Heart Disease Induced by AAS Abuse, Using Experimental Mice/Rats Models and the Role of Exercise-Induced Cardiotoxicity

I. Riezzo*[,1], D. De Carlo[1], M. Neri[2], A. Nieddu[2], E. Turillazzi[1] and V. Fineschi[1]

[1]*Department of Forensic Pathology, University of Foggia, Foggia, Italy*

[2]*Institute of Legal Medicine, University of Sassari, Sassari, Italy*

Abstract: The anabolic-androgenic steroids (AAS) are all synthetic derivates of testosterone and are commonly used as sport performance enhancers in athletes. The heart is one of the organs most frequently affected by administration of anabolic steroids. A direct myocardial injury caused by AAS is supposed to determine marked hypertrophy in myocardial cells, extensive regional fibrosis and necrosis. A number of excellent studies, using animal models, were performed to evaluate the cardiac effects of AAS. It is known that exogenous administration induced cardiac hypertrophy *in vitro* and *in vivo*, and when combined with exercise, anabolic steroid use has been shown to change exercise-induced physiological cardiac hypertrophy to pathophysiological cardiac hypertrophy. However the molecular mechanisms are still poorly understood. It's described that sudden cardiac death, myocardial infarct; ventricular remodelling and cardiomyopathy do to AAS is related to apoptosis and oxidative stress when associated with exercise. Mechanical stimuli and circulating humoral factors (TNF-α, HSP-70, IL-1β) released by the heart and peripheral organs are responsible.

Testosterone and derivates can work through genomic (activation of specific androgen receptor, interaction with co-activators and co-repressors transcription factors, gene regulation) and non-genomic mechanism (membrane-receptor-second messenger cascades).

Chronic AAS abuse results in different patterns of pathologic alterations, which depend on type, dose, frequency, and mode of use. The difficulty in interpreting experimental data on animals (mice and rats) lies in the diversity of experiments (the diversity of substances, which show different properties, different mice / rats by sex and age, duration of treatment with AAS, dosages used, type, scope and exercise duration).

Keywords: Apoptosis, colliquative myocytolysis, intracellular mechanisms, left ventricular hypertrophy, oxidative stress, troponin, ventricular remodelling.

INTRODUCTION

Anabolic androgenic steroids (AAS) are synthetic derivates of testosterone. Nandrolone decanoate (DECA) has been used by athletes and non-athletes for almost five decades in order to improve performance by increasing muscle mass and strength [1-7].

AAS can induce adverse cardiovascular effects, including hypertension, left ventricular hypertrophy (LVH), impaired diastolic filling, arrhythmia, erythrocytosis, altered lipoprotein profiles, and thrombosis [8,9]. In addition, abnormalities in vascular reactivity [10-15] and cardiovascular reflex control of the cardiovascular system [16-20] are also observed under AAS influence [21].

When administrated chronically to rats nandrolone has been associated with a significantly heightened heart rate response to cocaine [22], with accelerated development of hypertension in developing, spontaneously hypertensive rats [23], and with left ventricular hypertrophy [23,24] in sedentary rats. More recently an increase in myocardial susceptibility to ischemia/reperfusion injury has also been shown in isolated hearts prepared from rats treated chronically with nandrolone [7,25].

Anabolic agents have also been shown to enhance this pressor response to catecholamines in rodents [26]. Several animal studies have led to the speculation that AAS may interact with exercise-induced adaptations of the cardiovascular system to produce unfavorable effects [27]. Sympathetic neurons, instrumental in nervous control of the cardiovascular system, may be affected by AAS administration when it is combined with exercise [28]. Other studies have indicated that testosterone can both selectively inhibit extraneuronal uptake of neuroamines and increase the vascular response to norepinephrine [29].

Cardiac and metabolic effects of AAS abuse are particular unclear, although there are alarming reports of cardiac mortality and morbility [30].

Four hypothetical models of how AAS abuse might induce adverse cardiovascular effects (1) an atherogenic model involving the effects of AAS on lipoprotein concentrations; 2) a thrombosis model involving the effects of AAS on clotting factors and platelets; 3) a vasospasm model involving the effects of AAS on the vascular nitric

*Address correspondence to this author at the Department of Forensic Pathology, University of Foggia, Ospedale Colonnello D'Avanzo, Viale degli Aviatori, 1, 71100 Foggia, Italy; Tel: +39 0881 733195; Fax: +39 0881 736903; E-mail: ireneriezzo@tin.it

1389-5575/11 $58.00+.00

oxide system; and 4) a direct myocardial injury model involving the effects of AAS on individual myocardial cells) have been proposed [28].

