwatsu electric Co.Ltd, Japan). The distance between the transducers was measured with a Triton Technology Inc. sonomicrometer (San Diego, Cal, USA).

In the remaining rats in the two groups on which cardiac performance was assessed *in vivo* (steroid; n = 12, control; n = 14), LV short axis external diameter, rather than long axis segmental length changes were measured. The device designed to enable us to use sonomicrometry to measure short axis changes

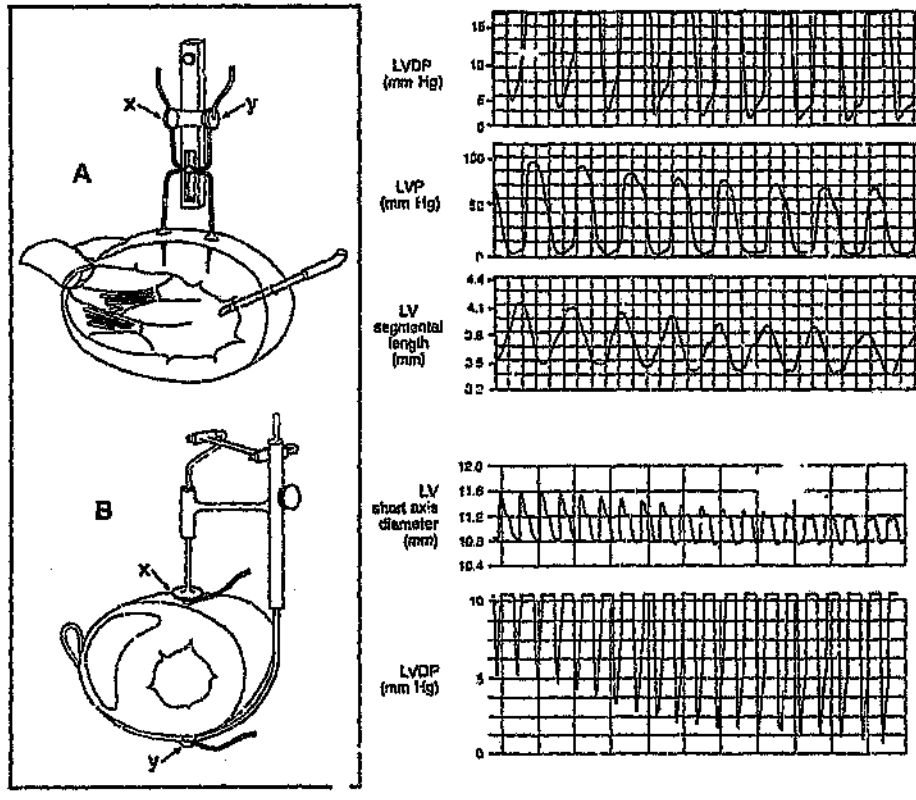

Figure 2. Schematic diagram of A; the device used for the measurement of LV long axis segmental length changes during the cardiac cycle using sonomicrometry and B; the device used for the measurement of LV short axis diameter changes during the cardiac cycle using sonomicrometry. "x" and "y" are the transmitting and the receiving ultrasonic transducers respectively. Typical traces of LVP, LV diastolic pressure and LV anterolateral wall segmental length or short axis diameter changes with inferior vena cava occlusion are provided. Original traces of short axis external diameter measurements during i.v.c. occlusion are provided on page 164 of appendix B.

throughout the cardiac cycle is illustrated in figure 2(B). This system allowed for ultrasonic signals to be transmitted directly across the short axis of the heart. At the end of each experiment, the positioning of the anterior and posterior wall transducers was checked at post-mortem to ensure that the positioning was similar in all experiments. The position of the anterior transducer was found to be 4-5 mm inferior to the pulmonary artery and to the left of the left anterior descending coronary artery. Using this device I was able to ensure that the piezoelectric crystals followed the myocardium closely during LV filling.

In a pilot experiment, our group has validated the use of this technique by correlating the LVED diameters (LVEDD) measured using this system with simultaneous LVEDD measurements obtained after suturing the transducers to the myocardium. The sutured transducers used were fixed to the myocardium adjacent to the pair attached to the device designed in our laboratory. In all of these experiments, I found that suturing the transducers resulted in a decrease in aortic flow as measured using a Narcomatic electromagnetic flow probe (Narco Bio-Systems, Houston, Texas, USA). However, the mean correlation co-efficient for "sutured" versus "cradle" measurements in 6 rats in which these measurements were successfully performed, was; $r = 0.989 \pm 0.001$ and $r^2 = 0.980 \pm 0.002$. The limits of agreement determined according to the method of Bland and Altman (1986) for the measurements obtained with the "cradle" were 0.078 mm below and 0.055 mm above the measurements obtained with the sutured transducers.

Recordings of LVP, LV long axis segmental length and LV short axis diameter were made on a Hellige Servomed recorder (Freiberg, FRG) and a

Beckman Type R411 Dynograph Recorder. Left ventricular pressures were amplified to a range of 0-15 mm Hg on a separate channel on the recorder in order to accurately measure diastolic pressures. Calibration of diastolic pressures in cm H_2O was achieved using a water filled "U-tube" and these values were subsequently converted into mm Hg. Further information on calibration techniques and equipment specifications is included in appendix E and F (pages 167-168). Cardiac dynamic responses were measured during inferior vena cava (i.v.c.) occlusion in order to obtain a range of end diastolic and end systolic lengths and pressures. Vena caval occlusion was achieved by compression of the vessel using a blunt edged artery forceps. A baseline (prior to i.v.c. occlusion) filling pressure of approximately 10 mm Hg was obtained in all rats by infusing the dextran 70 solution described above via the carotid catheter. Examples of typical traces of cardiac dynamic responses to vena cava occlusion using either the device constructed to measure long axis segmental length or the device constructed to measure external short axis diameter changes are illustrated in figure 2 and in the appendix B (page 164). No significant alterations in heart rate occurred while data used in the final analysis were recorded. If changes in heart rate were noted, blood volume was reduced by withdrawing 4-6 mls from the carotid arterial catheter until LVEDP values were approximately 5-6 mm Hg and the measurements repeated during i.v.c. occlusion over a lower range of preloads. Data was recorded during i.v.c. occlusion a minimum of three times on each rat to ensure reproducibility.

Diastolic cardiac performance

Left ventricular end-diastolic performance was assessed in a number of ways. Long axis segmental length values were corrected for the variability of unstressed LVED lengths (LVEDL at an LVEDP of 0 mm Hg = $LVEDL_0$) between individual rats using the following equation: $LVEDL\text{-}LVEDL_0/LVEDL_0$. Corrected LVEDL (which is equivalent to LVED strain) versus LVEDP relations were constructed and the slope of the linearised relations used as an index of long axis LVED stiffness.

Left ventricular ED chamber stiffness (k) was assessed from the slope of the linearised LVEDP versus LVED calculated internal diameter relation. Left ventricular ED internal diameter was determined as twice the internal radius (R), which was calculated using the equation given on page 161 of appendix A. As discussed on pages 42-47 of the literature review, LVED chamber k is influenced by a number of factors including cardiac geometry (Gilbert and Glantz, 1989, Mirsky, 1976). Left ventricular ED myocardial k, which takes into account possible differences in cardiac wall thickness and internal radius between the groups and the variability of cardiac geometry noted between individual animals, was determined from the slope of the linearised LVED stress versus LVED strain relation (Mirsky, 1976). Left ventricular ED strain was calculated using the equation given on page 162 of appendix A. A thick walled spherical model of cardiac geometry was assumed in the calculation of wall stress (Burns et al., 1991, Mirsky, 1976, Sagawa et al., 1988, Shroff and Motz, 1989, Weber et al.,

1988). Left ventricular ED wall stress was calculated from equation 1 of appendix A (page 161).

Systolic cardiac performance

Left ventricular systolic performance was also assessed using a number of approaches from LV long axis segmental length and LVP measurements. Left ventricular pressure-length area (P-L AREA) was used as an index of stroke work and was calculated as the area of the pressure-segmental length loop (Glower et al., 1985) for each beat during vena cava occlusion. The P-L AREA was related to both LVEDP and to LVED strain (indices of preload-recruitable stroke work) (Glower et al., 1985, Norton et al., 1993). The LV end-systolic pressure (LVESP) versus corrected LVES length (LVES strain) relation was used as an index of LV end systolic elastance (LV Ees) and thus LV contractility. Left ventricular ES strain (corrected length) was calculated from the equation given on page 162 of appendix A. The slopes of the linearised P-L AREA-LVEDP, P-L AREA-LVED corrected segmental lenght (LVED strain) and LVESP-LVES corrected segmental length (LVES strain) relations were used for statistical comparisons between groups.

2.2.4.2 Left ventricular performance as assessed *ex vivo*

At the end of the 3 month period of chronic androgenic steroid or vehicle administration, LV mechanical performance was assessed *ex vivo* in a sample of rats from each of the steroid treated (n=10) and vehicle treated control (n=9)

groups. Rats were anaesthetised with an intraperitoneal injection of ketamine HCl (75 mg/kg) and xylazine (15 mg/kg). The hearts were then excised and immediately rinsed in ice-cold physiological saline solution (PSS). Hearts were perfused retrogradely at a constant flow with 37°C PSS containing (in mM): NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $NaHCO_3$ 25, KH_2PO_4 1.2, $MgSO_4$ 1.2, and glucose 10 at a pH of 7.40 gassed with 95% O_2-5% CO_2. The coronary flow rate was determined volumetrically and adjusted to achieve an approximate flow of 12 ml/min/g heart weight, according to the estimated weight of the heart measured immediately after excision from the thorax with large vessels and pericardium still attached. The coronary perfusion pressure was monitored from a side arm of the aortic perfusion cannula with a Statham P23 transducer. The hearts were paced at 300 beats/min with the voltage at 10% above threshold via platinum wire electrodes attached to the left atrium and the apex of the heart.

Peak systolic LV pressure (LVP_{max}) and LV diastolic pressures were determined using a water filled balloon-tipped cannula (coupled to a Gould P50 pressure transducer) inserted via the left atrium into the LV cavity. A thin-walled, latex balloon with a filling volume beyond maximum LV lumen capacities was selected for this study in order to avoid the stiffness of the balloon wall contributing toward LVP at higher filling volumes. The volume of the balloon wall was assessed using a water displacement technique. Left ventricular pressures and coronary perfusion pressures were recorded using a Hellige Servomed (Freiberg, Germany) polygraph. Left ventricular pressures were amplified to a range of 0-30 mm Hg on a separate channel on the recorder in order to accurately

measute diastolic pressures. Calibration of diastolic pressures in cm H_2O was achieved using a water filled "U-tube" and these values were subsequently converted into mm Hg. A micromanipulator was used to gradually increase LV volumes to values which no longer resulted in increments in LV developed pressures. Left ventricular pressures were determined at as many multiple small increments in volume as were practically possible in order to improve on the accuracy of curve fitting during later analysis.

Systolic and diastolic cardiac performance

Left ventricular systolic chamber performance, as assessed from *ex vivo* measurements, was determined from the slope of the peak systolic pressure-volume (P_{max}-V) relationship (E_{max})(Sagawa et al., 1988, Weber et al., 1988). Left ventricular systolic myocardial performance, as assessed from *ex vivo* measurements, was determined from the slope of the linearised peak systolic stress-strain relationship (E^{n}_{max}) (Weber et al., 1988). Peak systolic stress and strain were calculated from previously described equations (Weber et al., 1988), assuming a thick-walled, spherical LV geometry (Burns et al., 1991, Sagawa et al., 1988, Shroff and Motz, 1989, Weber et al., 1988), using formulae as described on pages 161 and 162 of appendix A. Peak systolic pressures, as opposed to peak systolic developed pressures, were used to construct the LV P_{max}-V relation, as we assumed that diastolic stiffness would contribute toward systolic stiffness, and hence performance.

LV diastolic chamber and myocardial performance (stiffness-*k*) were determined using the same approach and using the same equations as for the assessment of systolic chamber and myocardial performance, except that diastolic pressures were used in place of systolic pressures in the relations constructed and the calculations performed.

Systolic and diastolic performance in the presence of an adrenergic stimulus

Subsequent to measuring P_{max} at incremental LV volumes in isolated perfused hearts in order to determine baseline E_{max} and E^n_{max}, isoproterenol (ISO-$10^{-6.5}$ M) (Sigma) was infused in a solution containing 0.1% sodium metabisulfate (Sigma) at 1% of the coronary flow rate in order to achieve a final concentration of ISO of $10^{-8.5}$ M. Left ventricular P_{max} and diastolic pressure measurements at incremental LV volumes were subsequently repeated in the presence of ISO in order to determine LV E_{max}, E^n_{max}, and LV diastolic and systolic chamber and myocardial *k* during a controlled adrenergic stimulus.

2.2.5 Ventricular diastolic geometry

In order to determine the effect of the androgenic steroid used in this study on left ventricular geometry, LV wall thickness (h) to radius (R) ratios were calculated over a range of LVEDPs and strains from measurements of short axis external diameter made on intact, beating rat hearts and post-mortem LV weights. Left ventricular "R" and "h" were calculated using equations provided in appendix A (pages 161-163).

In a randomly selected sample of 8 rats from each of the steroid treated and control groups, hearts were perfused with a PSS solution containing a K^+ concentration of 15 mmol/l in order to induce cardiac arrest in diastole. After fixing the K^+ arrested hearts in formalin, the LV was separated from the rest of the heart and maximum longitudinal and transverse diameters measured using callipers with a vernier scale. The ratio between the two diameters was then calculated in order to evaluate whether changes in LV long to short axis ratios had occurred subsequent to chronic administration of nandrolone decanoate.

2.2.6 Biochemistry, histology and heart weight.

At the end of the cardiac performance experiment conducted *in vivo*, rats were exsanguinated under anaesthesia and the hearts were extirpated and weighed. The LV and septum was dissected free from the right ventricle (RV) and weighed. A portion of the LV and RV was dried at 100°C for 48 hours to calculate dry heart weight.

Tissue samples were collected from the anterior lobe of the liver, fixed in formalin and embedded in paraffin in a random sample of rats from each of the steroid treated and control groups. Sections were cut and stained with haematoxylin, eosin and a reticulin stain for microscopic examination.

In a separate group of steroid (n = 9) and control (n = 7) rats, blood was taken from a PP50 polyethylene catheter implanted into the carotid artery under anaesthesia (0.02 mg fentanyl and 1 mg droperidol) for the measurement of plasma testosterone, blood glucose and free fatty acid concentrations. The blood samples were spun at 3000 g for 15 minutes, the plasma removed and stored at -

20°C. Plasma testosterone (Coat-A-Count, total testosterone, veterinary application: Diagnostics Products Corporation, Cal., USA), blood glucose (glucose oxidase, Glucoxact, Clinical Sciences Diagnostics, South Africa), and non-esterified fatty acid (NEFA) concentrations were measured using standard commercial kits and assay techniques. NEFA's were extracted from plasma as chloroform soluble copper salts and the concentration determined using a photometric technique.

2.2.7 Data analysis and statistical comparisons

Data analysis

Regression analysis was used to determine the lines of best fit for the cardiac function relations.

In vivo data. The LVEDP-LVED strain/or internal diameter and the LVED stress-LVED strain relations were found to best fit an exponential function: LVEDP (or LVED stress) = $b.exp^{(m.\ LVED\ internal\ diameter/strain)}$, which was linearised: ln LVEDP (or LVED stress)= ln b + m (LVED strain/internal diameter) for statistical analysis. The P-L AREA-LVEDP relation best fitted to a linear function (y = mx + b). The exponential function $y = mx^b$ was found to best fit the P-L AREA-LVED strain and LVESP-LVES strain relations, which were linearised (log y = mx + b) for statistical analysis.

Ex vivo data. The LVEDP-LVEDV and the LVED stress-LVED strain relations constructed from data obtained in isolated, perfused heart preparations were found to best fit exponential functions: LVEDP (or LVED stress) = $b.exp^{(m}$

$^{LVEDV/\ strain)}$, which were linearised: ln LVEDP (or LVED stress) = ln b + m (LVEDV or strain) for statistical analysis. The systolic LV P_{max}-V relations were linear. The systolic LV $stress_{max}$-strain relationships were found to best fit the following equation: $Stress_{max} = \ldots\ e^{(\ldots \ln strain)}$, which was linearised (ln stress = +m.strain+lnb) for statistical comparisons between the slopes (E^n_{max}).

Statistical comparisons and assessments

On regression analysis, an r value of > 0.95 was considered to be a good fit. Comparisons of the slopes (m) and the intercepts (b) of the cardiac function relations were made between groups using the unpaired Student's *t*-test. The same statistical procedure was used to establish differences between the groups for body weight (BW), baseline haemodynamics, heart weight, the radius to wall thickness ratio at increasing LVEDPs or LVED strains, the ratio between longitudinal and transverse LV diameters, systolic blood pressure (SBP) at the end of the three month treatment period, heart rate (HR), plasma testosterone, blood glucose and plasma free fatty acid concentrations, gastrointestinal energy absorption, food consumption, fat mass, lean body mass and % lean body mass. A paired Student's *t*-test was used to determine the effect of $10^{-8.5}$ M ISO on the slope of the cardiac function or linearised cardiac function relations assessed *ex vivo*. A repeated measures ANOVA followed by a Student-Newman-Keuls *post hoc* test was used to compare systolic blood pressures and body weight over the three month treatment period. All values in the text are presented as a mean ± SEM. Numbers in parentheses in the figures and the tables are sample numbers. A probability value of <0.05 was accepted as statistically significant.

2.3 Results

2.3.1 Chronic androgenic steroid administration decreases somatic growth

Nandrolone decanoate administration suppressed the growth of rats from week 7 until the end of the study as compared to control animals (Figures 3 and 4). The reduction in body weight was neither a consequence of differences in daily food intake (g of food/72 hours/100 g body weight) (steroid = 16.7 ± 0.3, control = 15.9 ± 0.3), nor differences in gastro-intestinal energy absorption (kJ/100 g body weight/24 hours) (steroid = 39 ± 7, control = 46 ± 5). Plasma testosterone concentrations (nmol/l) were markedly decreased as a result of exogenous steroid administration (steroid = 2.43 ± 0.33 ; control = 5.55 ± 0.53 : $p<0.01$) which may have contributed, in part, toward a decreased somatic growth. The decreased plasma testosterone concentrations indicated that a supraphysiological, rather than a physiological dose of the androgenic steroid was administered. The decreased growth was not associated with differences in fat versus protein metabolism, as the percentage lean body mass was similar between the steroid treated and control groups (steroid = 93.9 ± 0.91%, control = 93.4 ± 0.79%). The calculated lean body mass (g) and the calculated fat mass (g) were markedly decreased by steroid administration (lean body mass: steroid = 335 ± 7, control = 477 ± 15, $p<0.001$; fat mass: steroid = 21.9 ± 3.3, control = 33.4 ± 3.6, $p<0.05$). Histopathological analysis of the liver samples showed no hepatotoxic changes and hence alterations in body weight are unlikely to be attributed to a chronic active hepatitis.

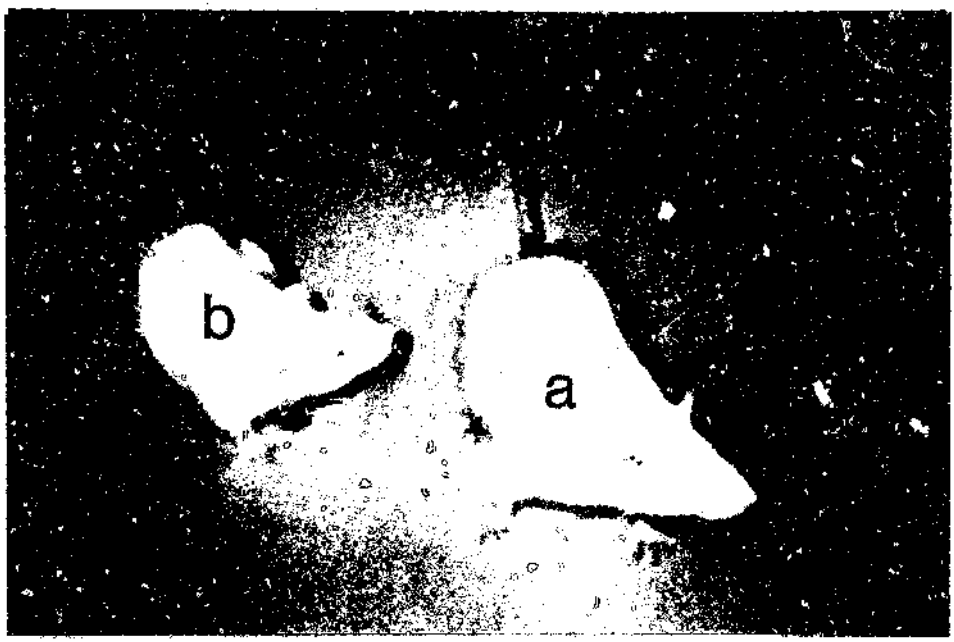

Figure 3. Photograph of a control (A) and nandrolone decanoate (steroid) treated (B) rat illustrating the effect of the androgenic steroid on body size.