Chronic AAS abuse results in different patterns of pathologic alterations, which depend on type, dose, frequency, and mode of use. The latency of sub-acute or chronic effects (multiple-dose treatments) can be the result of either drug accumulation or the sum of sub-toxic effects, which over time may become evident in clear clinical symptoms unrelated to the kinetics of the substance in question [31].

Animal studies provided the first evidence that the heart is a target organ for androgens. It is known that exogenous administration induced cardiac hypertrophy *in vitro* [32] and *in vivo* [33-35], but the molecular mechanisms are still poorly understood [36]. It's described that sudden cardiac death, myocardial infarct, ventricular remodelling and cardiomyopathy due to AAS is related to apoptosis [37].

In the mouse it has also been associated with inadequate vascularization of the hypertrophied myocardium [38], and in isolated rat ventricular myocytes it has been linked to increased apoptosis [37]. When combined with exercise, anabolic steroid use has been shown to change exercise-induced physiological cardiac hypertrophy to pathophysiological cardiac hypertrophy [6].

Some animal studies suggest that anabolic steroids compromise both contractile reserve capacity and the basal work capacity of the exercise-trained heart [39,40]. Furthermore the exercise induces increase in the pre-load (diastolic filling) on the heart [41].

Medei *et al.* [1] in their works established that DECA was unable to induce sudden death in rats treated weekly for 8 weeks with 10 mg/kg of nandrolone decanoate; however, as documented in the literature this phenomena is more frequently observed as a result of the combined effects of AAS use and exercise or vigorous weight training [42-44].

Supplementation of Anabolic Steroids (AS) exacerbates the normal cardiac adaptive mechanisms to exercise [45,46] and the ensuing physiologic insult [28,47,48]. These changes persist well after the cessation of AS use [2,49].

Mechanical stimuli and circulating humoral factors released by the heart and peripheral organs are responsible. Some studies have shown that circulating cytokines such as TNF-α may play a role in cardiac remodelling and that AAS strongly stimulate leukocyte TNF-α production [50,51].

In addition the exposure of cells to various sublethal stresses results in an adaptive increase in the inducible member of the 70-kDa family of heat shock proteins (HSP-70), the 72-kDa HSP-72, which confers cellular resistance to a variety of stresses [52]. Exercise is also a type of stressor that increases the content of HSP-72 in mammalian cardiac muscle [53,54] and some evidence indicates that exercise-induced HSP-72 plays a protective role in the mammalian heart against stresses [55,56].

Although myocardial hypertrophy associated with AS use has been quite reproducible in animals, the actual mechanisms by which this might occur are still vague [28].

This review is focused on direct cardiotoxicity mechanism (fourth hypothesis). The difficulty in interpreting experimental data on animals (mice) lies in the diversity of experiments (the diversity of substances, which show different properties, different mice / rats by sex and age, duration of treatment with AAS, dosages used, type, scope and exercise duration).

ANABOLIC ANDROGEN STEROID ABUSE AND EXERCISE: CARDIAC FINDINGS AND VENTRICULAR REMODELLING

Animal studies on ultrastructural findings since 1973 indicate that AS-induced-cardiomyopathy is the result of increased myocardial fibrosis, particularly in the subepicardium and central aspects of the left ventricle [42,57]. Administration of testosterone [58-60] or any of a host of other AS [61-65], has generated significant cardiomegaly in many different animal models, regulating cardiac myosin heavy chain (MHC) expression. Rats that were exposed to chronic hypoxia and at least 5 weeks of testosterone treatments generated ventricular hypertrophy [59]. In another rat study, nandrolone decanoate injections (6 weeks nandrolone decanoate treatment: total dose 30 mg kg^{-1}) stimulated cardiomegaly that reversed after cessation of treatment [63].

It is agreed that when AAS abuse is coupled with intense exercise training, concentric hypertrophy of the left ventricular wall and impaired diastolic function may result [66]. Drug-free vigorous weight training will also increase left ventricular wall thickness and mass but will not hinder cardiac function. However, when combined with anabolic steroids, cardiac hypertrophy could become pathologic [67].