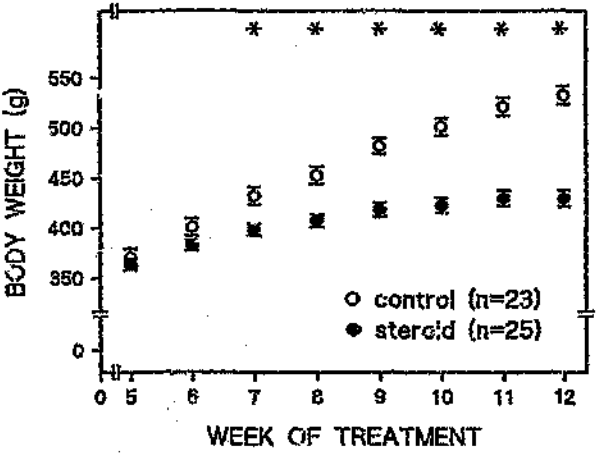

Figure 4. Graph shows the effect of nandrolone decanoate (steroid) on the growth

steroid versus control: * $p < 0.01$.

2.3.2 Androgenic steroid effects on heart weight, systolic blood pressure and heart rate.

Total wet and dry heart weight and LV weights were significantly decreased in the group receiving the androgenic steroid (Table 1). The change in heart weight is likely to reflect a decrease in growth induced by the anabolic steroid. However, there were no differences in RV weights between the groups (Table 1). The total heart weight expressed as a percentage of body weight was increased in the steroid treated group (Table 1).

Heart rate and systolic blood pressures were not different between the groups at any time period during the study, including at the termination of the experiment (Table 1).

2.3.3 Androgenic steroid effects on LV performance

Systolic and diastolic performance determined from long axis segmental length measurements *in vivo*.

Chronic androgenic steroid administration produced a leftward shift in the LVEDP-LVED corrected long axis segmental length (LVED strain) relationship as compared to the control group (Figure 5, upper panel). The slope of the linearised LVEDP-strain relation (LVED k) was enhanced in the steroid treated group of rats (Table 2). This did not occur because of differences in the baseline LVED needle distance as the unstressed LVED segmental length ($LVEDL_0$) was

Table 1. The effect of a biweekly 3 month period of nandrolone decanoate (steroid) administration on wet and dry heart weight (HW), systolic blood pressure (SBP) and heart rate (HR) in rats.

	HW	HW/BW	LVW		RVW		SBP	HR
	(g)	(%)	wet (g)	dry (g)	wet (g)	dry (g)	(mm Hg)	(beats.min^{-1})
Steroid group (n=25)	1.26 ± 0.03*	0.29 ± 0.004*	0.89 ± 0.03*	0.198 ± 0.005*	0.34 ± 0.02	0.077 ± 0.003	120 ± 3	411 ± 8
Control group (n=23)	1.44 ± 0.04	0.27 ± 0.005	1.04 ± 0.03	0.239 ± 0.006	0.37 ± 0.02	0.086 ± 0.003	123 ± 2	392 ± 11

LV, left ventricle; RV, right ventricle.

HW/BW, wet heart weight/body weight.

* $p<0.01$ Steroid versus Control

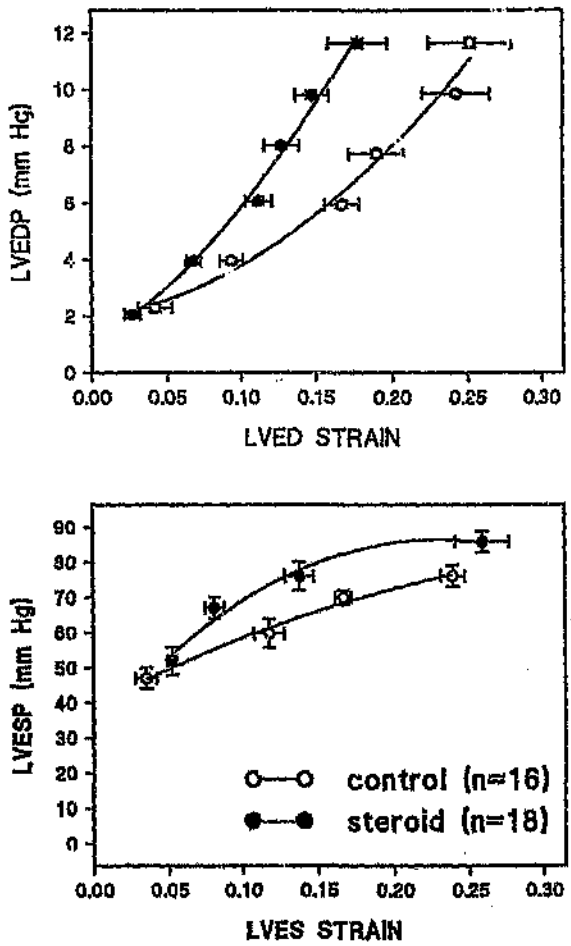

Figure 5. Line graphs show the effect of nandrolone decanoate (steroid) on LV diastolic performance as determined from the relations between LVEDP and LVED strain (top panel) and on LV systolic performance as assessed from the relations between LVESP and LVES strain (bottom panel) in rats. Strain values are calculated from long-axis segmental length measurements. The slopes of the linearised relations are summarised in Table 2.

Table 2. Slopes (m) and intercepts (b) of the linearised relations of figures 5, 6 and 8.

	Slope (m)	Intercept (b)
Figure 5		
ln LVEDP = ln b + m (LVED strain)		
Steroid group (n=18)	11.62 ± 0.83*	0.50 ± 0.10
Control group (n=16)	7.26 ± 0.44	0.60 ± 0.08
log LVESP = m (LVES strain) + b		
Steroid group (n=18)	0.90 ± 0.33	1.72 ± 0.05
Control group (n=16)	1.06 ± 0.15	1.65 ± 0.02
Figure 6		
ln LVEDP = ln b + m (LVED INT D)		
Steroid group (n=12)	1.48 ± 0.04*	8.1 ± 0.3
Control group (n=14)	1.06 ± 0.09	5.9 ± 0.7
ln LVED stress = ln b + m (LVED strain)		
Steroid group (n=12)	54.3 ± 4.0*	0.158 ± 0.13
Control group (n=14)	35.6 ± 3.1	0.407 ± 0.17
Figure 8		
P-L AREA = m (LVEDP) + b		
Steroid group (n=18)	1.76 ± 0.08*	3.94 ± 0.60
Control group (n=16)	2.25 ± 0.12	3.51 ± 0.88
log P-L AREA = m (LVED strain) + b		
Steroid group (n=18)	1.57 ± 0.24	1.02 ± 0.003
Control group (n=16)	1.01 ± 0.24	1.07 ± 0.05

LVED INT D, left ventricular end-diastolic internal diameter; LVEDP, left ventricular end-diastolic pressure; LVED strain, left ventricular end diastolic strain; P-L AREA, pressure length area (index of stroke work); LVESP, left ventricular end-systolic pressure; LVES strain, left ventricular end-systolic strain. * $p < 0.01$, Steroid versus control.

similar between the groups ($LVEDL_0$: steroid = 5.09 ± 0.24 mm; control = 5.2 ± 0.16 mm).

The slope of the LVESP versus LVES strain relation (Figure 5) was concave toward the LVES strain axis, consistent with the shape of the contractility curves obtained by Santamore et al. (1991). There were no statistically significant differences between the slopes of these linearised relations between the two groups (Table 2). Hence, this index of LV contractility was unaltered by androgenic steroid administration. An analysis of the heart rates between the two groups, immediately prior to and during i.v.c. occlusion revealed no significant differences between the groups. Thus, the effect of alterations in heart rate on cardiac contractility did not mask differences between the groups.

Diastolic performance determined from short axis dimension measurements *in vivo*

A left shift of the LVEDP versus LVED internal diameter relation was noted in the steroid treated group as compared to the control group (Figure 6, upper panel). The left shift in this relationship was the consequence of an increased slope of the relationship (Table 2). The increased slope of the linearised LVEDP-LVED internal diameter relationship indicates an increased LVED chamber *k* (LVED chamber stiffness-Figure 7). The LVED stress versus strain relationship was also shifted to the left following chronic androgenic steroid administration (Figure 6, lower panel). This altered relationship between LVED

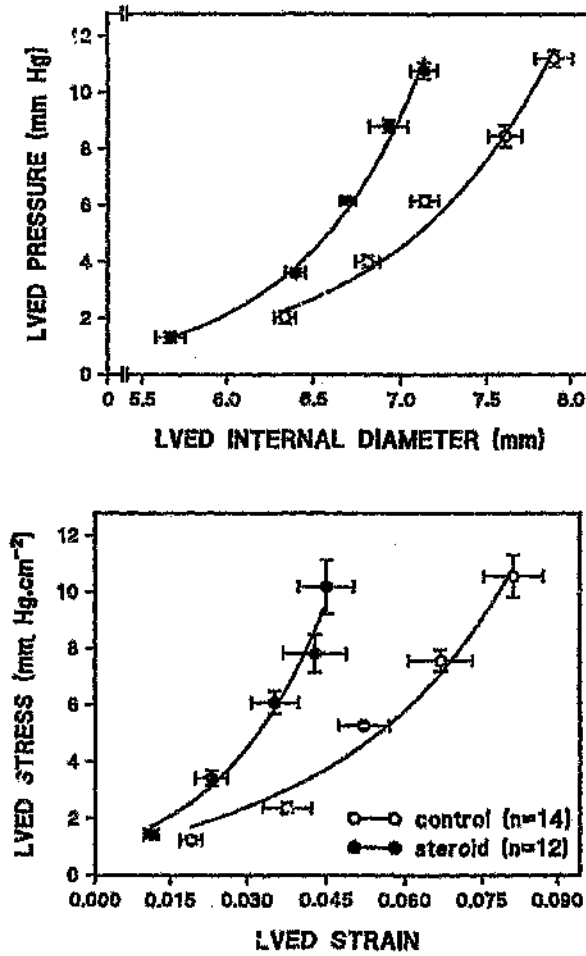

Figure 6. Line graphs show the effect of nandrolone decanoate (steroid) on LVED performance as assessed from the relationship between either LVEDP and LVED internal diameter (chamber performance) (upper panel), or LVED stress and LVED strain (myocardial perform ance) (lower panel) in rats. These were values calculated from LV short axis external diameter measurements. The slopes of the linearised relations are given in table 2 and figure 7.

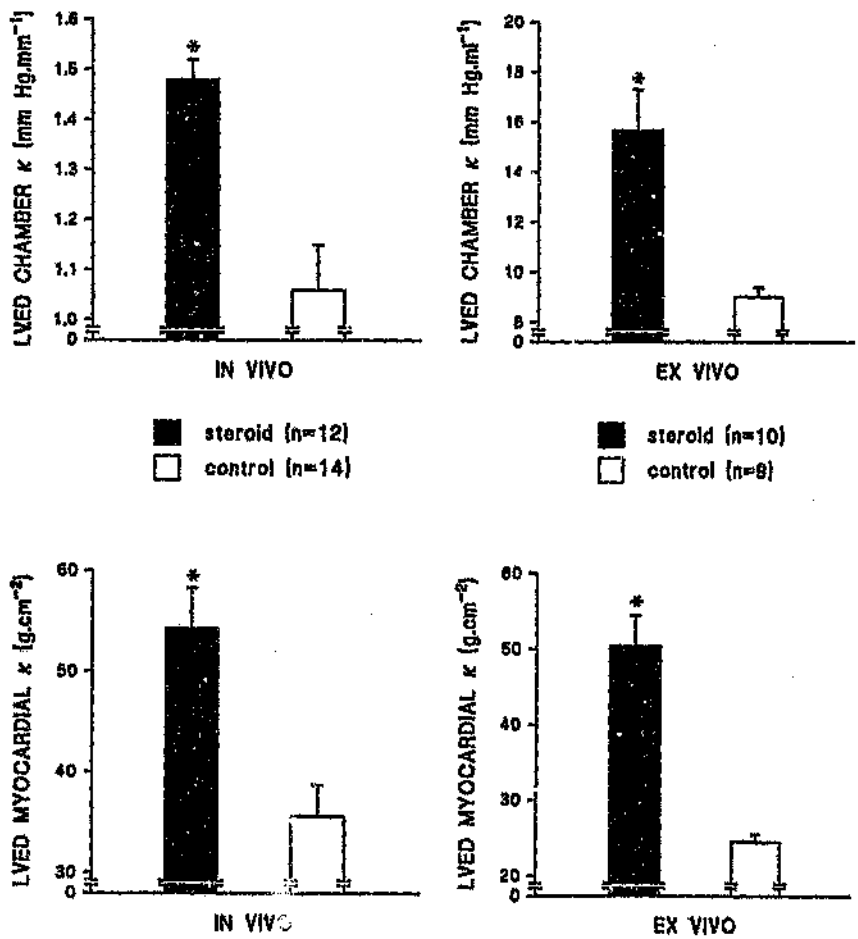

Figure 7. Effect of nandrolone decanoate (steroid) on LVED chamber and myocardial stiffness constants (k) in rats. *In vivo* (left panels) indicates stiffness constants calculated from LVEDP-dimension data obtained in anaesthetised, ventilated, open-chest rats, and *ex vivo* (right panels) indicates stiffness constants calculated from LVEDP-volume data obtained in isolated heart preparations.

stress and strain was also the consequence of an increased slope of the relationship (ED myocardial *k*) (Table 2 and Figure 7).

Preload-recruitable stroke work determined from long axis segmental length measurements *in vivo*.

The androgenic steroid decreased the slope of an index of preload-recruitable stroke work when LVEDP was used as an index of ventricular filling (Figure 8 and Table 2). However, no difference in the slope and intercept of the linearised function of the stroke work-LVED corrected segmental length (strain) relation was noted between the groups (Figure 8 and Table 2).

Left ventricular diastolic performance as determined *ex vivo*.

In keeping with results obtained in anaesthetised, artificially ventilated, open-chest rats, nandrolone decanoate administration produced a left shift in both the LV diastolic P-V and stress-strain relationship as determined in isolated, constant flow perfused, heart preparations (Figure 9). The left shift in the latter relations was in part a consequence of an increased slope of these relations (slope of the linearised diastolic P-V and stress-strain relations are given in Table 3 and Figure 7) and hence because of a steroid-induced increase in LVED chamber and myocardial *k* (Table 3 and Figure 7). In addition, the left shift in the LVED pressure-volume relationship following steroid administration was also as a consequence of smaller hearts, as evidenced by a reduced volume intercept

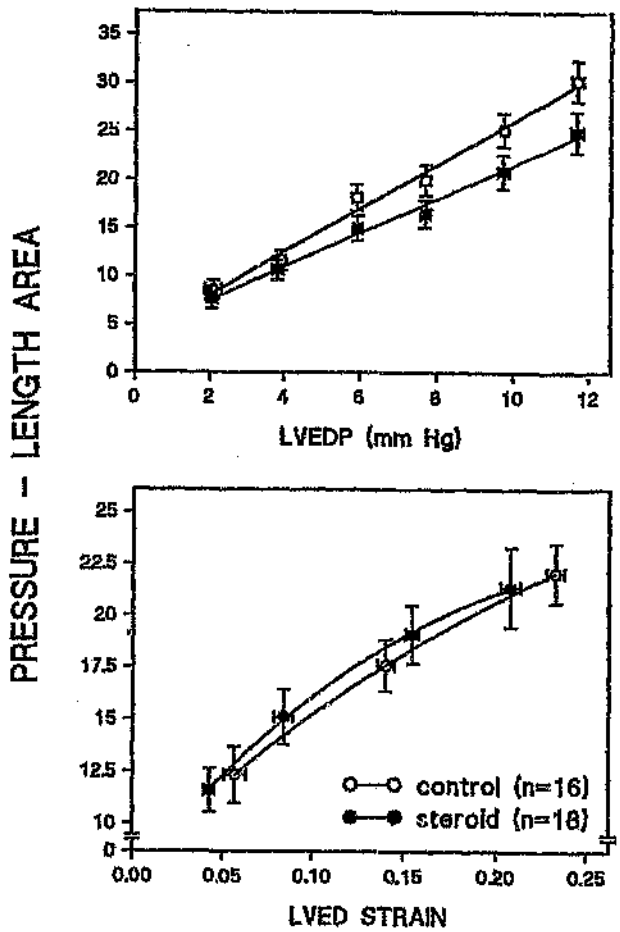

Figure 8. Line graphs show the effects of nandrolone decanoate (steroid) on the P-L AREA ~ stroke work relation with LVEDP (upper panel) and with LVED strain (lower panel) in rats. The myocardial length changes used in the calculation of indices of stroke work and strain were obtained from the long axis segmental length measurements. The differences in the slope of these relations are given in table 2.

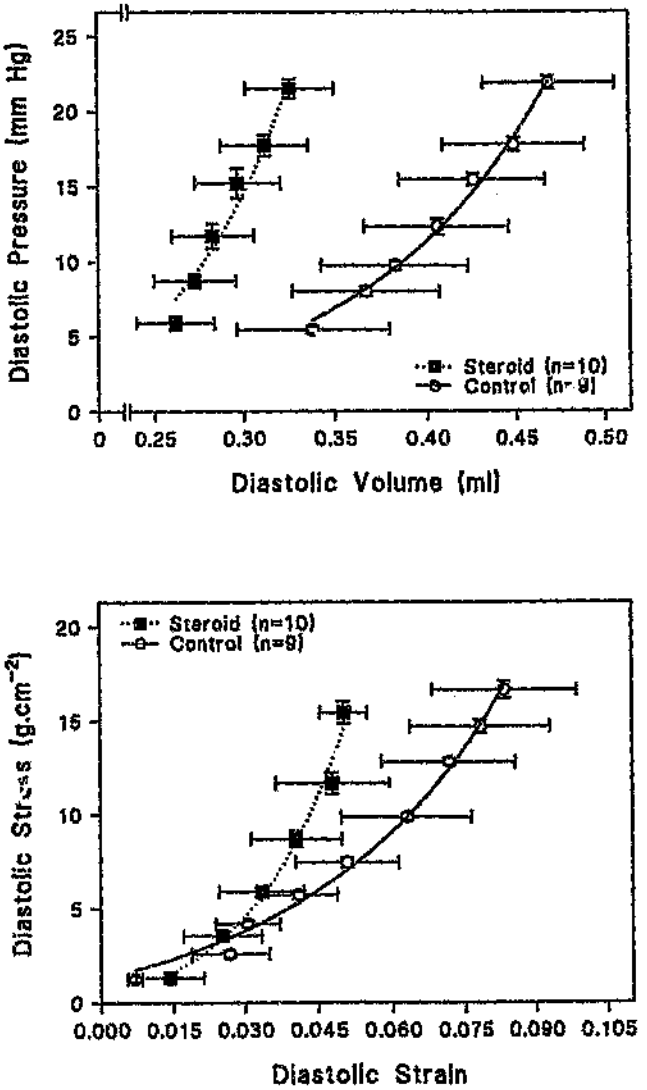

Figure 9. Line graphs show the effect of chronic nandrolone decanoate (steroid) administration on LV diastolic pressure-volume (upper panel) and stress-strain (lower panel) relations as determined in isolated perfused heart preparations. The slopes of the linearised relations are given in Table 3. Statistical comparisons between the slopes of the linearised relations are made in Table 3.

of the diastolic P-V relation (V at an LVEDP of 0 mm Hg -V_0) (steroid = 0.236 ± 0.001 mls, control = 0.294 ± 0.004 mls, $p<0.001$). The differences in diastolic performance noted between the groups cannot be attributed to discrepancies in coronary flows (mls/min/g heart weight) (steroid = 12.3 ± 0.5, control = 12 ± 0.5) or coronary perfusion pressures (mm Hg) (steroid = 82 ± 6, control = 78 ± 4) between the groups.

Left ventricular systolic performance as determined *ex vivo* in the absence of an adrenergic stimulus.

The LV peak systolic P-V relation (P_{max}-V) as assessed *ex vivo* at controlled coronary flows (coronary flow values are given above) was shifted to the left in rats receiving nandrolone decanoate (Figure 10, upper panel). This steroid-induced left shift in the P_{max}-V relation was partly as a consequence of a modestly increased slope (E_{max}) of the relationship (Table 3) indicating a beneficial action of nandrolone decanoate on systolic chamber performance. However, the major reason for the steroid-induced left shift in the P_{max}-V relationship was because of effects of the androgenic steroid on LV geometry. The volume intercept (V at a P_{max} of 0 mm Hg) of the P_{max}-V relationship was reduced in steroid treated rats (steroid = 0.098 ± 0.005 mls, control = 0.118 ± 0.003 mls, $p<0.05$). Furthermore, the modest differences in E_{max} noted between steroid treated and control rats was accounted for by converting the data to LV $stress_{max}$-strain relations (Figure 10, lower panel) as the linearised slopes of these relations (E^n_{max}) (Table 3) were the same when comparing the steroid treated and control

Table 3. Slopes (m) and intercepts (b) of the linearised relations of figures 9, 10, 11 and 12.