The exact myocardial cellular changes that occur when combining exercise training with anabolic steroid use is not well defined.

Du Toit *et al.* [7] found, in their study on rats subjected to swimming training with and without i.m. injection of nandrolone laurate (0.375 mg/kg) once weekly for 6 weeks, that chronic use of supra-physiological doses of anabolic steroids: 1) decrease mechanical function of the heart when in conjunction with exercise training program; 2) decreases post-ischemic mechanical function of the normal sedentary and exercise – trained heart; 3) increase myocardial cAMP and TNF-α concentrations in the normoxic heart under basal conditions, but they have no direct evidence to suggest that these elevations plays a causative role in the myocardial hypertrophy.

It has also been shown that anabolic steroids may prevent the exercise-induced increase in LV wall thickness to internal diameter ratio [6]. This change may cause an increase in the LV wall stress in the anabolic steroid-treated heart and contribute to the decreased cardiac performance seen in these hypertrophied hearts [40].

Woodiwiss *et al*. found that supraphysiological doses of an androgenic steroid (biweekly intramuscular injection 3.5 days apart (5 mg/kg) of the androgenic steroid nandrolone decanoate) modify exercise induced left ventricular remodelling and prevent exercise-mediated increases in

relative wall thickness without influencing the degree of cardiac growth in rats. Habitual exercise without steroid administration resulted in rightward shifts in left ventricular end diastolic pressure-internal diameter relationships but an increased left ventricular end diastolic relative wall thickness as a consequence of appropriate left ventricular hypertrophy. Alternatively, nandrolone decanoate given to exercised rats augmented the exercise-induced rightward shift in the left ventricular end diastolic pressure-internal diameter relationship as determined over a physiological range of filling pressures and consequently reduced relative wall thickness values despite the presence of a similar increase in left ventricular weight. However the steroid-mediated effects on exercise-induced left ventricular remodelling were not attributed to alterations in the left ventricular interstitium. Therefore supraphysiological doses of an androgenic steroid augment an exercise induced rightward shift in left ventricular end diastolic pressure-dimension relationships and subsequently reduce relative wall thickness values as determined over a physiological range of left ventricular filling pressures in rats. However in not clear whether the influence of the androgenic steroid on exercise-induced LV remodelling produces increases in LV diastolic wall stress when filling volumes increase during exercise [6].

Rocha FL *et al.* investigated the effects of AS administration associated with or without swimming in the cardiac remodelling process and in cardiac function in rats [68]. They found that swimming training associated with supraphysiological doses of AS (nandrolone decanoate: Deca - durabolin; Organon, Roseland, NJ, administered subcutaneously twice a week, in a dosage of 5 mg kg^{-1} per injection, equalling 10 mg kg^{-1} wk^{-1}) cause exacerbated cardiac hypertrophy with interstitial fibrosis. The improvement in LV function promoted by exercise training is loss by AS treatment.

These data support the work reported by Liang and co-workers that showed that hearts from rats trained on a treadmill and treated with nandrolone decanoate performed poorly when compared with their trained, vehicle-treated counterparts [7,40].

Fontana *et al.* used transgenic mice with a human lipaemic phenotype (CETP+/-LDLr-/+) to evaluate the effects of mesterolone treatment (2 μg/g body weight) with or without an aerobic exercise training protocol, on the cardiovascular system, on BP (blood pressure), plasma lipoprotein profile and cardiac remodelling. They found that mesterolone alone (Sed-M) promoted only slight changes in the cardiac structure, i.e. a borderline trend towards LV hypertrophy ($P = 0.054$). On the other hand, the exercise training induced favorable cardiac remodelling either in mice treated with mesterolone or vehicle. This is evidenced by a marked reduction in the cardiac interstitium (more prominent in Ex-C) and enlargement of cardiomyocyte size. The deleterious effect of mesterolone in sedentary mice was confirmed by increased levels of circulating TnT, a marker of myocardium lesion, and exercise training attenuated this adverse effect of mesterolone on the cardiac integrity. In addition, the exercise training enhanced significantly the cardiac vascularization in the Ex-C and, to a lesser extent, in Ex-M groups (compared with Sed-C), as a response to the increased myocardial oxygen demand imposed by physical activity [69].