	Slope (m)		Intercept (b)
Figure 9.			
ln LVEDP = lnb + m (LVEDV)			
Steroid (n=10)	16.7±1.6**		-1.96±0.41
Control (n=9)	9.0±0.4		-1.1±0.15
ln LVED stress = lnb + m (LVED strain)			
Steroid (n=10)	50±4**		0.21±0.18*
Control (n=9)	24.5±1		0.84±0.07
Figure 10.			
$LVSP_{max}$ = m (LVV) + b			
Steroid (n=10)	1086±91*	(E_{max})	104±14
Control (n=9)	850±38	(E_{max})	99±7
LV $stress_{max}$ = m (strain) + lnb			
Steroid (n=10)	18.3±2.4	(E^n_{max})	5.8±1.6
Control (n=9)	19.2±2.1	(E^n_{max})	5.4±1.3
Figure 11.			
$LVSP_{max}$ = m (LVV) + b			
Control (n=9)	850±38	(E_{max})	-99±7
Control ISO (n=9)	1203±41††	(E_{max}-ISO)	-147±7*
Steroid (n=10)	1086±91*	(E_{max})	-104±14
Steroid ISO (n=10)	1094±53#	(E_{max}-ISO)	-106±9
Figure 12.			
LV $stress_{max}$ = m (strain) + lnb			
Control (n=9)	19.2±2.1	(E^n_{max})	5.4±1.3
Control ISO (n=9)	25.6±1.9†	(E^n_{max}-ISO)	5.7±0.9
Steroid (n=10)	18.3±2.4	(E^n_{max})	5.8±1.6
Steroid ISO (n=10)	18.5±1.5#	(E^n_{max}-ISO)	6.4±1.1

E_{max} = slope of the LV systolic pressure-volume relation (chamber systolic performance); E^n_{max} = slope of the LV systolic stress-strain relation (myocardial systolic performance); ISO, isoproterenol; LVEDV, left ventricular end diastolic volume; LVEDP as in Table 2; $LVSP_{max}$, LV peak systolic pressure; LV $stress_{max}$, LV stress at peak systolic pressure; LVV, LV volume.* $p<0.05$, ** $p<0.01$, Steroid versus control.† $p<0.02$, †† $p<0.0001$, Control ISO versus control. # $p<0.009$, Steroid ISO versus control ISO.

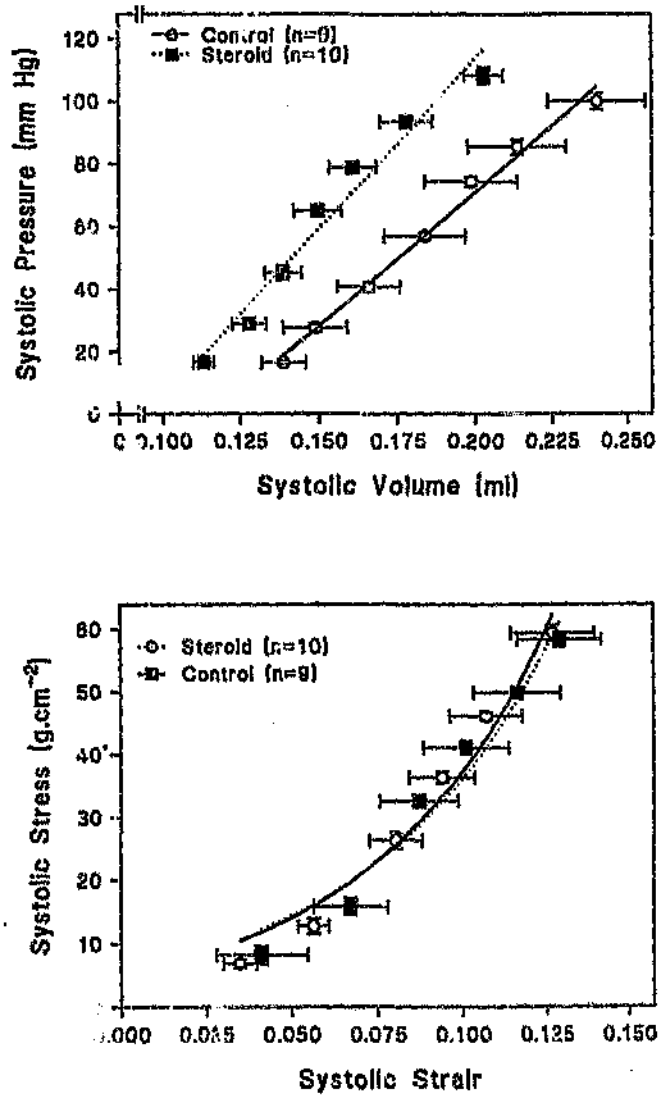

Figure 10. Line graphs show the effect of chronic nandrolone decanoate (steroid) administration on LV systolic pressure$_{max}$-volume (upper panel) and stress$_{max}$-strain (lower panel) relations as determined in isolated perfused heart preparations. The slopes of the relations or the linearised relations are given in Table 3. Statistical comparisons between the slopes of the relations or the linearised relations are made in Table 3.

groups.

Left ventricular systolic and diastolic performance as determined *ex vivo* in the presence of an adrenergic stimulus.

Isoproterenol ($10^{-8.5}$ M) infusion shifted the LV peak systolic pressure-volume relation (P_{max}-V) to the left and increased the slope of this relation (E_{max}) in control rats, but not in rats receiving nandrolone decanoate (Figure 11, upper panels and Table 3). In contrast to the steroid-mediated increase in E_{max} as assessed at baseline, as a consequence of the isoproterenol-induced increase in E_{max} in control rats, but not in steroid treated rats, no differences in E_{max} were noted between steroid treated and control groups in the presence of isoproterenol (Figure 11, Table 3).

Congruous with the response of LV systolic chamber performance to adrenergic stimulation, the isoproterenol infusion resulted in a left shifted LV systolic $stress_{max}$-strain relation and an increased slope of this relation (E''_{max}) in control rats, but not in steroid treated rats (Figure 12, upper panels and Table 3). As a consequence of differences in adrenergic responsiveness, chronic steroid administration resulted in a decreased E''_{max}, as determined in the presence of a controlled adrenergic stimulus in comparison to the control group (Figure 12, lower panel and Table 3).

Isoproterenol infusion to steroid treated rats failed to alter LV diastolic chamber or myocardial stiffness constants in steroid treated rats (LV diastolic

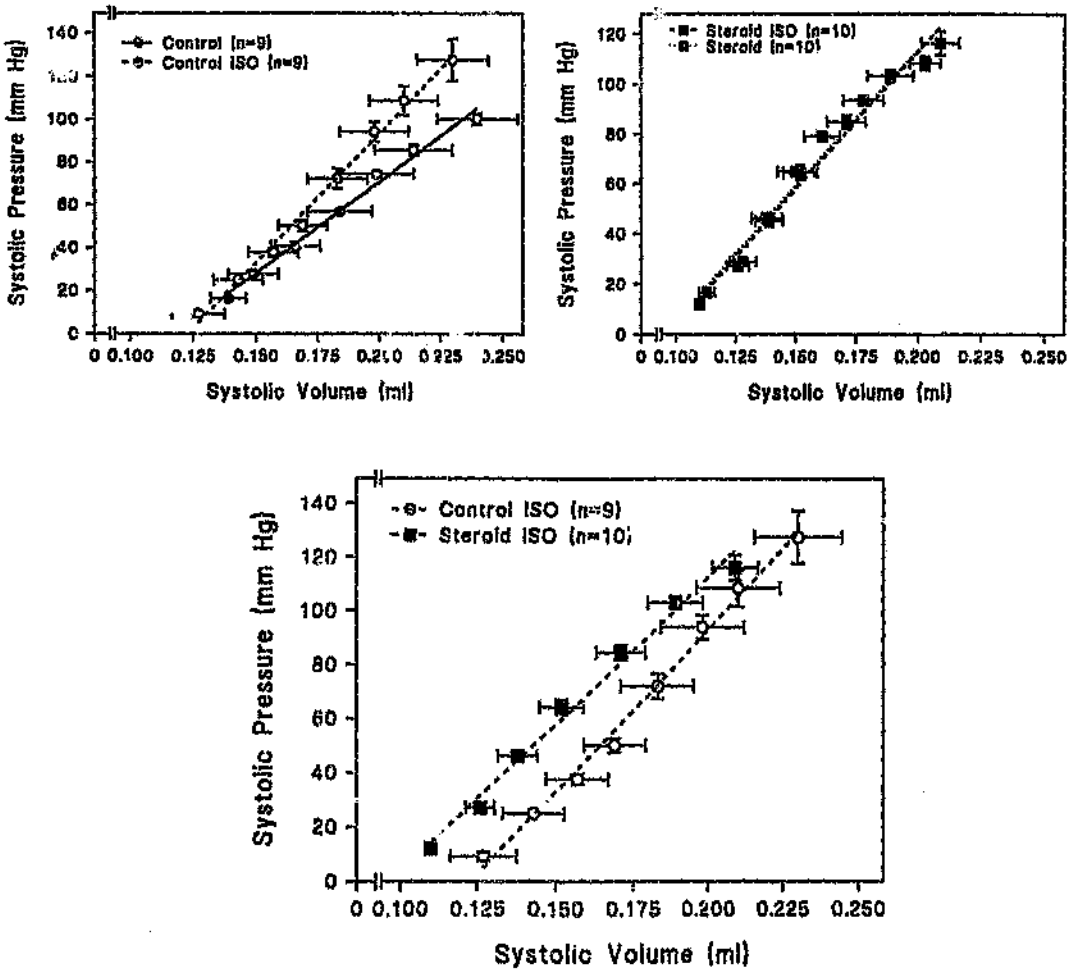

Figure 11. Line graphs show the effect of isoproterenol ($10^{-8.5}$ M) on LV systolic pressure$_{max}$-volume relations in chronic nandrolone decanoate (steroid) treated (upper right panel) and control (upper left panel) rats, as well as the differences in these relations between steroid treated and control rats as assessed in the presence of isoproterenol (lower panel). The slopes of the relations are given in Table 3. Statistical comparisons between the slopes of the relations are made in Table 3.

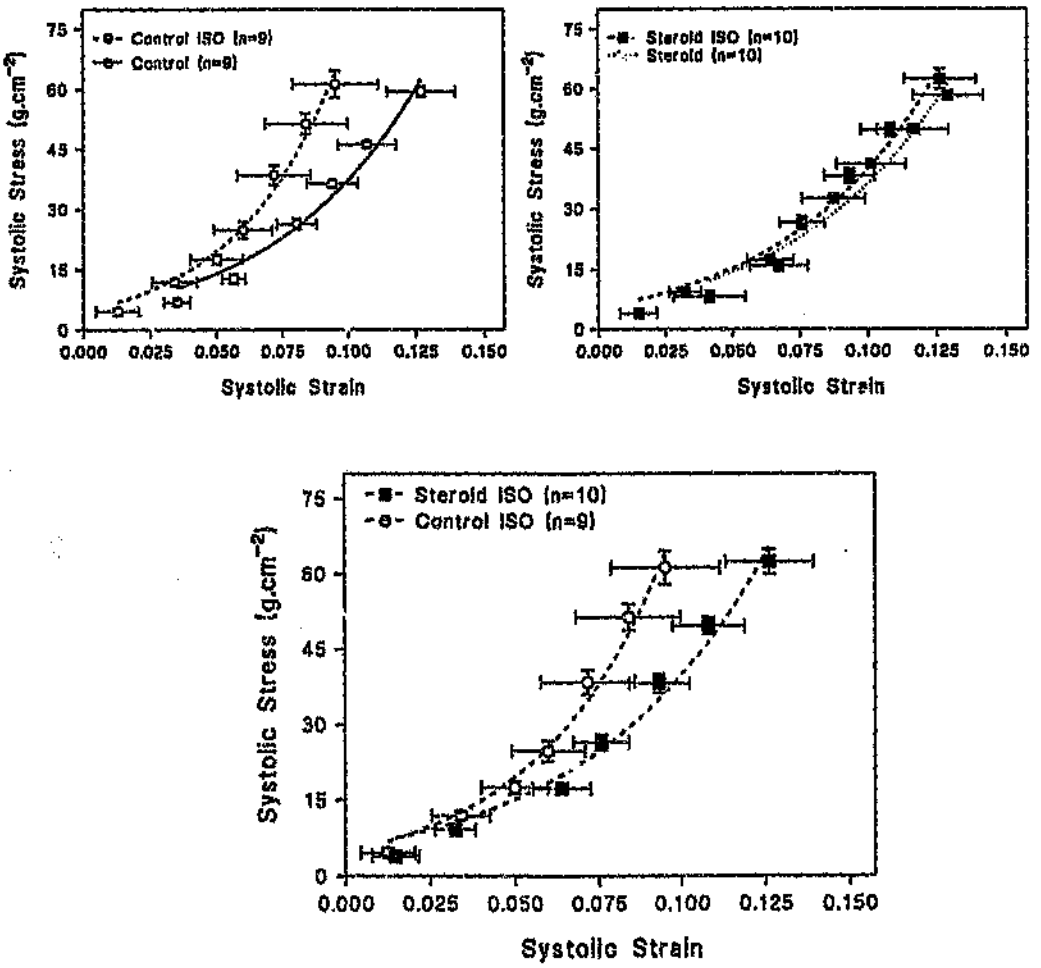

Figure 12. Line graphs show the effect of isoproterenol ($10^{-8.5}$ M) on LV systolic $stress_{max}$-strain relations in chronic nandrolone decanoate (steroid) treated (upper right panel) and control (upper left panel) rats, as well as the differences in these relations between steroid treated and control rats as assessed in the presence of isoproterenol (lower panel). The slopes of the relations are given in Table 3. Statistical comparisons between the slopes of the relations are made in Table 3.

chamber *k*- mm Hg. ml^{-1}, before ISO = 15.7 ± 1.6, after ISO = 12 ± 1.7; myocardial diastolic *k*- $g.cm^{-2}$, before ISO = 50.4 ± 4.3, after ISO = 42.4 ± 2.8).

LV geometry as determined in diastole.

Predictably, calculated LV wall thickness to radius ratios (h/R), as determined over a range of LVEDPs and strain values, decreased with increasing filling pressures in both the steroid treated and the control groups (Figure 13). However, no significant differences in h/R were noted between the groups over the range of filling pressures or strains examined (Figure 13). In addition, no differences in long to short axis internal radius ratios were noted between steroid treated and control groups as determined from longitudinal and transverse LV internal diameter measurements in K^+ arrested hearts (longitudinal to transverse LV diameter ratio: steroid = 1.2 ± 0.05, control = 1.12 ± 0.04). Hence, the decreased cardiac growth that occurred subsequent to chronic nandrolone decanoate administration failed to influence LV geometry as determined at end diastole.

2.3.4 Myocardial energy substrates

The androgenic steroid failed to alter either blood glucose (mmol/l) (steroid= 4.2 ± 0.2; control= 3.9 ± 0.3) or NEFAs (mmol/l) (steroid= 0.37 ± 0.055; control= 0.44 ± 0.065) concentrations.

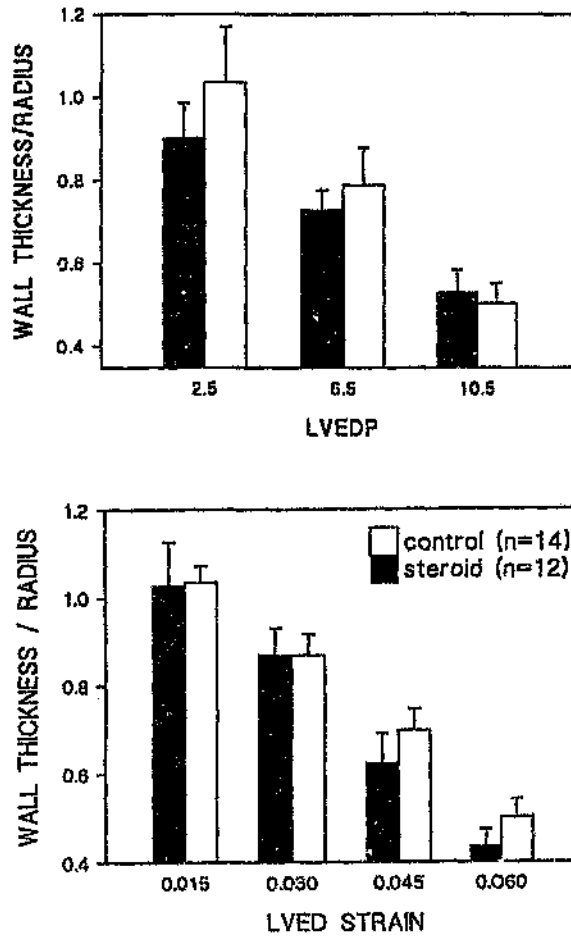

Figure 13. Histograms show the effect of chronic nandrolone decanoate (steroid) administration on LV wall thickness (h) to radius (R) ratios as determined over a range of LVEDPs (upper panel) or LVED strains (lower panel) in rats. No significant differences in the h/R were noted between the groups at incremental LVEDPs or LVED strains.

2.4 Discussion

The main findings of this study are as follows. Supraphysiological doses of an androgenic steroid (i.e. sufficiently high enough to produce suppression of circulating testosterone concentrations) resulted in a decrease in somatic and cardiac growth, a marked increase in LV diastolic stiffness as assessed both *in vivo* and *ex vivo*, but a preserved myocardial systolic mechanical performance as determined *in vivo* as well as *ex vivo* in the absence of a β-adrenoceptor stimulus in sedentary rats. Alternatively, myocardial systolic mechanical performance as determined *ex vivo* in the presence of a controlled β-adrenoceptor stimulus was reduced following chronic androgenic steroid administration. Androgenic steroid-induced alterations in LV chamber diastolic stiffness (slopes of the linearised diastolic P-D and P-V relations) were attributed to increases in myocardial stiffness (slopes of the linearised diastolic stress-strain relations) and not to alterations in LV geometry (LV wall thickness to radius ratios) which were similar between steroid treated and control groups as determined over a range of LV filling pressures. These data do not preclude the possibility that similar effects on LV mechanical performance might occur with longer periods of treatment at lower doses of androgenic steroids.

Androgenic steroid effects on growth.

An androgenic steroid-induced decrease in somatic growth as demonstrated in this study is in agreement with studies conducted in male rats treated with

androgenic steroids (Yu-Yahiro et al., 1989). In the study described in this chapter, the steroid-induced decrease in growth was not as a consequence of a reduced food consumption or a decreased gastrointestinal energy absorption. Koschakian and Endahl (1959) suggested that excess androgens leading to a greater use of body fat may be responsible for weight reduction. This suggestion is however unlikely as an equivalent decrease in both lean and fat mass occurred in rats treated with an androgenic steroid.

Other factors responsible for the decreased growth include a suppression of endogenous sex steroids, such as testosterone, as demonstrated in this study, or suppression of other growth factors such as somatomedins. Other authors have also demonstrated a decreased plasma testosterone concentration in response to androgenic steroid administration (Remes et al., 1977, Stromme et al., 1974). Exogenous oral administration of androgenic steroids is known to produce hepatotoxic changes (Mooradian et al., 1987), which in turn may decrease the rate of hepatic synthesis of somatomedins. The latter explanation, is however unlikely, as no histological evidence of hepatotoxicity was noted in my study. Other likely explanations for the decreased growth that I have not explored include either down-regulation of cytoplasmic steroid receptors in target cells or a decreased receptor response to exogenous as compared to endogenous androgens.

Androgenic steroid effects on blood pressure.

Androgenic steroid abuse is associated with hypertension in humans (Kuipers et al., 1991, Lenders et al., 1988). Previous studies have demonstrated

that treatment of rats with methyltestosterone and methylandrostenediol can induce hypertension (Molteni et al., 1969). My study failed to show alterations in systolic blood pressure in response to androgenic steroid administration. I therefore cannot attribute the increase in LVED stiffness to chronic alterations in ventricular systolic loading conditions although LV systolic stress was not determined in intact rats. The most likely reason for the disparity between my results and other authors with regards to blood pressure effects, is the difference in the type of androgenic steroid and the treatment regime used. The hypertension produced in other studies may in part be explained as a consequence of renal damage in the steroid treated rats (Molteni et al., 1969, Skelton, 1953). In my study I did not assess renal function.

Androgenic steroid effects on cardiac weight and geometry.