Therefore treatment of rats with supraphysiological doses of AASs induced pathological myocardial hypertrophy, and when combined with exercise, these steroids reduced the beneficial effects of exercise on left ventricular hypertrophy and cardiac circulation [6,38,69,70].

In our experimental study conducted on twenty-five male CD1 mice (8-10 weeks old; 35g initial body weight) treated with intramuscular Nandrolone Decanoate (DECA-DURABOLIN), for 42 days, twice per week, with different dosages, some free to move in their animal rooms (two groups) other exercised by running on a motor-driven treadmill (three groups) studying plasma lipid analysis, cardiac histopathological features, cardiac β-1 adrenergic receptor expression, and the effects of the myocardial expression of inflammatory mediators (IL-1β , TNF-α) on the induction of cardiomyocytes apoptosis (HSP-70, TUNEL), using proteomic and immunohistochemical analysis, we found that recurring high dose AAS administration and physical training in mice produce a moderate increase of heart weight, morphologically extensive cardiac hypertrophy and wide colliquative myocytolysis which could result in significant heart failure, were observed in the high dose AAS administration and physical training group [31].

Interestingly, the hearts increase in weight, suggesting the heart enhanced protein synthesis for response to the AAS administration.

This data was speculated also by Du Toit [7] besides possible changes in collagen crosslink formation [71]; anabolic steroids evidently change collagen synthesis and distribution in the left ventricle [72].

Morphological alterations of the myocardium as direct consequences of AS have been well documented [28,73-75]. Medei E. *et al.* demonstrated the presence of approximately 25% less nuclei and greater cardiomyocyte nuclei diameter in the ventricles of the DECA group with heart weight similar to that in the control group together suggest cardiac hypertrophy [1].

The extracellular space of rat myocardium treated with Dianabol or others AS is occupied by bundles of collagen fibrils, and the cells appear separated as during the early stages of fibrosis [31,68,76].

In the Rocha's study, when the cardiac collagen of the T+S (trained + steroid) group was investigated, a higher amount of collagen was found, as determined by the hydroxyproline method and histological quantification of the CVF (collagen volumetric fraction). This group also had an increase in collagen type I and type III cardiac expression compared with these in the trained group. These findings may contribute to the larger cardiac hypertrophy in this group [68].

Given this experimental background, our finding [31] of myocardial disarray (from hypertrophic myocytes with bizarre forms characterized by nuclei that exhibit nuclear enlargement, pleomorphism and hyperchromasia to wide

fields of disarray with star-like disposition of adjacent myocytes, aligned obliquely or perpendicular to one another, and joined together by short, generally hypertrophic myobridges, with interconnecting myofibrils layer of the cardiac wall) associated with contraction band necrosis, present in all forms, from early to late healing stages, and of focal myocardial fibrosis, is highly significant. It may provide a substrate for the occurrence of potentially lethal arrhythmias and sudden, unexpected cardiac death [42].

In their study Rocha F.L. *et al.* determined by histological quantification of the CVF and hydroxyproline methods the cardiac hypertrophy establishing that it was due to higher collagen concentration, and that it was not detected in the diameter of the myocytes. The myocyte diameter was similar in the T+S and T groups. This result shows that the AS were not involved in the hypertrophy of the myocytes, suggesting that another component contributed to the increase in cardiac mass. However they found that the cardiac hypertrophy and collagen syntheses induced by steroid treatment and by physical exercise associated with AS treatment were totally prevent by losartan (AT-receptor blocker) treatment [68].

It has been suggested that the different changes in the β-adrenoceptor-mediated mechanism in the failing heart may be due to differences in the type and stage of hypertrophy [77], since heart failure is linked to cardiac hypertrophy [78].

At the early stage of hypertrophy due to pressure overload, an unaltered [77] or increased number of β1-adrenoceptors was shown [79,80]; however, the β1-adrenoceptor density was augmented in hypertrophied hearts because of volume overload [77]. Nonetheless at the late stage of both types of hypertrophy, the number of β1-adrenoceptors was reduced. We observed a weak positive reaction in scattered and sparse foci in the subendocardial layer of the myocardium in group A; a moderate diffuse positive reaction was observed in group B, while in group C, an intense and massive positive reaction was found in the deep layers of the myocardium and in the subendocardial layers [31].