The heart weights of the steroid treated rats were significantly smaller than that of the control rats, probably reflecting a decreased total body growth. However, the heart weight to body weight ratio increased with steroid administration. It has previously been demonstrated that a decrease in the growth rate of rats is associated with a normal heart weight to body weight ratio (Van Liere and Northup, 1957). Hence, it is likely that the androgenic steroid has produced effects on cardiac protein turnover that may be different to the effects on the rest of the body. Androgenic steroids have been demonstrated to alter cardiac growth by modifying protein anabolism and catabolism (Kinson et al., 1991). However, in the study by Kinson et al. (1991), the effects of anabolic

steroid treatment on prepubertal cardiac growth and on cardiac hypertrophy in response to chronic aortic constriction was investigated. In the study described in this thesis, rats were treated post-pubertally, and surgery to induce an increase in afterload to the LV was not performed.

Data obtained from echocardiographic studies suggests that androgenic-anabolic steroid abuse in body-builders augments cardiac hypertrophy induced by their training regimes (De Piccoli et al., 1991, Urhausen et al., 1989), this being accompanied by diastolic cardiac dysfunction (Urhausen et al., 1989). As the study by Urhausen et al. (1989) was in weightlifters and body-builders, it is difficult to separate the effects of steroids from those of exercise training. However, other authors have reported on no changes in LV morphology in athletes using androgenic steroids (Salke et al., 1985). Nevertheless, the study by Urhausen et al. (1989) raises the question as to whether diastolic abnormalities subsequent to steroid abuse occur as a consequence of an enlarged heart or because of alternative changes. In the study described in this thesis, nandrolone decanoate administration alone was able to produce diastolic mechanical abnormalities without changing LV geometry as assessed in diastole.

Androgenic steroid effects on diastolic mechanical performance

A steroid-induced increase in the slope of the LVEDP versus LVED corrected segmental length relations (long axis segmental length measurements *in vivo*), LVEDP versus LVED internal diameter relations (short axis dimension

measurements *in vivo*), LVEDP versus LVEDV relations (*ex vivo* measurements) and LVED stress versus strain relations calculated from both *in vivo* and *ex vivo* measurements, indicates unequivoc ..y that the androgenic steroid used in this study has produced a marked increase in LV stiffness. This in turn was responsible for the reduced overall cardiac performance as assessed by the P-L AREA versus LVEDP relation (preload-recruitable stroke work index).

The increased LV diastolic stiffness following chronic nandrolone decanoate administration to sedentary rats in this study is in contrast to the lack of effect noted by Liang et al. (1993). Possible explanations for this discrepancy include differences in the effectiveness of the androgenic steroid used in the two studies, as well as an inadequacy in the analytical approach employed by Liang et al. (1993) (see chapter 1, pages 35-37). In our study nandrolone decanoate produced marked and reproducible decreases in somatic and cardiac growth which were not apparent in sedentary rats in the study by Liang et al. (1993). In addition, the diastolic P-V relations obtained by the latter authors (Liang et al., 1993) showed an upward and leftward shift in the steroid treated group, but the authors failed to assess the slopes of these relations. Furthermore, these authors (Liang et al., 1993) failed to assess diastolic stress-strain relations.

Left ventricular end diastolic stiffness may be influenced by a number of different factors, including pericardial, pleural and lung forces, the interaction between ventricular forces in diastole, viscoelastic forces, diastolic suction forces, coronary vascular engorgement, alterations in ventricular geometry, and changes in intrinsic myocardial passive or active properties. As LV chamber and

myocardial performance was assessed in open-chest rats, after a parietal pericardectomy, as well as in isolated, perfused heart preparations, the influence of pericardial, pleural and lung forces, as well as the effect of RV diastolic forces on the LV diastolic P-V relation may be ignored (rev- Gilbert and Glantz, 1989). The extent to which changes in viscoelastic forces contribute toward ventricular diastolic stiffness in normal or diseased hearts has not been determined (rev- Gilbert and Glantz, 1989). As suction forces are likely to contribute toward diastolic P-V relations mainly in the early, but not in the later phase of diastole (rev- Gilbert and Glantz, 1989), and the androgenic steroid increased LV stiffness *in vivo* at end diastole, it is unlikely that suction forces can account for the differences in diastolic performance noted between the groups in this study. Coronary engorgement is determined by aortic perfusion pressures at the orifice of the coronary vessels (rev- Gilbert and Glantz, 1989). Arterial blood pressures were not significantly different between the groups for any given LVED strain (index of filling volume) as determined *in vivo* (data not shown) and coronary perfusion pressures were matched between groups in the *ex vivo* preparation. Hence alterations in coronary vascular engorgement are unlikely to explain the steroid-induced increments in diastolic stiffness noted in this study. Consequently, the factors which need to be considered that might explain the diastolic performance changes that occur following chronic administration of nandrolone decanoate include alterations in either myocardial passive and active properties or geometric influences.

An increase in the slope of the LVED stress-strain relation (the myocardial elastic stiffness constant), as a consequence of chronic administration of nandrolone decanoate, indicates that the androgenic steroid increased myocardial stiffness. The LVED myocardial elastic stiffness constant is a measure of LV stiffness which accounts for the influence of alterations in ventricular geometry. Hence, it is likely that the changes in LVED chamber stiffness subsequent to steroid administration were in part the result of alterations in LV myocardial properties.

Steroid administration failed to influence ventricular wall thickness to radius ratios as determined over a range of LVEDPs and LVED strain values. In addition, there were no significant differences between the steroid treated and control groups in the ratio between longitudinal and transverse LV diameters as determined in hearts arrested in diastole. Hence, the steroid-induced increase in LV stiffness is not attributed to an impact of ventricular geometry. Consequently, the increased diastolic stiffness constants produced by chronic administration of the androgenic steroid can only be attributed to changes in myocardial wall properties.

The possible changes in myocardial characteristics responsible for the increase in myocardial diastolic stiffness include alterations in either passive (collagen) or active (relaxation) properties. Androgenic steroids have been demonstrated to increase myocardial collagen content (Takala et al., 1991). In addition, androgenic-anabolic steroids increase the mobilisation of intracellular calcium stores and calcium influx from the extracellular space (Koenig et al.,

1989). An increase in the cytoplasmic calcium concentration during diastole may decrease the extent of ventricular relaxation. This in turn would impair ventricular filling and thereafter diastolic stiffness (rev- Gilbert and Glantz, 1989). The possible explanation for the increased Ca^{2+} fluxes produced by androgenic steroids has been suggested by Koenig et al. (1982, 1989). These authors demonstrated that rapid, transient polyamine synthesis is essential for androgenic stimulation of Ca^{2+} influxes. These alterations in polyamine synthesis are as a result of androgenic steroids acting on their receptors in cardiac myocytes.

A possible down-regulation of β_1-adrenoceptors or changes in post-receptor events may also explain the diastolic abnormalities noted in this study. Data obtained by Ranno (1987a) and myself (this study) have demonstrated decreased inotropic responses to isoproterenol as a result of androgenic steroid administration. If these changes are the consequence of a decreased myocyte intracellular cAMP concentration, one may speculate that this will lead to a depressed SR Ca^{2+} ATPase activity. This in turn could produce an increased cytosolic Ca^{2+} concentration during diastole and decrease ventricular relaxation. However, in this study an increased myocardial stiffness constant was noted when assessed in the *ex vivo* preparation in the absence of an adrenergic stimulus. Hence, in our study, it is unlikely that changes in β_1-adrenoceptors or post-receptor events would explain the alterations in diastolic performance noted.

It may be argued that a decreased energy availability as a result of androgenic steroid administration may alter the active relaxation properties of the myocardium and hence decrease the extent of LV relaxation. Cardiac relaxation

relies on the energy requiring Ca^{2+} ATPase dependent process of SR Ca^{2+} uptake during diastole. However, in my study, blood glucose and plasma free fatty acid concentrations were unaltered by steroid administration. In addition, previous studies in rats have demonstrated that chronic starvation does not influence the end diastolic P-V relation (Penpargkul et al., 1980).

Androgenic steroid effects on systolic mechanical performance

LVESP versus LVES strain, P-L AREA versus LVED strain, LVP_{max} versus LVV and LV $stress_{max}$ versus strain relations and the slopes of these relations were either unchanged or modestly enhanced following chronic androgenic steroid administration to rats. Thus cardiac contractility, as assessed in the intact, but anaesthetised rat, as well as *ex vivo* in the absence of an inotropic stimulus, was not attenuated by anabolic steroid treatment. These data are consistent with the findings obtained in an isolated, isovolumically beating left ventricular preparation in androgenic steroid treated rats (Liang et al., 1993).

Although the slope of the P-L AREA versus LVEDP relation was reduced subsequent to steroid administration, this finding is unlikely to be attributed to a decreased myocardial contractility. The index of LV Ees used in this study, which was determined from data obtained simultaneously with the values used to assess the PL-AREA-LVEDP relation was unchanged in the steroid treated group. It is therefore likely that the decreased slope of the index of preload-recruitable stroke work relation following chronic administration of an androgenic steroid, is the

result of an increased LVED stiffness, rather than a depressed cardiac contractility.

Left ventricular E^n_{max} was reduced in steroid treated rats as assessed in the presence of a controlled adrenergic stimulus. The latter results are consistent with the decreased inotropic response to isoproterenol infusion noted in dog hearts after chronic nandrolone decanoate administration as demonstrated by Ramo (1987a). However, unlike Ramo (1987a) who used load and heart rate-dependent measures of systolic cardiac performance in their study, I was able to show that the steroid-induced decrease in adrenergic-inotropic responsiveness was a consequence of alterations in myocardial contractile performance as opposed to an effect on the LV mediated through differences in load or heart rate. The steroid-induced decrease in myocardial contractility, as assessed in the presence but not in the absence of an adrenergic stimulus, suggests that changes in β-adrenoceptor sensitivity/affinity or post-receptor events, but not myofilament performance have occurred.

Potential study limitations.

No attempt was made in my study to block neurohumoral responses to acute decreases in preload when systolic performance was assessed *in vivo*. This may have masked changes in myocardial contractility. However, I adopted an approach designed to avoid the influence of these responses on measures of systolic performance. Data was collected over a brief time period within which autonomic responses are unlikely to produce cardiovascular effects. Indeed, heart

rate did not change for the duration of data collection, indicating minimal neurohumoral influences.

No attempt was made in my study to assess systolic myocardial mechanical performance over a range of isoproterenol concentrations. However, in a pilot study I noted that higher concentrations of isoproterenol resulted in LVP_{max} values at a balloon volume of 0 mls that were too high to allow for accurate P_{max}-V and $stress_{max}$-strain relations to be constructed. In addition, by assessing myocardial performance at baseline I was able to draw conclusions about myofilament contractile performance as opposed to β-adrenergic receptor or post-receptor (second messenger) events.

In my study I did not measure indices of ventricular relaxation. These measures indicate alterations in the rate rather than the extent of ventricular relaxation. The rate of ventricular relaxation influences the P-V relation during early diastole, whereas the extent of relaxation influences this relationship at the end of diastole (rev- Gilbert and Glantz, 1989). In addition, measures of the rate of ventricular relaxation are often difficult to interpret as they are not only indicative of changes in myocardial diastolic properties. The rate of ventricular relaxation depends to a large extent on the ability of myocytes to shorten to less than equilibrium lengths at the end of systole. This stores energy, which during isovolumic relaxation generates suction forces (rev- Gilbert and Glantz, 1989). The ability of myocytes to shorten is in turn dependent on myocardial contractile (systolic) properties. In addition, the rate of ventricular relaxation is also

decreased by an increased ventricular afterload, a decreased heart rate and a decreased preload.

The calculation of ventricular wall stress is based on a thick walled spherical model of ventricular geometry (Mirsky, 1976, Sagawa et al., 1988, Weber et al., 1988). This assumption may influence the interpretation of my results as this model of ventricular geometry underestimates wall stress (Janz et al., 1980). However, it has also been demonstrated that the thick walled spherical versus elliptical models of ventricular geometry yield similar values for the slope of diastolic stress versus strain relations (regional myocardial elastic stiffness constant) (Fester and Samet, 1974, Janz et al., 1980). I was not interested in comparing the absolute diastolic wall stress between the steroid treated and control groups, but rather the elastic stiffness constant. In addition, the same errors in calculating ventricular wall stress would have been made in both groups by assuming a spherical as opposed to an elliptical geometry.

2.5 Conclusions

The results obtained in this study indicate that chronic administration of supraphysiological doses of an androgenic steroid that is commonly used by athletes to improve muscle strength increases LVED chamber stiffness through alterations in diastolic myocardial properties in rats. Despite these changes, the androgenic steroid failed to influence indices of myocardial contractility as measured in anaesthetised, open-chest, artificially ventilated rats and in isolated perfused hearts in the absence of an adrenergic stimulus. However, in the

presence of a controlled adrenergic stimulus *ex vivo*, a decrease in myocardial contractile performance was noted in steroid treated rats. The increase in cardiac stiffness may be a consequence of changes in either myocardial passive (collagen) or active (relaxation) properties produced by the steroid. The steroid-induced decrease in myocardial systolic performance as assessed in the presence of, but not the absence of an adrenergic stimulus, suggests a steroid-mediated decrease in β-adrenergic receptor responsiveness or alterations in post-receptor events.

CHAPTER 3

High dose androgenic steroid-induced effects on ventricular diastolic stiffness and geometry following habitual exercise in rats.

3.1 Introduction

Exercise training results in an enhanced LV systolic performance (rev-Blomqvist and Saltin, 1983) possibly through changes in LV size (De Maria et al., 1978) mediated through an increased blood volume (Convertino et al., 1980). Chronic high dose androgenic steroid administration, an approach which is frequently used by athletes or bodybuilders to enhance muscle strength or bulk (Bower and Reardan, 1972, Casner et al., 1971, Crist et al., 1983, Fahey and Brown, 1973, Golding et al., 1974, Johnson and O'Shea, 1969), has been shown to attenuate the improved systolic mechanical performance that occurs subsequent to exercise training in animal models (Karhunen et al., 1988, Liang at al., 1993, Ramo et al., 1987b). However, except for one study, which was limited by the inadequate analytical techniques employed, and hence failed to adequately address the question (Liang at al., 1993) there are no investigations examining the effect of high dose androgenic steroid administration on LV chamber and myocardial diastolic mechanical performance in exercised animals.

An attenuated LVED chamber and myocardial stiffness has recently been shown to occur following chronic habitual exercise in both rodents (Woodiwiss and Norton, 1995) and in humans (Levine et al., 1991). These exercise-mediated decreases in LVED chamber and myocardial stiffness are thought to be beneficial during acute bouts of exercise as they allow for increments in filling volumes without producing marked changes in filling pressures (Levine et al., 1991, Woodiwiss and Norton, 1995). However, the potential beneficial effects of

habitual exercise on LV diastolic performance might be reduced in athletes that abuse androgenic steroids. As indicated in chapter 2 of this thesis, the most profound cardiac mechanical change that occurs subsequent to chronic administration of supraphysiological doses of androgenic steroids in sedentary rats is an increased LV diastolic chamber and myocardial stiffness.

Therefore, the aim of this study was to examine the effect of chronic administration of high dose androgenic steroids on the diastolic performance changes that occur subsequent to prolonged exercise. I used a rat model of habitual exercise which has previously been shown by investigators in our laboratory to decrease diastolic chamber and myocardial stiffness (Woodiwiss and Norton, 1995).

3.2 Methods

3.2.1 Experimental groups

Male Sprague Dawley rats (OLAC, UK) weighing 75-90 g were placed in voluntary exercise wheels for training selection. Training selection was carried out as previously described (Woodiwiss and Norton, 1995). Forty of seventy rats which ran on average more than 2 km/day over a 10-day period were chosen for the study. Selected rats were randomly divided into an exercised group receiving the androgenic steroid (n=11) and an exercised group receiving the vehicle of the androgenic steroid (n=10), a sedentary steroid treated group (n=10) and a sedentary vehicle treated group (n=10). The exercise steroid treated and exercised

control group were allowed to voluntarily exercise for 16 weeks before cardiac function was measured. The steroid treated group and exercise steroid treated group received a biweekly injection of the androgenic steroid nandrolone decanoate, as previously described (chapter 2, pages 53-54). The exercised and sedentary control groups received a biweekly injection of arachis oil with 10% (vol/vol) benzyl alcohol, the vehicle for the androgenic steroid, as previously described in chapter 2 (page 54). Nandrolone decanoate and vehicle injections were started when rats reached 150-250 g, approximately 3 weeks after exercise had been initiated. The sedentary steroid and control groups were housed without access to exercise wheels. Steroid and vehicle administration continued for a 3 month period and rats received standard laboratory rat chow and water *ad libitum* throughout this period. All rats were housed in a room that was lighted between 0600 and 1800. Body weight, systolic blood pressure and heart rate were measured in all rats using techniques previously described in chapter 2 (page 55).

3.2.2 Habitual exercise protocol.

The exercised steroid treated and exercised control rats were housed in separate cages attached to exercise-training wheels designed in our laboratory (see Figure 14). Rats had free access to the training wheels through an opening between the cage and the wheel. The circumference of the wheel was 1 m, and the wheels were designed to allow rotation in only one direction to prevent coasting or unmeasured running. Distance, speed and duration of exercise were monitored using Cateye Micro Cyclocomputers (CC-6000, Cat Eye Co. Ltd,

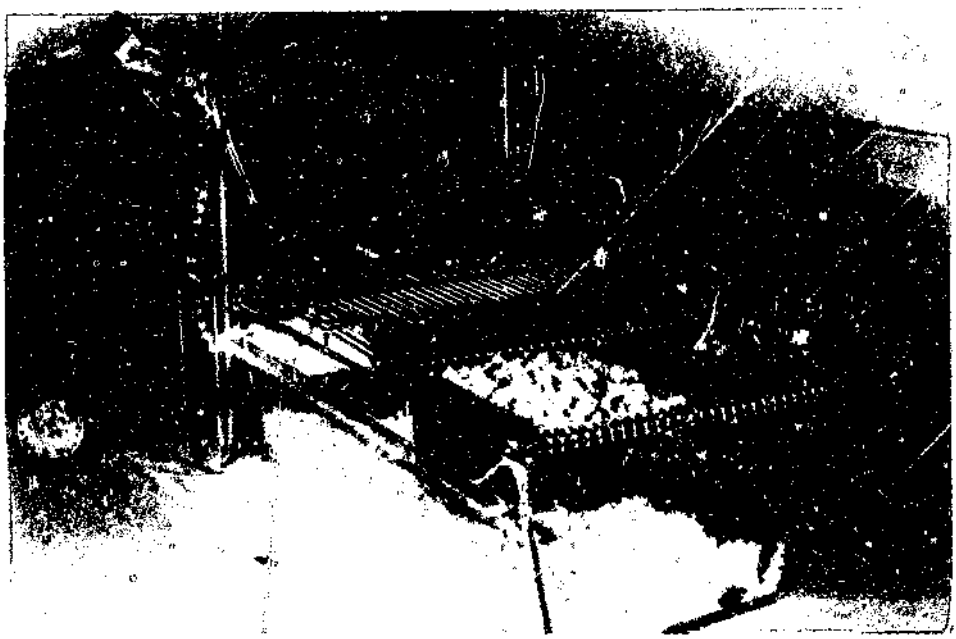

Figure 14. Photograph of an example of one of the exercise training wheels used in this study. It consists of a cage, a wheel with stopper allowing rotation in only one direction and a counter to measure distance and speed.

Japan). The average distance run over the 16 weeks was 2.15 ± 0.13 km/day for the exercised steroid treated group and 2.30 ± 0.24 km/day for the exercised control rats. The average speeds were 1.09 ± 0.05 km/h and 1.02 ± 0.09 km/h, for the exercised steroid treated and exercised control groups respectively. Neither the average distance run, nor the average speed were statistically different between the groups. Rats were allowed to exercise for four months and no significant decrease in daily performance was noted. It has been shown that moderately high levels of chronic exercise can be maintained for up to 7 months in rats that are preselected for their desire to run (Mondon at al., 1985). Lambert and Noakes (1990) showed that a significant training effect by increasing maximal oxygen consumption would occur in rats that ran more than 1.66 km/day.

3.2.3 Assessment of LV diastolic performance and LV geometry.

Because of the limited number of training wheels available, it was not possible to employ larger sample numbers and hence a variety of techniques to measure LV performance and geometry. Therefore, in this study I chose to use the anaesthetised, artificially ventilated, open-chest approach to assessing LV diastolic performance and geometry in rats. The isolated perfused heart preparation was not selected as the measurement technique of choice because of the unphysiological nature of assessing mechanical performance and geometry in isovolumic preparations. Only LV short axis diameters and LVEDPs were measured, rather than long axis segmental length changes together with LVEDPs because of insufficient sample numbers available. LV short axis rather than long

axis dimensions were measured as LVED stress can be calculated from short axis dimension, but not long axis segmental length measurements. The surgery, instrumentation, experimental techniques and calculations used in the present study to examine diastolic cardiac performance in anaesthetised, open-chest, ventilated rats have previously been described in chapter 2 (pages 56-62). At the end of the three month period, those haemodynamic parameters that were necessary to calculate left ventricular chamber and myocardial stiffness were successfully measured in all rats.