The high levels of circulating catecholamine due to chronic stress also become available for oxidation to produce aminolutins and oxyradicals generating oxidative stress. Both oxyradicals and the oxidized form of catecholamine have been shown to produce toxic effects such as coronary spasm, arrhythmias, ultrastructural cell damage, and contractile failure in the heart [81]. The effect of catecholamine on the density β-adrenoceptors is considered to be an important feature which, depending on the receptor selectivity, may affect the myocardial function differently. Catecholamine-mediated increase in the total population, binding capacity and affinity of β1-adrenoceptors may promote the effects of sympathetic stimulation on the myocardium. Furthermore, increasing the β1-adrenoceptor and G protein coupling for the catecholamine-induced cAMP synthesis may intensify the catecholaminergic action [81, 82].

Myofibrillar disarray, interstitial fibrosis and hypertrophy have all been shown to be a consequence of direct overexpression of human β1-adrenergic receptors in the heart of transgenic mice [83].

Penna C. *et al*. found that 14 days treatment only with nandrolone decanoate induces an overexpression of β2-adrenoreceptors, and reverses the depression of the contractile response induced by acute stress. In particular short-term nandrolone decanoate treatment induces an overexpression of β2-adrenoceptors without cardiac hypertrophy. They describe that, few minutes after an acute stress, the heart of vehicle-treated animals responds with a reduced increase in inotropic force under sympathetic stimulation [84]. This reduced response has been interpreted as a defense mechanism of the heart against over-stimulation, which has been attributed to a β1-receptor down-regulation [85-87]. However, hearts of treated animals with ND do not show this reduced response. Since an acute sympathetic over-stimulation may induce a "physiological" sympathetic β-receptor down-regulation within minutes [85-87], it is possible to establish that a component of post-stress blood pressure recovery may be the reduced response to the adrenergic stimulation, *via* 1-AR/G(s) protein uncoupling. Therefore they confirm that this post-stress reduced inotropic response occurs. However, after 2 weeks of treatment with ND, this reduced response to sympathetic stimulation does not occur. With this data it could be argued that the absence of this down-regulation may expose nandrolone addicted to an exaggerated increase in contractility and pressure after acute stress. Therefore ND-pretreatment alters not only the stress-activated central circuits [88,89], but also peripheral β-receptor expression and function.

Instead the downregulation of β-adrenoceptors has now been well established to occur in the failing human heart [90-92]. Indeed, heart β1-receptors would be directly influenced to some degree by AAS [28].

The histological changes that occur are exacerbated by endurance exercise and are similar to those observed in the early phases of left ventricular failure [45,74].

In fact we found colliquative myocytolysis from grade 1 (occasional or small groups of disappearance of myofibrils with intramyocardial oedema resulting in empty sarcolemmal tube and with any type of reaction) to grade 3 (interesting more than 50% of myocells and was prominent in the subendocardial half of the cardiac wall). It's defined a progressive loss of myofibrils paralleled by intramyocellular oedema [93]. This process starts around apparently normal nuclei with myofibrillar disappearance producing an increasing vacuolization of myocardial cells until a histologic pattern of empty sarcolemmal tubes without any cellular reaction or signs of healing results. This lesion is generally present in the subendocardial half of the cardiac wall [31].

ANABOLIC ANDROGEN STEROID ABUSE AND EXERCISE: OXIDATIVE STRESS

Start from the data that exercise-induced cardioprotection is impaired by supraphysiological doses of DECA in treadmill-exercised rats, Chaves E.A. *et al.* presented for the first time that the increased antioxidant enzyme levels promoted by exercise is impaired by DECA treatment (10

mg kg^{-1} body weight during 8 weeks), which could be associated with the cardiac deleterious effects of this drug [94].

The hearts of DT (DECA trained) animals exhibited lower SOD (superoxide dismutase), GPx (Glutathione peroxidase) and GR (glutathione reductase) activities when compared with CT (control trained) group indicating that in DT animals, DECA could be acting through the blockage or down regulation of the mechanism(s) involved in the improved antioxidant defences, which would explain the reduced % LVDP (left ventricular developed pressure) and increased infarct size in this group.