3.2.4 Data analysis

Statistical and analytical methods used in this study have been described in chapter 2 (pages 68-69). Briefly, regression analysis was used to determine the lines of best fit for LVEDP versus LVED internal diameter and LVED stress versus strain relations. These relations were linearised for statistical analysis as previously described (chapter 2, page 68). Left ventricular stress and strain values were calculated as described in appendix A (pages 161-163). Left ventricular wall thickness (h) and internal radius (R) were calculated at incremental LVEDPs using those formulae provided in appendix A (pages 161 and 163). Comparisons between the groups were made using a Tukey's test. Values in the text are mean $\pm$ SEM. A p value of < 0.05 was accepted as statistically significant.

3.3 Results

Heart rate and systolic blood pressures were not significantly different between the groups at any time period during the study, including at the termination of the experiment (Table 4).

Androgenic steroid and exercise-induced effects on LV morphology.

Habitual exercise resulted in an increased heart weight, and LV wet and dry weights in both exercised steroid treated and control groups (Table 5). However, consistent with data described in chapter 2, androgenic steroid administration alone resulted in a reduced heart weight, and LV and RV (data not shown) weight in comparison to the other groups (Table 5).

The exercised steroid treated group had larger unstressed LVED internal diameters (i.e. LVED internal diameter at an LVEDP of 0 mm Hg = $LVEDD_0$) in comparison to the other groups (Figure 15), indicative of eccentric LV remodelling. In addition, although wall thickness to radius ratios (h/R-relative wall thickness) were increased following exercise training in the untreated group (Figure 15), which indicates the presence of concentric LV remodelling, h/R in the exercised steroid treated group was similar to sedentary rats (Figure 15), despite the increased LV weights. The lack of change in relative wall thickness in exercised rats receiving an androgenic steroid, despite an exercise-induced increase in LV weight, indicates that the androgenic steroid has changed the exercise-mediated concentric LV geometry to an eccentric pattern.

Table 4. Systolic blood pressure (SBP) and heart rate (HR) in exercise steroid treated, exercised control, sedentary steroid treated and sedentary control groups of rats.

	SBP (mm Hg)	HR (beats/ min)
Exercise steroid (n=11)	114±3	384±12
Exercise control (n=10)	116±3	421±10
Steroid (n=10)	127±6	397±23
Control (n=10)	121±4	413±23

Table 5. Body weight (BW), wet heart weight (HW), heart weight-to-body weight ratio (HW/BW), wet and dry left ventricular weight (LVW) in exercised (Exerc.) nandrolone decanoate (steroid) treated, exercised (Exerc.) control, sedentary steroid treated and sedentary control rats.

	BW (g)	HW (g)	LVW		HW/BW
			wet (g)	dry (g)	(%)
Exerc. steroid (n=11)	548± 16	1.59± 0.06**	1.17± 0.02**	0.23± 0.005**	0.287± 0.002**
Exerc. control (n=10)	505± 15	1.44± 0.03†	1.11± 0.04†	0.21± 0.006†	0.291± 0.004†
Steroid (n=10)	433± 17*	1.13± 0.04*	0.88± 0.02*	0.17± 0.003*	0.262± 0.001#
Control (n=10)	528± 36	1.30± 0.02	0.99± 0.02	0.19± 0.003	0.245± 0.004

* $p < 0.05$; steroid versus exercise steroid, exercise control and control
** $p < 0.01$; exercise steroid versus steroid
† $p < 0.05$; exercise control versus control
\# $p < 0.01$; steroid versus control

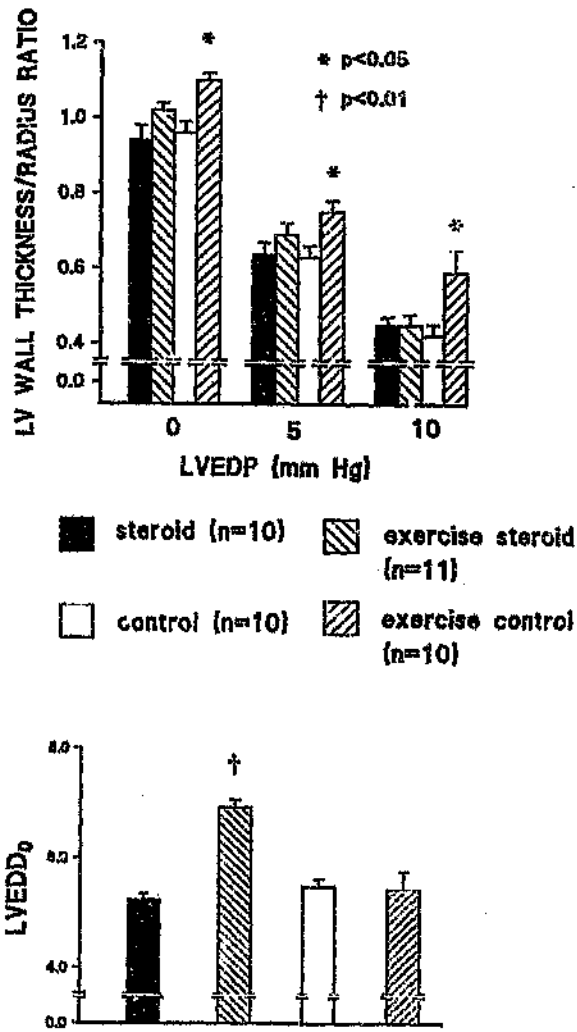

Figure 15. Effect of nandrolone decanoate (steroid) on LVED geometry in exercised rats. Bar graphs show the wall thickness to radius ratio (relative wall thickness) as determined at incremental LVEDPs (upper panel) and LVED diameter at a LVEDP of 0 mm Hg ($LVEDD_0$) (lower panel) in steroid treated, exercised steroid treated, sedentary control and exercised control rats. * and †, versus the other three groups.

Androgenic steroid and exercise-induced effects on LV diastolic performance.

Consistent with data described in chapter 2, androgenic steroid administration resulted in a left shift in the LVEDP-internal diameter and LVED stress-strain relationship (Figure 16) largely as a consequence of an increased slope of these relations (Figure 18). In contrast, habitual exercise mediated a right shift in the LVEDP-internal diameter and LVED stress-strain relationship (Figure 16), because of a decreased slope of these relations (Figure 18).

Steroid administration to exercised rats resulted in a right shift in the LVEDP-LVED internal (Figure 17, upper panel) and external (appendix C, page 165) diameter relations as compared to exercised and sedentary control rats. This right shift in the diastolic P-D relations subsequent to steroid administration to exercised rats was attributed to an altered LV geometry, as the $LVEDD_0$ was right shifted (Figure 15, lower panel). Nevertheless, consistent with the previously described effect of nandrolone decanoate on LVED performance (chapter 2), the slope of the LVEDP-internal diameter and the LVED stress-strain relations was increased in steroid treated exercised rats in comparison to exercised untreated rats (Figures 17 and 18). The effect of the androgenic steroid on LVED chamber and myocardial k in exercised rats resulted in similar values for LVED k when compared with sedentary control rats (Figure 18). Thus, chronic steroid administration nullified the beneficial effect of habitual exercise on chamber and myocardial diastolic stiffness in rats.

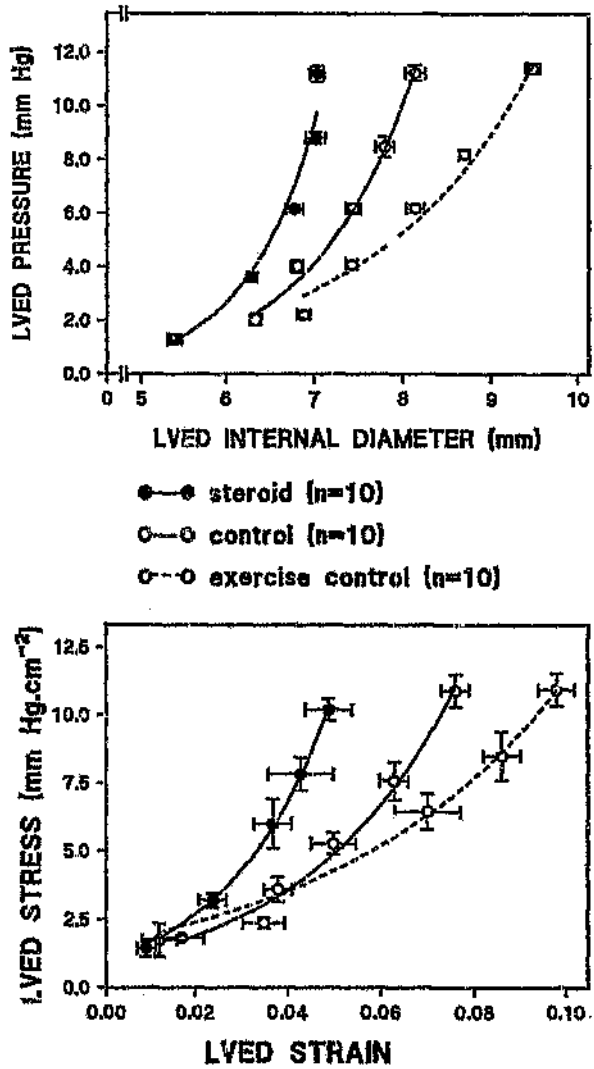

Figure 16. Line graphs illustrate the effect of nadrolone decanoate (steroid) and the effect of habitual exercise on LVEDP versus LVED internal diameter (upper panel) and LVED stress versus strain relations (lower panel). A statistical comparison of the slopes of the linearised relations of these curves is made in Figure 18.

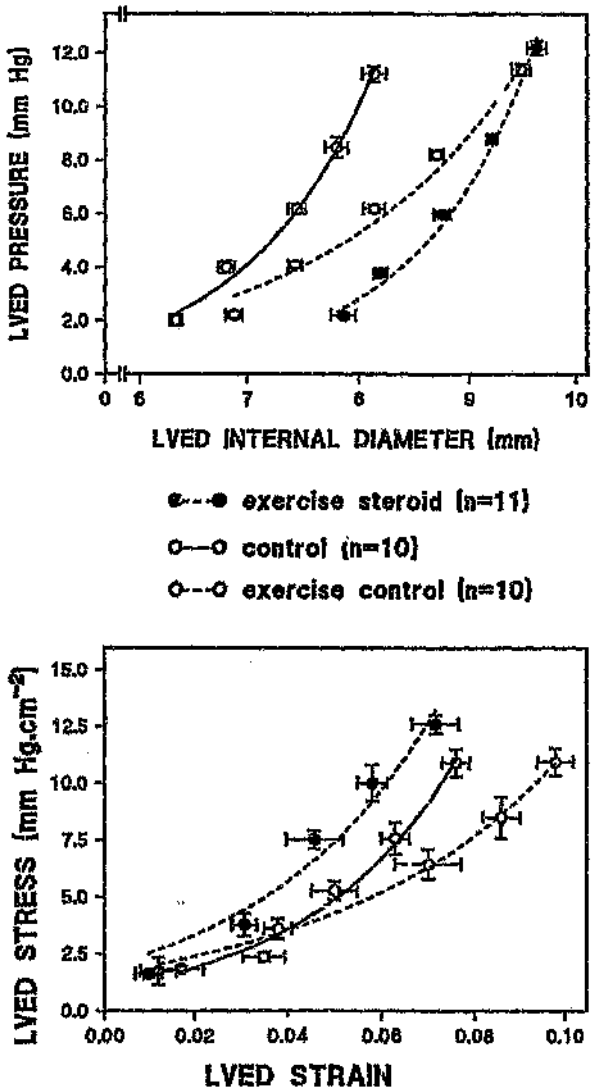

Figure 17. Line graphs illustrate the effect of nandrolone decanoate (steroid) on LVEDP versus LVED internal diameter (upper panel) and LVED stress versus strain relations (lower panel) in exercised rats. A statistical comparison of the slopes of the linearised relations of these curves is made in Figure 18.

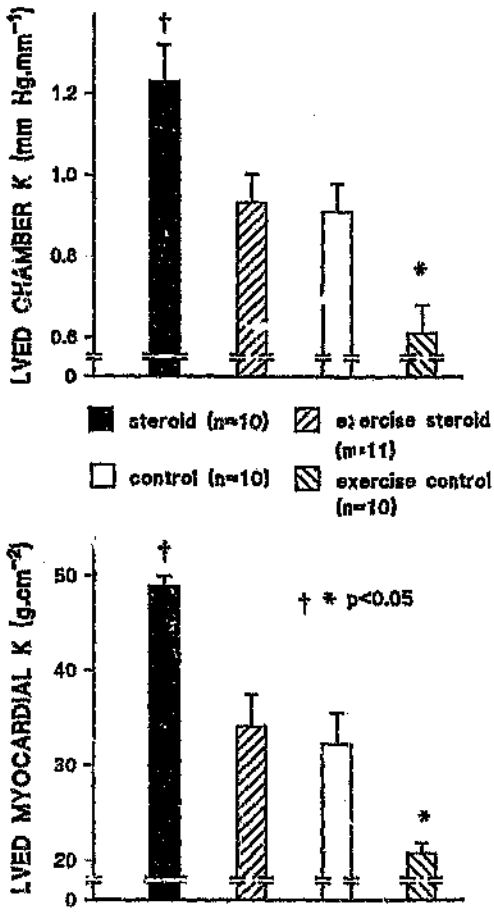

Figure 18. Bar graphs illustrate the effect of nandrolone decanoate (steroid) administration on the slopes of the linearised LVEDP versus LVED internal diameter relations (LVED chamber *k*) (upper panel), and the LVED stress versus strain relations (LVED myocardial *k*) (lower panel) in exercised rats. * exercised control versus the other three groups, † sedentary steroid versus the other three groups.

3.4 Discussion

The main findings of this study are that the beneficial effects of habitual exercise on LV chamber and myocardial diastolic stiffness are abolished following androgenic steroid administration to rats. However, the detrimental effects of the androgenic steroid on LVED chamber and myocardial stiffness in exercised rats is apparently offset by an altered LV geometry in steroid treated rats on an exercise programme. Chronic administration of the androgenic steroid resulted in eccentric LV remodelling, as determined by increased unstressed LV internal diameters, as well as normal relative wall thickness values in the presence of an increased LV weight in exercised rats. This was in contrast to concentric LV changes noted in exercised untreated rats (increased LV weight and LV relative wall thickness values determined over a range of filling pressures). The consequence of the eccentric remodelling process produced by the androgenic steroid in exercised rats was a right shift in the LVEDP-internal diameter relationship and a reduced LVEDP for a given LVED internal diameter despite the increased LVED chamber and myocardial stiffness constants.

Exercise and steroid-mediated effects on somatic and LV growth and remodelling.

Consistent with data described in chapter 2, steroid administration to sedentary rats suppressed both somatic and cardiac growth. In contrast, steroid administration failed to alter the development of physiological LVH or somatic

growth in exercised rats. Hence, androgenic-anabolic steroids have the ability to produce potential catabolic as well as anabolic effects on cardiac and somatic growth depending on the experimental conditions. My data are congruous with Karhunen et al. (1988), who described an increase in heart weight associated with an exercise programme in rats receiving an androgenic steroid. However, Liang et al. (1993) found no differences in heart weights subsequent to either steroid administration or chronic exercise. This disparity is likely to be due to differences in the exercise protocols employed (voluntary training wheel as used by us versus forced treadmill exercise as used by Liang et al., 1993) and the frequency of the steroid administration (biweekly as used by us versus once weekly used by Liang et al., 1993).

In this study habitual exercise resulted in LV hypertrophy (LVH) in both the steroid treated and the untreated groups of rats. However, LVH in the exercised steroid treated group was associated with an eccentric remodelling process as compared to the concentric LVH changes noted in the exercise untreated group of rats. Although LVH following running training is thought to produce eccentric, rather than concentric remodelling (De Maria et al., 1978), the LV geometric changes that are measured at rest might simply reflect differences in blood volumes (Convertino et al., 1980) and hence LV filling volumes at the time of measurement, rather than myocardial remodelling. Following habitual running in rodents, our group has previously shown that LV relative wall thickness is increased after habitual exercise (Woodiwiss and Norton, 1995). The exercised-induced concentric remodelling noted in the study described in this

chapter confirms the data previously obtained by our group (Woodiwiss and Norton, 1995). Concentric LVH is thought to be the consequence of high intensity, short duration exercise (Huston et al., 1985). In my study, it is difficult to determine whether rats were performing short duration, high intensity exercise, or endurance exercise. However, with regards to total distances run (greater than 2 km on average each day), it is difficult to believe that the exercise was high intensity exercise. In addition, the mean 24 hour running speeds noted in each group are likely to reflect low rather than high intensity exercise. Furthermore, Lambert and Noakes (1990) have shown endurance training effects following voluntary running in rats that run on average more than 1.66 km/day, a distance well below that achieved by all rats recruited for the study described in this thesis.

The mechanism by which steroid administration converted the exercise-induced concentric LVH to an eccentric geometry was not determined in this study. In addition, whether this has potential detrimental effects on cardiac morbidity and mortality was also not determined. However, it is well known that concentric LV remodelling represents a beneficial process which according to Laplace's Law results in decreases in LV stress when LV pressures are increased. In contrast, eccentric remodelling and LV dilatation represents an alteration which fails to maintain normal or relatively normal LV stress values when LV pressures are increased such as occurs during exercise. Hence, it is likely that exercise-induced increases in LV pressures will result in greater LV stress values if androgenic steroids are administered in the face of physiological LVH.

Steroid-induced effects on exercise-mediated changes in LV diastolic performance.

The beneficial effect of exercise on LVED chamber and myocardial stiffness was prevented by concomitant androgenic steroid administration to rats. Exercise-mediated improvements in diastolic stiffness are thought to provide for a reduced LV filling pressure when filling volumes are increased during acute exercise (Levine et al., 1991, Woodiwiss and Norton, 1995). Clearly, an androgenic steroid-induced increase in LV chamber stiffness would oppose this effect. However, as the androgenic steroid produced an eccentric LV geometry, as determined in diastole, LVED filling pressures were noted to be lower in the steroid treated exercised group for a given filling dimension. Hence, the potentially deleterious effect of the androgenic steroid-induced increase in LVED chamber stiffness in exercised rats was offset by changes in LV diastolic geometry.

3.5 Conclusions

In a model of exercise that results in LV remodeling consistent with strength training, I have shown that the beneficial effects of habitual exercise on LV chamber and myocardial stiffness are opposed by concomitant androgenic steroid administration to rats. In addition, the androgenic steroid modified the geometric changes associated with physiological LVH. Chronic administration of the androgenic steroid converted physiological LVH from a concentric to an eccentric geometry. This change in LV diastolic geometry, produced by the

androgenic steroid in exercised rats, resulted in greater filling diameters for a given LVEDP, and hence nullified the potential detrimental actions of the androgenic steroid on diastolic P-V or P-D relations in exercised rats. Although the altered LV geometry produced by the androgenic steroid is likely to be detrimental by producing increases in LV wall stress when LV systolic pressures increase (such as occurs in exercise), this has yet to be determined.

CHAPTER 4

Novel attributes of an androgenic steroid-induced increase in left ventricular end diastolic stiffness in rats

4.1 Introduction

The LVED, as opposed to early or mid-diastolic period of the cardiac cycle, has always been considered unique in that alterations in the slope of the linearised stress-strain relations calculated from pressure and volume (or dimension) measurements determined during this period alone (LVED myocardial stiffness), are thought to rely exclusively on variations in myocardial interstitial constituents that govern passive properties, rather than on changes in myocyte active properties. Indeed, alterations in LVED k in cardiac disease are usually linked to modifications in myocardial collagen characteristics (Brilla et al., 1991, Mukherjee et al., 1990, Iiomoto et al., 1988, Norton et al., 1995, Norton et al, 1997). The dependence of the LVED stiffness constant on passive properties in cardiac disease is also exemplified by the lack of data showing an impact of acute changes in cardiac dynamics (load, systolic performance and heart rate) on LVED myocardial stiffness, despite the load and heart rate-sensitivity of measures of performance obtained over all other systolic and diastolic portions of the cardiac cycle. Alterations in cardiac load have only been shown to modify myocardial diastolic stiffness in instances where stiffness is calculated from measurements obtained over early and mid-diastolic periods of the cardiac cycle (Komamura et al. 1992).

Despite the remarkable stability of LVED myocardial stiffness constants in the face of profound acute modifications in LV dynamics, in recent years the dependence of LVED stiffness on myocardial passive properties alone in cardiac

disease has been questioned. In certain circumstances, poorly defined myocyte cellular events that determine the extent rather than the rate of myocardial relaxation induce increases in LVED stiffness (rev- Gilbert and Glantz, 1989). However, to date, only one pathological state (pacing-induced ischaemia) has been shown to involve alterations in the myocardium that impact on LVED stiffness, that cannot be ascribed to changes in the interstitium (Appelgate et al., 1990, Bristow et al., 1970, Dwyer, 1970, Grossman and Mc Laurin, 1976, Takano and Glantz, 1995). The cellular changes that might, in part, be responsible for the increase in LVED stiffness that occurs subsequent to pacing-induced ischaemia is a decreased activity of the SR Ca^{2+} ATPase (SERCA) pump (Kihara et al., 1989). Indeed, alterations in performance in most other portions of the diastolic period of the cardiac cycle are often attributed to changes in Ca^{2+} handling (Gwathmey and Morgan, 1985) through alterations in SERCA activity.

In the previous chapters I have described how an androgenic steroid, nandrolone decanoate, when administered at pharmacological doses, produces an increased LVED chamber stiffness. This increase in chamber stiffness was shown to be the consequence of an enhanced myocardial stiffness. As androgenic steroids mediate effects through an action on nuclear receptors, it is possible that the steroid-mediated increase in end diastolic myocardial stiffness results from changes in the properties of the myocardial ECM i.e. changes in either total myocardial collagen concentrations or the relationship of collagen phenotypes following chronic androgenic steroid administration. It follows that if modifications in myocardial passive properties account for the steroid-induced

change in end diastolic performance, reminiscent of most other pathological states that induce alterations in LVED performance, LVED k would not be altered in steroid treated rats following an acute intervention that changes cardiac dynamics. Alternatively, if alterations in myocardial passive properties cannot account for the steroid-induced change in end diastolic performance, the most likely cellular mechanism that would explain this change is a decrease in SERCA activity.

In the current study I report on three unique attributes of the steroid-induced increment in LVED k. First, the steroid-mediated increase in LVED k was not associated with alterations in the myocardial ECM. Second, unlike the changes in LVED k noted in spontaneously hypertensive rats (SHRs) and rats with diabetes mellitus (DM), the steroid-induced increase in LVED k was sensitive to acute alterations in cardiac dynamics induced by administration of the Ca^{2+} channel blocker, verapamil. Third, the steroid-mediated increment in LVED k could not be attributed to changes in SERCA activity. Taken together, these results indicate that the steroid-induced change in LVED k is the result of alterations in active and not passive myocardial properties, but cannot be attributed to modifications in SERCA activity.

4.2 Methods

4.2.1 Experimental groups

Seventy eight Sprague Dawley rats (OLAC, UK) weighing 150-2[illegible] g were used for this study. Rats were randomly divided into two groups: steroid and

control. The steroid group (n=40) received an intramuscular injection of the androgenic-anabolic steroid, nandrolone decanoate as previously described (chapter 2, pages 53-54). The control group (n=38) received a biweekly injection of arachis oil with 10% vol/vol benzyl alcohol, the vehicle for the androgenic steroid. Steroid and vehicle administration continued for a 3 month period and rats received standard laboratory rat chow and water *ad libitum* throughout this study. All rats were housed in a room that was lighted between 0600 and 1800.

Six 45 week old SHRs, six 45 week old Wistar Kyoto controls (WKY), 5 Sprague Dawley rats with streptozotocin-induced DM and 8 euglycaemic controls were used to evaluate the action of verapamil on LVED *k* in animal models with an increased LVED *k* associated with alterations in the cardiac interstitium. Diabetes mellitus was induced with a single dose of streptozotocin, as previously described (Norton et al., 1996) and cardiac performanc assessed after 4 months.

4.2.2 Acute verapamil infusion in androgenic steroid treated rats and in rats with an altered cardiac interstitium.

The effect of verapamil on systolic and diastolic cardiac performance was examined after 3 months of either nandrolone decanoate or vehicle injection in 12 steroid treated and 12 control rats, as well as in the SHRs, WKYs, rats with DM and the euglycemic control rats. The haemodynamic effects of verapamil were assessed in anaesthetised, ventilated, open-chest rats using both piezoelectric ultrasonic transducers placed across the short axis of the heart and a fluid-filled

catheter system to measure LVESPs and LVEDPs (chapter 2, pages 56-62). In a pilot study conducted subsequent to the study described in chapter 2, we were able to show that piezoelectric transducers placed across the short axis of the heart follow the heart closely during ejection (Norton et al., 1997). Hence, I used LV short axis external diameter measurements to assess the effect of verapamil on systolic performance in either steroid treated rats, SHRs, rats with DM, or their appropriate controls.

Briefly, LV systolic and diastolic performance were determined from LVES stress versus strain, LVEDP versus LVED internal diameter and LVED stress versus strain relations, constructed from a range of LVESPs and LVES external diameters as well as a range of LVEDPs and LVED external diameters obtained during brief periods of i.v.c. occlusion as described in chapter 2 (pages 56-63). The slopes or linearised slopes of the LVES stress-strain, LVEDP-internal diameter, and LVED stress-strain relations were used to assess myocardial contractility (E^n), LVED chamber stiffness (k) and diastolic myocardial stiffness (k) respectively as described in chapter 2, pages 62-63, and 68-69.

The effect of verapamil on blood pressure, heart rate, LVED chamber and myocardial k, and E^n was determined in steroid treated and control rats, SHRs, and in rats with DM after the collection of baseline values. Baseline measurements during i.v.c. occlusion were repeated 3 times after the injection of the saline vehicle of verapamil. Verapamil-HCl (Isoptin, Knoll Pharmaceuticals, Whippany, NY) at a dose of 5×10^{-4}mmol/kg and made up in 0.3 mls of a normal saline solution, was injected through the carotid arterial catheter. At

between 4-5 minutes post-injection, when haemodynamic changes had stabilised, cardiovascular parameters were measured during i.v.c. occlusion. At 5 minutes, a second injection of the same dose was administered and the assessments of cardiac performance repeated 9-10 minutes from baseline measurements. Cardiac performance was therefore determined at baseline, after a 5 x 10^{-4}mmol/kg dose, and after a 10^{-3}mmol/kg dose of verapamil-HCl in each rat.

4.2.3 Myocardial collagen characteristics.

At the end of the 3 month treatment period, the LV of 10 steroid treated and 10 control rats was formalin fixed. The collagen-specific stain, Sirius Red F3BA (Brilla et al., 1991), was used on 5 μm thick, paraffin-embedded coronal sections of the LV obtained from the equator. An entire cross-section of the LV wall was used for morphometric analysis. The equator was selected as representative of the whole LV. From each section 15 fields were randomly selected and analysed for collagen volume fraction (CVF) and 8 fields for perivascular CVF.

Collagen volume fraction as well as the perivascular CVF was determined using videodensitometry. This analysis was undertaken on a computer based flexible image processing system (FIPS, CSIR, SA) (Figure 19) and an example of a histological section is provided in Figure 19. By using grey level thresholding, the stained areas were selected and the area fraction (CVF) of stained versus non-stained tissue determined.

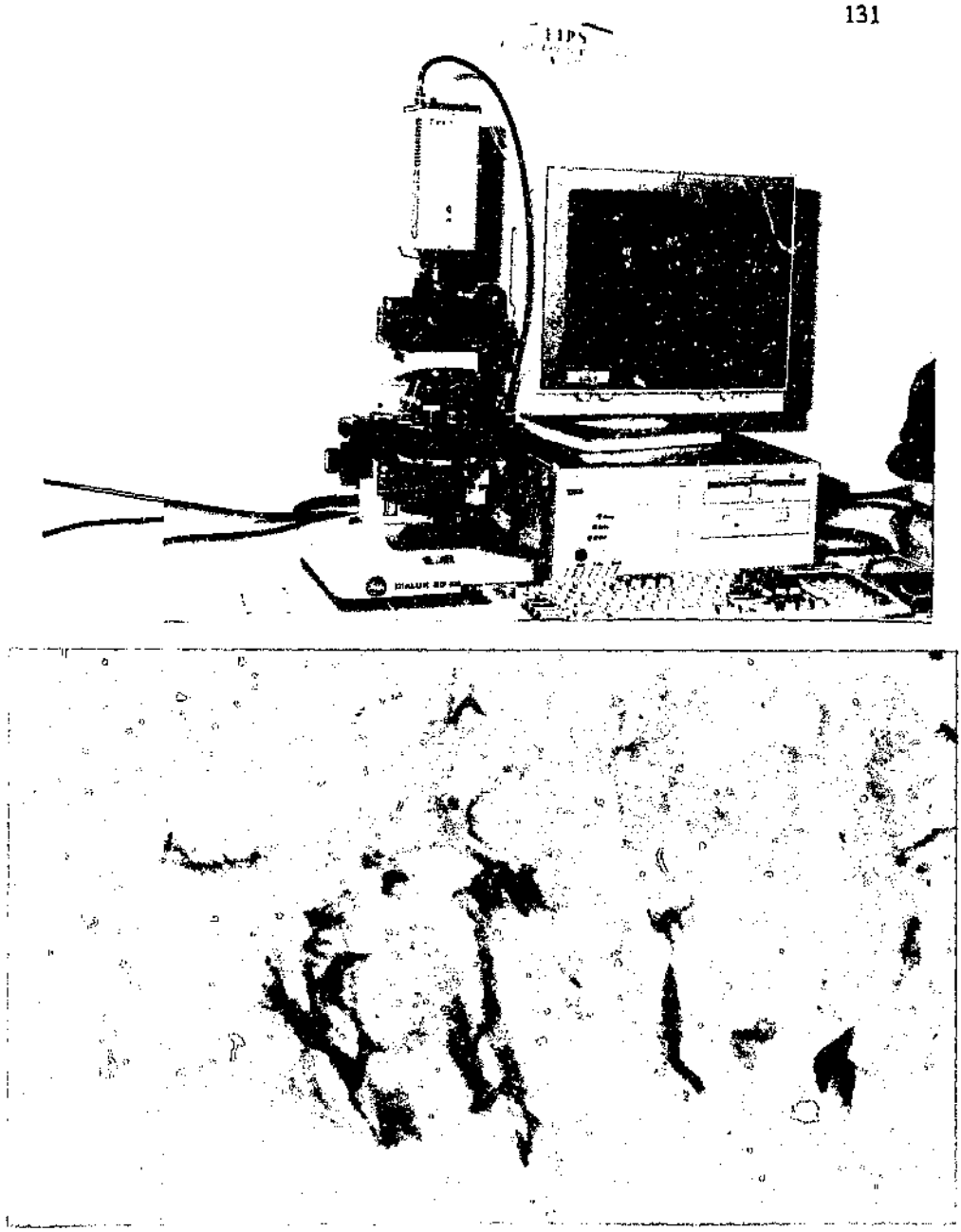

Figure 19. Photograph of a computer based flexible image processing system (FIPS, CSIR, SA) (upper panel) and an example of a histological section scanned with the imaging system (lower panel). The pink stained tissue represents that portion of the myocardium made up of collagen. The remainder of the myocardium is non-collagenous material.

At the end of the 3 month treatment period the LV of a separate group of 15 steroid treated and 13 control rats as well as all SHRs and their controls was weighed and stored at -70° for hydroxyproline (HPRO), collagen subtype, and collagen solubility analysis. To determine myocardial HPRO content, tissue was hydrolysed with 6N HCl at 107°C for 16 hours in evacuated sealed tubes. HCl was evaporated off from hydrolysed tissue under nitrogen, and the remaining tissue was redissolved in a buffer (pH 6.0) that has been previously described (Stegemann and Stalder, 1967). After centrifugation to remove insoluble material, myocardial 4-*trans*-hydroxyproline concentration was determined by the method of Stegemann and Stalder (1967).

The extraction and phenotyping of myocardial collagen was performed following the procedure described by Mukherjee and Sen (1990) as modified from Laurent et al. (1981). Briefly, myocardial tissue was first homogenised with phosphate buffered saline (PBS) at 4°C and centrifuged. This was followed by five sequential homogenations and centrifugations with 2% sodium dodecyl sulfate (SDS) to remove non collagenous proteins, three sequential homogenations and centrifugations with PBS to remove excess SDS, and two sequential homogenations and centrifugations with acetone. Thereafter the pellet residue was dried under vacuum. Cyanogen bromide (CNBr)(20 mg/ml) was added to a 1.5 ml suspension of the purified myocardial collagen residue (70% vol/vol formic acid per 100 mg of original tissue). Nitrogen gas was bubbled through the mixture, tubes were sealed and the reaction allowed to proceed for 18 hours at 25°C. The supernatant was removed and dried under vacuum. Polyacrylamide gel

electrophoresis was performed on vertical gels (Mini-Protean II, BioRad Laboratories, Richmond, California) 1.5 mm thick with 15 sample wells, by stacking and separating gel concentrations of 3% and 12.5% respectively. Samples of 15 μg of collagen (determined by HPRO measurements of the supernatant obtained after CNBr digestion) were loaded using a microsyringe. Stacking was allowed to occur at a current of 25 mA after which the current was increased to 35 mA. When electrophoresis was complete (i.e. once the dye maker had reached 0.5 mm from the bottom of the gel), the gels were stained overnight in an aqueous solution (fixative: 50% distilled water, 40% methanol, 10% acetic acid) containing 0.1% Coomassie Blue (R250), and destained in fixative for several hours. Gels were subsequently scanned using a Helena Laboratories EZ scan (Beaumont, TX).

Electrophoretic patterns of myocardial collagen extracts similar to those previously published by Mukherjee and Sen (1990) were obtained and the bands corresponding to collagens type I and III identified from collagen type I (Sigma) and III (Calbiochem) standards (Figure 20). The relative amounts of type I:III collagen were determined from the relationship between the amount of collagen applied to the gel and the relative area under the densitometry curve corresponding to bands G [α_1 (I) - CB-8] (type I) and H[α_2 (I) - CB-3](type I) (Laurent et al., 1981) and band M[α_1 (III) - CB-5 plus α_1 (III) - CB-9] (type III) (Laurent et al., 1981, Mukherjee and Sen, 1990). Bands G,H and M were chosen because they contain very little interference from comigrating peptides of other collagen types. To validate the densitometry technique used to calculate the ratio

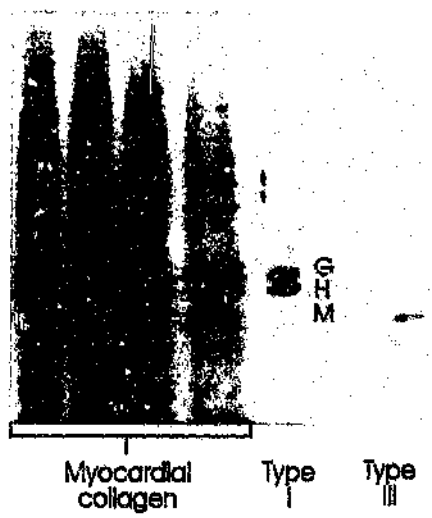

Figure 20. Typical separation pattern on SDS-polyacrylamide gel electrophoresis of cyanogen bromide digested fragments of myocardial collagen (lanes 1-4 from left to right), a collagen type I standard (lane 5), and a collagen type III standard (lane 6-7). G [α_1 (I) -CB-8] (type I), H[α_2 (I) - CB- 3] (type I) and M [α_1 (III) - CB-5 plus α_1 (III) -CB-9] (type III) bands are the bands used for densitometry analysis.

of type I:III collagen, I correlated the peak areas measured under the densitometry curves obtained from incremental amounts of collagen standards, with the amount of collagen loaded onto the polacrylamide gel. These correlations are illustrated in figure 21 and the correlation coefficients are given in the figure.

The amount of total collagen, type I collagen and type III collagen extracted after CNBr digestion was expressed as a percentage of the collagen extracted after acid hydrolysis. This was used as a measure of myocardial collagen solubility and subsequently as an index of the degree of myocardial collagen cross-linking (Brownlee et al., 1986). I chose to examine the degree of collagen cross-linking using CNBr digests as opposed to neutral salt precipitation (Iiomoto et al., 1988) or pepsin digests, as both neutral salt (Medugorac and Jacob, 1983) and pepsin (Mukherjee and Sen, 1990) result in low myocardial collagen yields even in normal rats.

Myocardial collagen advanced glycosylation end product formation, the collagen changes thought to be responsible for the stiff myocardium in DM (Norton et al., 1996) was determined by collagen fluorescence as previously described (Norton et al., 1996) in rats with DM and their appropriate controls.

4.2.4 Sarcoplasmic reticulum Ca^{2+} ATPase activity.

At the end of the 3 month treatment period with the androgenic steroid (n=15) and the vehicle (n=15) rats were euthenased by cervical dislocation. The hearts were immediately removed and washed with ice-cold saline and weighed. Sarcoplasmic reticulum vesicles were prepared by the method of Harigaya and

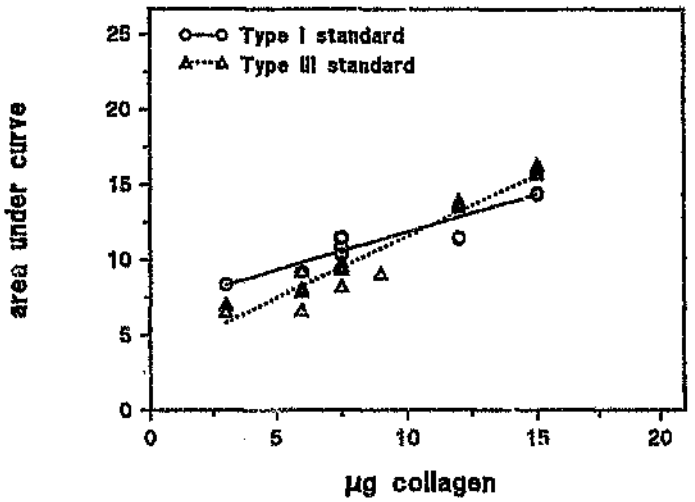

Figure 21. Correlation between the area under the densitometry curve and the amount of collagen standard loaded on the SDS-polyacrylamide electrophoretic gel. The bands used for densitometry analysis are shown in figure 20.

Schwartz (1969) with a few modifications. Briefly, sliced ventricular muscle was placed in a 4-5 m volume of solution containing 10 mM $NaHCO_3$ and 5 mM Na azide, homogenised on ice, and centrifuged at 8700 g for 20 minutes. The supernatant was centrifuged twice more (8700 g for 20 minutes and then at 37000 g for 30 minutes) before suspending the precipitate in a 20 mM Tris-maleate buffer (pH 6.8) containing 0.6 M KCl. The suspension was recentrifuged at 37000 g for 20 minutes and the harvest precipitate subsequently resuspended in a 20 mM Tris-maleate buffer (pH 6.8) containing 50 mM KCL.

Calcium-dependent ATPase activity was measured spectrophometrically using an NADH-coupled enzyme assay (Lopaschuk et al., 1982) within 3 hours of vesicle isolation. The incubation medium (final volume of 2.5 ml) was preincubated at 25°C for 3 minutes and contained; 40 mM histidine-HCl (pH 6.8), 5 mM $MgCl_2$, 5 mM Tris-ATP, 110 mM KCl, 0.22 mg phosphonenolpyruvate, 0.28 mg NADH, 7 U lactate dehydrogenase and 42 U pyruvate kinase. The reaction was started by the addition of 20-40 μg of SR protein. The total Ca^{2+}-Mg^{2+} ATPase activity was measured in the presence of 0.2 mM enthyleneglycolbis (β-aminoethylether)-N,N'-tetraacetic acid (EGTA) and incremental $CaCl_2$ concentrations. Mg^{2+} ATPase activity was measured in the presence of 0.2 mM EGTA without Ca^{2+}. Ca^{2+}-dependent ATPase activity was calculated by subtracting Mg^{2+} ATPase activity from the total Ca^{2+}-Mg^{2+} ATPase activity. The protein concentration of extracted SR vesicles was measured using a colorimetric Bio-Rad DC Protein Assay (Lowry et al., 1951).

4.2.5 Data analysis

Correlations between the area under the densitometry curve and the amount of collagen standard loaded on the SDS-polyacrylamide electrophoretic gel was determined using regression analysis. An r value of > 0.95 was considered to be a good fit. An unpaired Student's *t*-test was used to compare CVF, myocardial HPRO concentrations, collagen phenotype ratios and myocardial collagen solubility between experimental groups and their respective .ol groups. A repeated measures ANOVA was used to compare Ca^{2+} and Mg^{2+}-ATPase activity between groups.

Analytical methods used to assess cardiac performance in this study have been described in chapter 2 (pages 68-69). Briefly, regression analysis was also used to determine the lines of best fit for the cardiac function relations. These relations were linearised for statistical comparisons of the slopes (chapter 2, page 68-69). Left ventricular stress and strain values were calculated as described in appendix A (pages 161-162). Comparisons of the slopes of the linearised cardiac function relations were made between groups using an unpaired Student's *t*-test. The slopes of these functions before versus after verapamil administration were compared using a paired Student's *t*-test with a Bonferroni correction. The same statistical procedures were used to compare cardiovascular haemodynamic parameters, body weights and heart weights. Values in the text are mean $\pm$ SEM. A p value of < 0.05 was accepted as statistically significant.

4.3 Results

Consistent with results described in chapters 2 and 3, chronic nandrolone decanoate administration resulted in a reduced cardiac and somatic growth, but a normal LV relative wall thickness as determined over a range of filling pressures in the rats used in this study (Appendix D, page 166).

Effects of an androgenic steroid on the cardiac interstitium.

Chronic androgenic steroid administration failed to alter the myocardial CVF or perivascular CVF as determined using flexible image videodensitometry in rats (Table 6). Moreover, there were no differences between the steroid and control group of animals in total myocardial HPRO concentrations, phenotypic expression of myocardial collagen, total collagen solubility after CNBr digestion, i.e. an index of collagen cross-linking, as well as the CNBr solubility of type I and type III collagen solubility (Table 6).

Effect of verapamil on LVED performance in nandrolone decanoate treated and control rats.

Consistent with data described in chapter 2, chronic androgenic steroid administration resulted in a left shift in the LVEDP-LVED internal diameter (Figure 22, upper panel) and the LVED stress-strain (Figure 22, lower panel) relations as compared to control rats. The left shift in these curves was associated with an increased myocardial and chamber stiffness (LVED myocardial and chamber *k*) (Figure 23). Acute verapamil administration at a dose of 10^{-3}

Table 6. Effect of nandrolone decanoate (steroid) on interstitial characteristics of the rat myocardium.

	Steroid	Control
Collagen volume fraction (%)	5.6±1.1 (10)	5.5±0.6(10)
Perivascular Collagen volume fraction (%)	19.5±1.7 (10)	18.1±2.2 (10)
Hydroxyproline (HPRO) (μg/mg dry LV)	2.98±0.13 (15)	2.71±0.12 (13)
Type I/Type III collagen	2.73±0.24 (15)	2.53±0.17 (13)
Collagen solubility in CNBr*	40±4 (15)	40±4 (13)
Type I collagen solubility in CNBr**	0.30±0.03 (15)	0.28±0.03 (13)
Type III collagen solubility in CNBr**	0.10±0.01 (15)	0.12±0.01 (15)

Values are means ± SEM. Values in parentheses represent sample numbers. LV; left ventricle, CNBr; cyanogen bromide. * is the amount of HPRO measured after digestion with CNBr expressed as a percentage of the HPRO extracted with acid hydrolysis; ** is the amount of type I or III collagen extracted with CNBr digestion per mg of total collagen extracted after acid hydrolysis. No significant differences were noted between the groups.

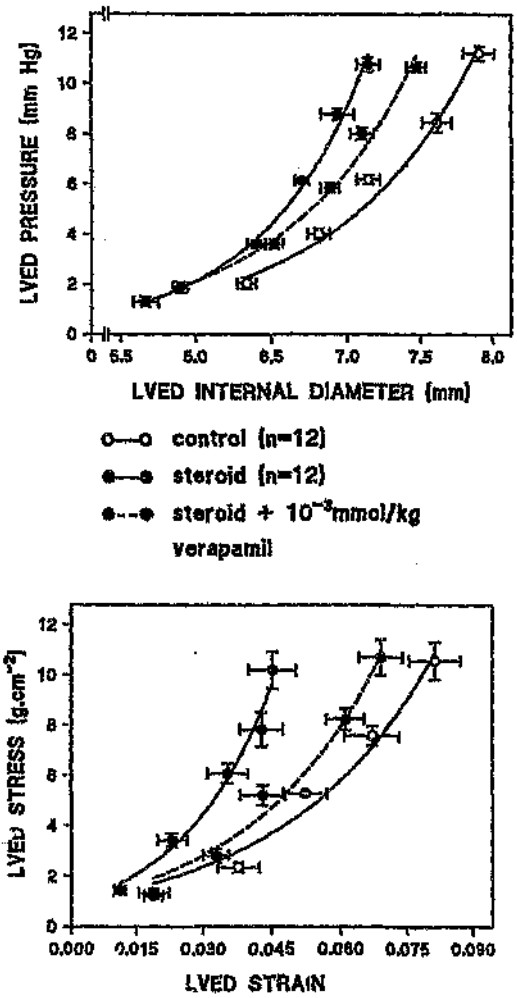

Figure 22. Line graphs illustrate the effect of nandrolone decanoate (steroid) and the action of an acute infusion of verapamil (10^{-3} mmol/kg) in nandrolone decanoate treated rats on LVEDP versus short axis internal diameter and LVED stress versus strain relations. A statistical comparison of the linearised relations of these curves is made in figure 23.

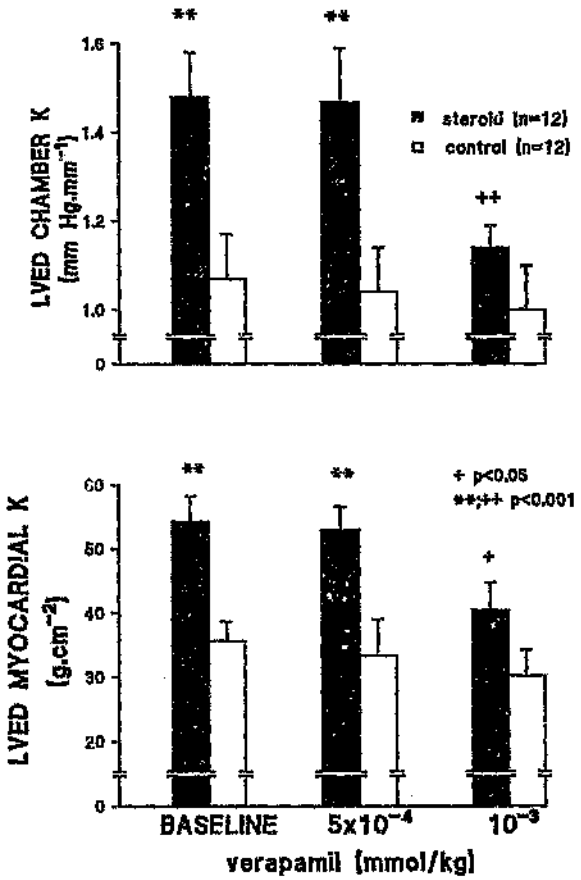

Figure 23. Bar graphs illustrate the effect of nandrolone decanoate (steroid) and the action of verapamil infusions in nandrolone decanoate treated and control rats on the slopes of the linearized LVED pr[illegible] versus short axis internal diameter relations (upper panel) (LVED chamber stiffness constant - *k*) and the LVED stress versus strain relations (lower panel)(LVED myocardial stiffness constant - *k*). **refers to differences between steroid and control group. †, †† refers to differences after verapamil administration as compared to baseline.

mmol/kg, but not at a dose of $5x10^{-4}$ mmol/kg (not illustrated), resulted in a right shift in the LVEDP-LVED internal diameter and LVED stress-strain relations in steroid treated rats relative to data obtained prior to verapamil administration (Figure 22). The shift in these curves was the result of a decrease in LVED *k* to values not significantly different from controls (Figure 23). Alternatively, verapamil failed to influence the LVEDP-LVED internal diameter or the LVED stress-strain relations in control rats (not illustrated). Indeed, LVED *k* was unaltered by verapamil administration in control rats (Figure 23). Also, no time-dependent changes in cardiac diastolic performance were noted following administration of the vehicle of verapamil.

Verapamil produced a decrease in BP, HR, LVES stress and myocardial systolic performance (E^a) and an increase in LVEDP in both steroid treated and control groups (Table 7). The effect of verapamil on these haemodynamic parameters was similar between the groups. Hence, differences in the response of diastolic cardiac performance to verapamil administration in the steroid treated as compared to the control group, cannot be attributed to differences in either cardiac systolic responsiveness and vascular sensitivity to Ca^{2+} channel blockade, or to differences in the effect of verapamil on systolic loading conditions or HR. However, the verapamil-induced decrease in LVED myocardial stiffness in the steroid-treated rats occurred at a dose associated with a decrease in E^a, LVES stress and heart rate. The latter association suggests that the androgenic steroid has induced an increase in LVED stiffness that is sensitive to alterations in cardiac dynamics.

Table 7. Haemodynamic changes following infusion of verapamil in 12 nandrolone decanoate treated (steroid) and 12 control rats.

	Steroid			Control		
Verapamil dose (mmol/kg)	Baseline	$5x10^{-4}$	10^{-3}	Baseline	$5x10^{-4}$	10^{-3}
MAP (mm Hg)	81±3	71±1*	59±2*	77±2	69±2*	62±2*
Heart rate (beats/min)	347±7	305±6*	265±4*	368±10	323±12*	277±6*
LVEDP (mm Hg)	10.8±0.5	10.4±0.3	12.7±0.3*	10.7±0.4	11±0.3	12.4±0.3*
E* ($g.cm^{-2}$)	460±53	441±56	274±25*	446±62	420±64	256±18*
LVES Stress ($g.cm^{-2}$)	117±3	112±4	97±3*	123±5	119±3	100±6*

Values are means ± SEM. MAP; mean arterial pressure, LVEDP; left ventricular end diastolic pressure. E*; myocardial end systolic elastance (the slope of the linearised LVES stress-strain relation). *$p < 0.05$ as compared to baseline. No significant differences were noted in hemodynamic values between control and steroid treated rats at baseline or at both doses of verapamil injected.

Effect of verapamil on LVED *k* in SHRs and in rats with DM.

Although verapamil at a dose of 10^{-3} mmol/kg failed to influence LVED *k* in control rats, it may be argued that the action of verapamil on diastolic performance in steroid treated animals reflects a modulating effect on animals with an enhanced myocardial stiffness as opposed to a normal myocardial stiffness. However, verapamil at the same dose, failed to alter the left shifted LVED stress versus strain relations (Figure 24) or the increased LVED *k* (Table 8) noted in SHRs and in rats with DM, despite similar effects as those noted in steroid-treated rats on BP, HR, LVES stress and E^1 (data not shown). The lack of effect of verapamil on the left shifted LVED stress-strain relations and the increased LVED *k* noted in SHRs and in rats with diabetes mellitus (DM) is likely to reflect an inability of a verapamil-induced change in cardiac dynamic-sensitive active myocyte processes to impact on collagen-mediated alterations in LVED *k*. Indeed, myocardial collagen changes were apparent in both SHRs and in rats with DM (Table 8). In SHRs, increases in myocardial HPRO concentrations, type I/III collagen phenotype ratios and collagen solubility were noted (Table 8). In rats with DM, an increase in myocardial collagen fluorescence, an index of advanced glycosylation collagen end-product formation, was measured (Table 8).

The effect of an androgenic steroid on SERCA activity.

The activity of SERCA, as assessed in vesicle membranes of the SR over a range of Ca^{2+} concentrations (Figure 25), was not significantly altered by

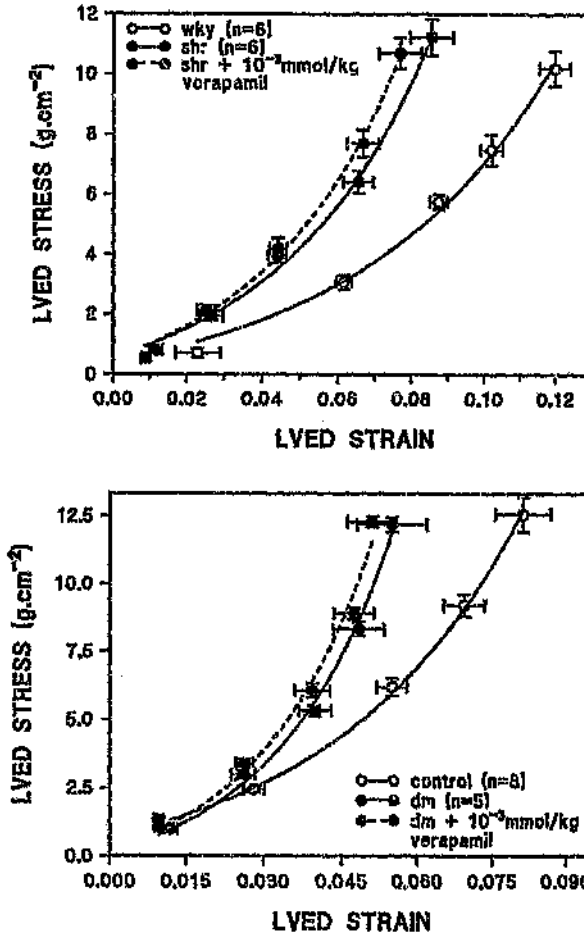

Figure 24. Line graphs illustrate LVED stress versus strain relations in spontaneously hypertensive rats (shr) (top panel) and in rats with streptozotocin-induced diabetes mellitus (dm) (lower panel) as well as the effect of verapamil (10^{-3} mmol/kg) on these relations. wky = Wistar Kyoto control. A statistical comparison of the linearised relations of these curves (LVED myocardial *k*) is made in Table 8.

Table 8. Myocardial stiffness (LVED myocardial *k*), the effect of verapamil on myocardial stiffness and myocardial collagen characteristics in spontaneously hypertensive rats (SHR), Wistar Kyoto controls (WKY), rats with streptozotocin-induced diabetes mellitus (DM) and euglycemic controls (CON).

	LVED myocardial *k* ($g.cm^{-2}$)				
Verapamil dose (mmol/kg)	0	10^{-3}		0	10^{-3}
SHR (n=6)	24.1±1.31*	26.3±1.17*	DM (n=5)	43.6±2.71*	45.3±5.3*
WKY (n=6)	17.5±0.98		CON (n=8)	24.8±1.07	

	Myocardial collagen characteristics				
	HPRO (μg/mg dry LV)	Type I/III	Collagen solubility (%)	Collagen flourescence (units/μg HPRO)	
SHR (n=6)	4.13±0.30*	9.3±1.3*	17.0±1.9*	DM (n=5)	10.08±0.67*
WKY (n=6)	2.96±0.12	1.9±0.06	34.6±2.0	CON (n=8)	7.14±0.76

LVED; Left ventricular end diastolic, myocardial *k*; linearized slope of the LVED stress-strain relationship, HPRO; hydroxyproline, Type I/III; ratio between type I and III collagen phenotypes. Collagen solubility; the amount of HPRO measured after digestion with cyanogen bromide (CNBr) expressed as a percentage of the HPRO extracted with acid hydrolysis. *$p<0.05$; SHR and DM versus their respective controls.

Table 8. Myocardial stiffness (LVED myocardial *k*), the effect of verapamil on myocardial stiffness and myocardial collagen characteristics in spontaneously hypertensive rats (SHR), Wistar Kyoto controls (WKY), rats with streptozotocin-induced diabetes mellitus (DM) and euglycemic controls (CON).

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WKY (n=6)	17.5±0.98		CON (n=8)	24.8±1.07	

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	HPRO (μg/mg dry LV)	Type I/III	Collagen solubility (%)	Collagen flourescence (units/μg HPRO)	
SHR (n=6)	4.13±0.30*	9.3±1.3*	17.0±1.9*	DM (n=5)	10.08±0.67*
WKY (n=6)	2.96±0.12	1.9±0.06	34.6±2.0	CON (n=8)	7.14±0.76

LVED; Left ventricular end diastolic, myocardial *k*; linearized slope of the LVED stress-strain relationship, HPRO; hydroxyproline, Type I/III; ratio between type I and III collagen phenotypes. Collagen solubility; the amount of HPRO measured after digestion with cyanogen bromide (CNBr) expressed as a percentage of the HPRO extracted with acid hydrolysis. *$p<0.05$; SHR and DM versus their respective controls.

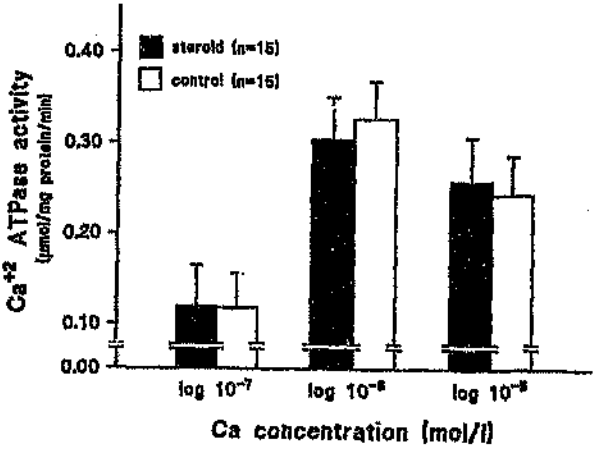


Figure 25. Myocardial sarcoplasmic reticulum Ca^{2+} ATPase activity in nandrolone decanoate treated (steroid) and control rats. No significant differences were noted between the groups.

androgenic steroid administration. In addition, neither Mg^{2+} ATPase, nor Ca^{2+}-Mg^{2+} ATPase activity were altered by nandrolone decanoate (data not shown).

4.4 Discussion

The main findings of this study are that chronic androgenic steroid administration produces changes in end diastolic myocardial stiffness that are unique in comparison to alterations in LVED stiffness constants reported on in other cardiac diseases. Unlike the increased end diastolic myocardial stiffness constants noted in classical pathological models of diastolic cardiac disease (hypertensive and diabetic cardiomyopathies), the steroid-induced increase in end diastolic stiffness is not associated with changes in the cardiac ECM. In addition, the steroid-mediated increment in myocardial stiffness was not associated with alterations in SERCA activity, but is sensitive to acute changes in either systolic LV performance, LV stress or heart rate.

LV passive processes and diastolic performance.

As indicated in chapter 2, myocardial factors that may contribute toward LVED stiffness include changes in either myocardial material properties (interstitial) or the active properties which determine the extent of myocardial relaxation (rev- Gilbert and Glantz, 1989). The interstitial material properties that may influence passive stiffness include changes in total collagen content, its subtypes, and the extent of collagen cross-linking (Brilla et al., 1991, Mukherjee

and Sen, 1990, Iimoto et al., 1988, Norton et al., 1996, Norton et al., 1997). However, chronic androgenic steroid administration failed to influence CVF, perivascular CVF, myocardial HPRO concentrations, the relation between collagen type I and type III phenotypes, and the CNBr solubility of total, type I and type III collagen. Therefore the influence of alterations in those interstitial material properties thought to determine passive myocardial stiffness were excluded as possible changes that explain the effect of chronic steroid administration on cardiac diastolic performance in rats.

It might be argued that changes in the myocardial collagen arrangement, rather than alterations in total collagen, it's phenotypes, or it's cross-linked properties may explain the steroid-induced increase in myocardial stiffness. However, I have excluded this possibility, as changes in the arrangement of myocardial collagen are usually associated with an increased CVF (Jalil et al., 1989), and the latter value was unchanged by steroid administration.

LV active processes and diastolic performance.

Consistent with the notion that in normal hearts and in many pathological circumstances, end diastolic stress-strain relations depend exclusively on myocardial passive characteristics, verapamil failed to alter LVED stiffness in normal animals and in animal models with an enhanced stiffness associated with changes in myocardial collagen. Alternatively, following acute administration of the Ca^{2+} channel blocker, verapamil, to steroid treated rats, LVED myocardial stiffness returned to values similar to those noted in control animals. The ability

of verapamil to improve end diastolic performance in steroid treated rats indicates that the androgenic steroid produced a unique effect on myocardial properties that has allowed changes in cardiac dynamic-sensitive myocardial active characteristics to impact on end diastolic stress-strain relations.

Verapamil has been shown to influence measures of LV early or mid-diastolic performance (Arrighi et al., 1994), but not end diastolic stiffness in cardiac disease. The mechanism by which verapamil may induce these effects has not been established. In this study, the specific mechanism (s) by which verapamil altered LVED myocardial stiffness constants in steroid-treated rats was also not examined. A number of mechanisms may explain the verapamil-mediated decrease in LVED stiffness in steroid treated rats, including alterations in cardiac systolic load, systolic performance, heart rate or changes in intracellular Ca^{2+} concentrations. Regardless of the specific cellular or mechanical change responsible for the verapamil-mediated modification in diastolic performance in my study, all of these are likely to alter LVED stiffness through an impact on myocyte active rather than passive processes.

Although end diastolic myocardial stiffness calculated from P-V or P-D relations obtained over the early (as opposed to late) diastolic period of a single cardiac cycle is load-sensitive (Komamura et al. 1992), LV myocardial stiffness calculated from end diastolic (as opposed to early diastolic) P-V or P-D relations are in most circumstances remarkably stable in the face of alterations in systolic cardiac load. Indeed, LVED myocardial stiffness constants were unchanged in control animals, SHRs and rats with DM, despite the modifications in systolic

cardiac load induced by verapamil infusion. As such, the argument that verapamil produced an effect on LVED stiffness in steroid treated rats through changes in loading conditions, is only valid in conjunction with an hypothesis that chronic nandrolone decanoate administration induces myocyte cellular alterations that sensitise LVED stiffness to changes in LV systolic load. The latter steroid-induced cellular effect is unlikely to involve modifications in passive myocardial processes, as LVED stiffness constants in SHRs and in rats with DM, which are attributed to alterations in myocardial interstitial properties, are insensitive to changes in LV systolic load.

Acute decreases in systolic performance, such as those noted following verapamil administration, may be responsible for a right and downward shift in indices of LVED stiffness relations in cardiac disease (Appelgate et al., 1990), through actions that can only be ascribed to alterations in myocardial active processes. An improved myocardial stiffness following verapamil administration in steroid-treated rats may therefore have occurred as a consequence of changes in LV systolic performance. However, verapamil produced a similar decrease in LV E^n in control animals, SHRs and in rats with DM, without altering end diastolic performance. Therefore, the acute effect of verapamil on LVED performance in androgenic-steroid treated rats can only be attributed to alterations in systolic performance if nandrolone decanoate had produced a myocardial change that sensitised LVED stiffness to acute changes in those active myocyte processes associated with a decrease in systolic performance.

Other possible explanations for the effect of verapamil on end-diastolic myocardial stiffness in the steroid treated rats in my study include either an action on intracellular Ca^{2+} concentrations (verapamil being an L-Ca^{2+} channel blocker) or an influence on heart rate. A decrease in intracellular Ca^{2+} concentrations may alter the extent of ventricular relaxation through a reduced period during which myofilament coupling occurs. Alternatively, a bradycardia will lengthen the time period for relaxation to occur following an extended diastolic period. The latter change might allow for prolonged relaxation to terminate prior to end diastole and hence prevent active myocardial properties impacting on end diastole.

The results of this study showing firstly a unique ability of acute changes in cardiac dynamics caused by verapamil administration to impact on LVED myocardial stiffness in steroid-treated rats and secondly that the steroid-induced increase in diastolic stiffness is not associated with alterations in myocardial collagen, supports the notion that changes in the myocardium other than those that determine passive properties are responsible for the stiff myocardium following androgenic steroid treatment. By exclusion, I can only attribute the steroid-induced increase in LVED stiffness to alterations in the extent of ventricular relaxation (rev- Gilbert and Glantz 1989).

Changes in the extent of relaxation, mediated by modifications in SERCA activity, are thought to be partly responsible for changes in LVED stiffness that occur subsequent to pacing-induced myocardial ischaemia (Kihara et al., 1989). However, SERCA activity was unchanged by chronic steroid administration. Therefore, an alternative cellular mechanism is likely to explain the steroid-

induced increase in LVED stiffness in rats. As androgenic steroids lead to a polyamine-mediated increase in the influx of extracellular Ca^{2+} into cardiac myocytes (Koenig et al. 1989), it is possible that nandrolone decanoate produced a decrease in the extent of ventricular relaxation and a subsequent increase in myocardial stiffness via alterations in Ca^{2+} cycling. However, other cellular changes need to be considered, such as possible modifications in the sarcolemmal Na^{+}-Ca^{2+} exchanger and alterations in stretch-activated cationic channels, the former considered as being important in decreasing Ca^{2+} concentrations during diastole, and the latter shown to be influential in mediating the increased end diastolic stiffness that occurs subsequent to pacing-induced ischaemia (Takano and Glantz, 1995).

4.5 Conclusions

In conclusion, I have been able to demonstrate that the increase in end diastolic myocardial stiffness that occurs subsequent to chronic administration of pharmacological doses of androgenic steroids, is not as a consequence of alterations in total collagen, collagen phenotypes or collagen cross-links. The unique sensitivity of the steroid-induced increase in end diastolic myocardial stiffness to acute changes in either cardiac loads, heart rate, or systolic performance suggests that the steroid-mediated effects on myocardial diastolic properties are through alterations in active myocardial processes. In this study, I have excluded the possibility that changes in SERCA activity, which is commonly affected in cardiac pathology, explains the steroid-induced change in

diastolic performance. Further studies are required to identify the cellular system that is modified subsequent to nandrolone decanoate administration that might explain the increased end diastolic stiffness constant produced by high doses of androgenic steroids.

CHAPTER 5

Conclusions

Androgenic steroid abuse is a common problem in athletes. Despite the well known influence of supraphysiological doses of androgenic steroids on other organ systems, the effect of similar doses of androgenic steroids on cardiac mechanical performance is currently unresolved.

I investigated the effect of a biweekly intramuscular injection of nandrolone decanoate (5 mg/kg) (steroid) for three months on LV mechanical performance in sedentary rats. Although the steroid did not affect blood pressures, chronic administration decreased somatic and cardiac growth probably as a consequence of a supressed testosterone production. However, steroid administration increased heart weight to body weight ratios which suggested the presence of relative left ventricular (LV) hypertrophy (LVH). Nevertheless as LV wall thickness/radius, determined over a range of LV end diastolic pressures (LVEDP), remained unchanged following chronic steroid administration, it is unlikely that the androgen used produced clinically important LV remodelling in sedentary rats.

Chronic steroid administration also increased LVED chamber stiffness (k), as determined both *in vivo* in anaesthetised, open-chest animals, using piezoelectric ultrasonic transducers to measure LV dimensions, as well as *ex vivo*, using an isolated, perfused, constant flow, isovolumic LV preparation. The

increased LVED chamber *k* was attributed to changes in myocardial properties as myocardial ED *k* was also increased. The altered LVED chamber performance mediated by chronic steroid administration subsequently resulted in a decreased preload-independent index of LV systolic performance (slope of the LV stroke work-LVEDP relation) as determined *in vivo*. However, load-insensitive indices of LV systolic performance (chamber and myocardial contractility), determined *in vivo* as well as *ex vivo* in the absence of an adrenergic stimulus (slopes of LV end systolic or peak systolic pressure-dimension, pressure-volume and stress-strain relations) were either modestly increased or unchanged following steroid administration. Alternatively, a β-adrenoceptor agonist, isoproterenol (at $10^{-8.5}$ M, which is approximately the EC_{50} established for isoproterenol in isolated, perfused LV preparations), produced a left shift and increased slope of LV peak systolic pressure-volume and stress-strain relations in control, but not steroid treated rats. As a consequence of the steroid-mediated alterations in adrenergic-inotropic responsiveness, LV systolic chamber and myocardial contractility were reduced in the steroid treated rats, as assessed in the presence of an equivalent adrenergic stimulus.

As the consistent LV mechanical performance change noted in both *ex vivo* and *in vivo* preprations that occurred as a consequence of chronic steroid administration was in the diastolic as opposed to the systolic period of the cardiac cycle, subsequent efforts in this thesis were focussed on both defining the mechanisms of the steroid-induced change in diastolic performance, and the

impact of the latter change on LV diastolic performance subsequent to an exercise programme.

Contrary to the effects noted in sedentary rats, steroid administration failed to influence an exercise-(voluntary running wheels for 3 months)-mediated increase in LV weight (physiological hypertrophy). However, the androgenic steroid attenuated an exercise-induced decrease in LVED myocardial and chamber *k*. Nevertheless, the steroid also produced an increased LV internal diameter as determined over a low-normal range of LVEDPs in exercised rats. Hence, despite the steroid-mediated alteration in LVED chamber *k*, ventricular remodelling (dilatation) offset the potential detrimental actions of the androgenic steroid on the relationship between diastolic pressures and dimensions in exercised rats. Nevertheless, the presence of ventricular dilatation in nandrolone decanoate treated exercised rats might indicate a deleterious effect of androgenic steroids on LV remodelling processes in exercise.

Although nandrolone decanoate's effect on LVED performance did not impact on exercise-mediated changes in diastolic pressure-volume relations in a pernicious fashion, the increased myocardial stiffness constant produced by the androgenic steroid suggested the potential presence of changes in the cardiac interstitium. However, two lines of evidence indicate that alterations in the myocardial interstitium cannot account for the steroid-induced increase in LVED *k*. Neither the myocardial hydroxyproline concentration, perivascular collagen volume fraction, the ratio of type I to type III phenotypes of myocardial collagen, nor the percentage of myocardial total, type I or type III collagen extracted after

cyanogen bromide digestion (an index of collagen cross-linking) were altered by nandrolone decanoate administration. Furthermore, acute administration of verapamil (10^{-3} mmol/kg)(a cardiovascular Ca^{2+} channel blocker) to rats *in vivo*, which resulted in decreases in LV loads, systolic performance and heart rate, was able to attenuate the increased LVED chamber and myocardial *k* produced by the steroid. In contrast, verapamil infusion failed to alter increments in LVED stiffness produced in rats with streptozotocin-induced diabetes mellitus and in spontaneously hypertensive rats. The increased myocardial *k* in both of the latter animal models was ascribed to changes in myocardial interstitial properties.

An enhanced myocardial diastolic stiffness subsequent to chronic androgenic steroid administration which could not be attributed to changes in the cardiac interstitium, but was sensitive to acute alterations in either heart rate, systolic performance, or cardiac loads, suggested the presence of modifications in a myocyte active cellular process which could contribute toward changes in the extent of LV relaxation. A cellular change that is frequently noted in cardiac disease and that is thought to contribute toward alterations in cardiac diastolic properties is a decrease in the sarcoplasmic reticulum (SR) Ca^{2+} ATPase pump which normally sequesters Ca^{2+} during diastole. However, chronic administration of nandrolone decanoate failed to alter the activity of the SR Ca^{2+} ATPase pump.

In summary, the results described in this thesis indicate that supraphysiological doses of androgenic steroids produce functional LV abnormalities of mechanical performance in rats. Marked changes in diastolic stiffness were noted in both sedentary and exercised rats. The changes in diastolic

performance are ascribed to modifications in myocardial properties and are relatively unique in that they are not attributed to remodelling of the cardiac interstitium or to changes of the activity of the SR Ca^{2+} ATPase pump, but are sensitive to alterations in either heart rate, systolic performance, or load. The steroid-induced increase in myocardial stiffness prevents the beneficial actions of habitual exercise on LV diastolic function, but this detrimental influence is offset by a steroid-induced eccentric LV remodelling which results in greater filling dimensions for a given filling pressure. Systolic mechanical alterations in sedentary rats were noted only in the presence of a controlled adrenergic stimulus, but were not apparent in either intact rats or in the absence of an adrenergic stimulus. The steroid-mediated increase in myocardial stiffness, decrease in adrenergic-inotropic responsiveness and LV remodelling may be of clinical importance in that these changes could contribute toward cardiac pathology in athletes who abuse androgenic steroids.

APPENDIX A

A. Cardiac Function Formulae

In vivo stress calculation

$$\text{Stress (LVED or LVES)} = \frac{1.36 \times \text{LVP} \times (2R)^2}{\text{LVESD or LVEDD}^2 - (2R)^2} \quad 1)$$

where LVP is LVEDP or LVESP, LVESD and LVEDD are LV end systolic or end diastolic external diameter and R is either end diastolic or end systolic internal radius and is calculated from

$$R\ (ED \text{ or } ES) = \sqrt[3]{\text{LVEDV (or LVESV)}} \times (3/4\pi) \quad 2)$$

where $\text{LVEDV or LVESV} = [(4\pi/3) \times (\text{LVEDD or LVESD}/2)^3] - V_m$ 3)

and Vm is the ventricular wall volume and is calculated from

$$V_m = 0.943 \times \text{LV wet weight} \quad 4)$$

Ex vivo stress calculation

$$\text{Stress}_{\text{max or min}} = \frac{1.36 \times \text{LVP}_{\text{max or min}} \times V^{2/3}}{(V + V_m)^{2/3} \times V^{-1/3}} \quad 5)$$

where $\text{LVP}_{\text{max or min}}$ is LV peak systolic or minimum diastolic pressure, V is volume and V_m is as indicated above (equation 4).

1) Sagawa et al. (1988)

2) Schiable and Scheuer (1981)

3) Sagawa et al. (1988)

4) and 5) Weber et al. (1988)

In vivo strain calculations

$$\text{Strain} = \frac{\text{LVEDD} - \text{LVEDD}_0}{\text{LVEDD}_0} \quad 6)$$

$$\text{Strain} = \frac{\text{LVEDL} - \text{LVEDL}_0}{\text{LVEDL}_0} \quad 7)$$

where $LVEDD_0$ and $LVEDL_0$ = the unstressed LVEDD and LVEDL, i.e. the LVEDD and LVEDL recorded at an LVEDP of 0 mmHg during inferior vena cava occlusion.

and strain at end systole is calculated from 8)

$$\text{Strain} = \frac{\text{LVESL} - \text{LVESL}_0}{\text{LVESL}_0}$$

where $LVESL_0$ is the unstressed LVESL i.e. the LVESL at which LVESP = 0 mm Hg determined by extrapolating the line of best fit of the LVESP versus LVESL relation.

Ex vivo strain calculation

$$\text{Strain}_{\text{(systolic or diastolic)}} = \frac{V^{1/3} + (V + V_m)^{1/3} - 1}{V^{1/3} \times (V_0 + V_m)^{1/3}} \quad 9)$$

where V is volume, V_m is as defined above (equation 4), and V_0 is the unstressed V at which LV pressure (systolic or diastolic) is = 0 mm Hg.

6), 7) and 8) Gilbert and Glantz (1989)

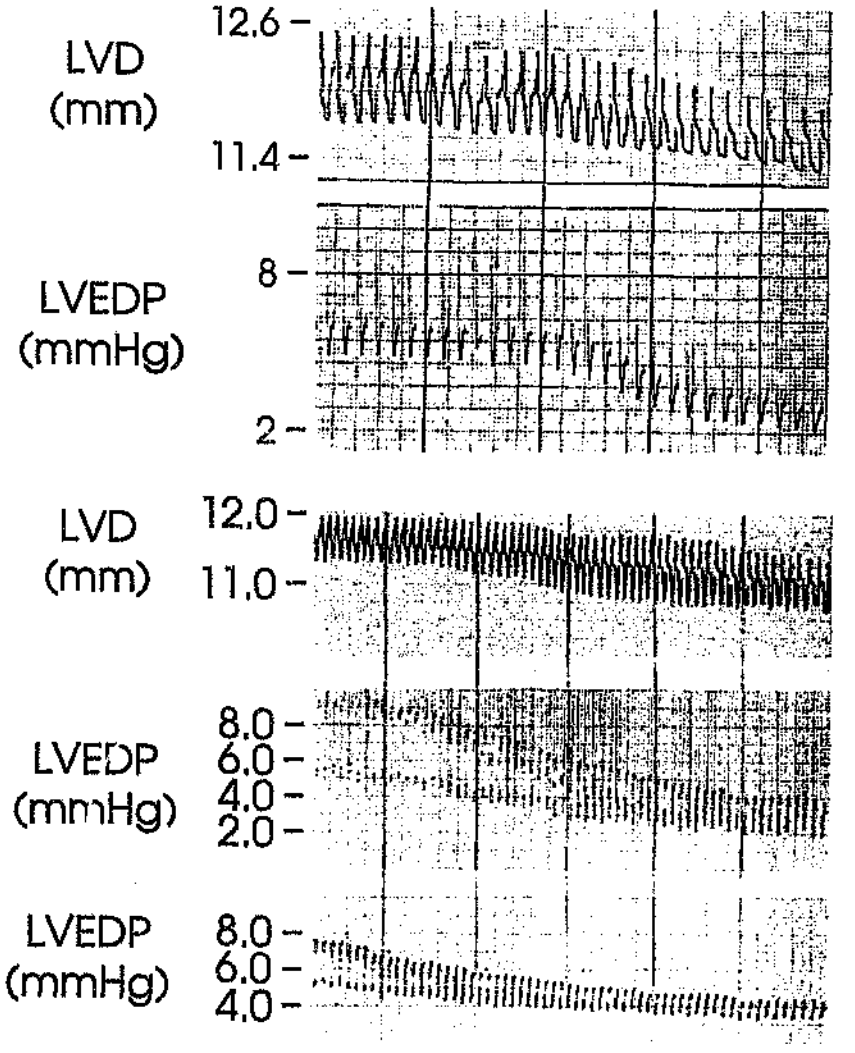
9) Weber et al. (1988)

LVED geometry

Radius (R) was calculated using equation (2) above and h was calculated from the standard equation:

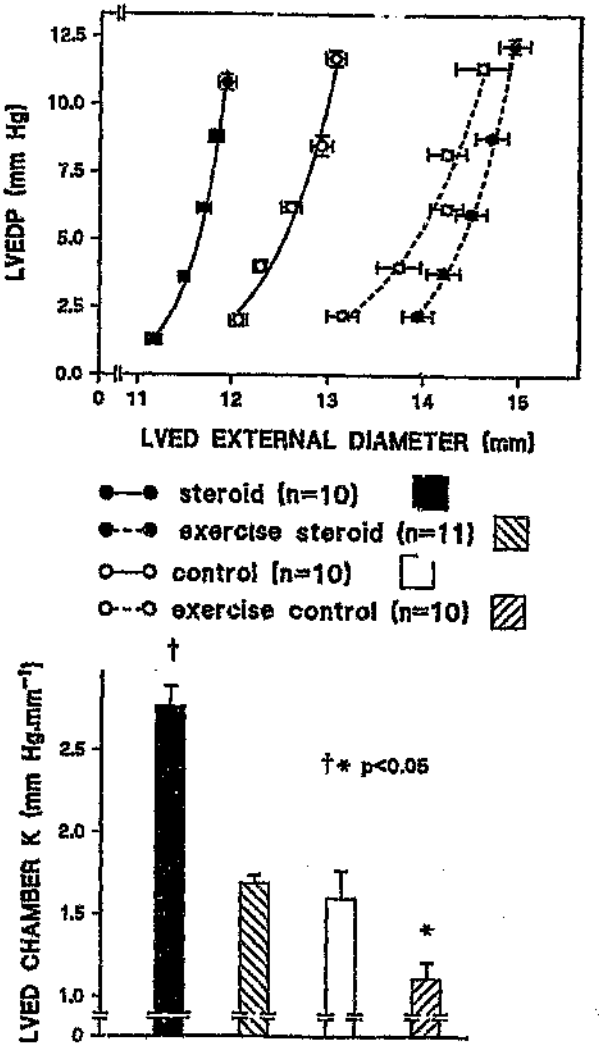
$$h = \frac{LVEDD - 2R}{2}$$

APPENDIX B



Two original traces of LV end diastolic pressure (LVEDP) and LV short axis external diameter changes (measured using sonomicrometry) obtained during i.v.c. occlusion in two separate rat LVs. LVD is the LV external diameter, LVEDP is LV end diastolic pressure. LVEDP is defined as the point of inflection of the LV pressure trace (at the beginning of isometric contraction). In the bottom example the pressures were recorded on two channels amplified over a slightly different range in order to ensure accuracy of the LVEDP measurement at low pressures while not missing higher values.

APPENDIX C



Line graphs in the upper panel and bar graphs in the lower panel illustrate the effect of nandrolone decanoate (steroid) and habitual exercise on LVEDP-LVED external diameter relations (upper panel) and the slope of the linearised LVEDP-LVED external diameter relations (LVED chamber *k*)(lower panel) respectively.* exercised control versus the other three groups, † sedentary steroid versus the other three groups.

APPENDIX D.

Body weight (BW), wet heart weight (HW), wet left ventricular weight (LVW), wet right ventricular weight (RVW), and wall thickness (h) to radius (R) ratio at an LV end diastolic pressure of 10 mmHg in nandrolone decanoate (steroid) treated and control rats used in the study described in chapter 4.

	BW (g)	HW (g)	LVW (g)	RVW (g)	h/R
Steroid (n=12)	441±16*	1.2±0.04*	0.88±0.03*	0.31±0.02	0.63±0.07
Control (n=12)	511±14	1.38±0.03	1.02±0.03	0.35±0.02	0.58±0.05

* $p < 0.01$; steroid versus control

APPENDIX E.

Specifications of Measuring Devices

a) Hellige Servomed Recorder

The channels used for recording LVP and carotid blood pressure have a sensitivity of 40mm/V, and the channel used for recording LVEDP has a sensitivity of 80mm/V. The frequency range is 0 to 80 (100) Hz for an amplitude frequency response within +0.5 and -1dB (rated values for 3-dB attenuation quoted in brackets), referred to a recording height (peak to peak amplitude) of 26 mm at 10 Hz.

b) Dynograph Recorder (R511A) - Rectilinear Writing Bandwidth

The frequency response range is from dc to 70 Hz ± 30% for 10 mm peak to peak pen deflection. The rise time of the pen is less than 11sec for 10-90% of a 40 mm deflection.

c) Triton Technology Sonomicrometer

The frequency response has a lower cutoff of -3 dB at 0.1 Hz and an upper cutoff of -6 dB at 65 Hz.

APPENDIX F.

Calibrations

a) Pressure Transducers

The pressure transducers were calibrated between 0 and 200 mm Hg against a mercury manometer for carotid blood pressure and LVP measurements. To ensure accuracy of LVDP measurements calibrations between 0 and 15 mm Hg were performed using a graduated (cm) column of water.

b) Piezoelectric Transducers

The 0.2 mm incremental internal calibration of the sonomicrometer was checked against a vernier calliper:- The piezoelectric transducers were placed in water as a transmitting medium and the distance between the transducers measured by means of a vernier calliper. The distance read off the vernier calliper was within 0.05 mm of the sonomicrometer reading.

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