In different reports exercise training in rats has been shown to improve myocardial resistance to ischemia/reperfusion injury [95-97] as physiological cardiac hypertrophy modifies the heart's susceptibility renders it more resistant to ischemia/reperfusion injury in *in vivo* rat hearts [98].

Despite the intensive research efforts, the molecular mechanisms associated to exercise-induced cardioprotection are still controversial. The variables such as the rat strain used and the exercise type, which would determine the level, may explain the divergences found in the literature and the pathways involved in these adaptive responses [99-109].

Exhaustive exercise can produce significant damage in the myocardium [110,111]. In animal exercise models, heart injury occurs, resulting in disruption of fiber ultrastructure [111], decreased Ca^{2+}uptake in sarcoplasmic reticulum [112], and loss of whole-heart force production capability [113].

Oxidative stress may result in cellular alterations including a depression in the activity of sarcolemmal Ca^{2+} pump ATPase and Na^{+}–K^{+} ATPase activities. The sum of these changes led to decreased Ca^{2+} efflux and increased Ca2+ influx, respectively. Oxidative stress has also been reported to depress the sarcoplasmic reticulum Ca^{2+} pump ATPase and thus inhibits Ca^{2+} sequestration from the cytoplasm in cardiomyocytes [114,115].

It is well established that ROS are involved in exercise-induced damage in several tissues [110,116-119]. Although the evidence is still fragmentary, available data suggest that the heart is also affected by the oxidative challenge imposed by acute exercise [110].

Kumar *et al*. showed that an acute bout of exhaustive endurance exercise increased the generation of free radical signals as well as malondialdehyde (MDA) production in the myocardium of female albino rats [120].

Venditti and Di Meo in 1996 and 1997 described the increase of MDA and hydroperoxides in the myocardium of young and adult rats swimming to exhaustion [119,121].

A decrease of reduced glutathione (GSH) content in the myocardium of mice swimming to exhaustion has been described [122].

Numerous reports on animals [124] have observed elevated serum cTnT/cTnI after prolonged exercised that can exceed clinical cut – off value for acute myocardial infarction [123,125].

This fact can be interpreted in two different ways: 1) as this happens in every animal performing a similar exercise bout this is likely a normal and physiological response, or 2) elevated levels of serum cTnT in these animals provide evidence that prolonged exercise provides a significant insult to the myocardium that at the very least disrupts myocardial membrane permeability.

Starners and Bowles have speculated that cTnT/cTnI release during prolonged exercise is mediated through myocardial stunning [126]; while Hickman *et al*. [127] and Lippi and Banfi [128] described the ischemic development of blebs. Finally Neumayr *et al.* referred to a transient changes in membrane permeability [129].

It is possible that any or all of these potential mechanisms could be related to elevated reactive oxygen species (ROS) production that occurs with prolonged exercise [130].

Nie J. *et al.* focus on determine if prolonged exercise resulted in the appearance of cardiac troponin T in serum and whether this was associated with elevated levels of myocardial oxidative stress. They assess the temporal association of serum cTnT with markers of ROS damage actually in the myocardium. In fact the strenuous swimming exercise (3h) resulted in the appearance of cTnT in all animals that disappeared by 24 h post-exercise. They also found an increase in myocardial tissue concentration of MDA (malondialdehyde, a marker of free-radical related lipid peroxidation). The temporal association of cTnT and MDA suggests a role for exercise-induced increases in ROS in the mediation of cTnT release from human cardiomyocytes that is commonly witnessed after prolonged exercise [125].

Increased levels of ROS could be harmful to the cells [131] specifically by triggering lipid cell membrane peroxidation, which may result in myocyte cell membrane disjunction and/or damage [132].

The mechanism by which cTnT is released from within cardiomyocyte to the intravascular space during prolonged exercise and recovery is unknown.

In our work we have identified the morphological aspect of the troponin release in exercised males treated with supraphysiological dose of DECA. The immunohisto-chemical study revealed a progressive depletion of troponin C and troponin I from group A to Group C where we describe a more extensive myocardial damage, a high degree of depletion of troponin C, troponin I, confirming the diagnosis of myocardial necrosis (myocytolysis and CBN). In this study, the myocardial lesion indicates a necrosis of the myocardial cells in a hypercontracted state (tetanic death) characterised by rhexis of the myofibrillar apparatus, anomalous hyper-eosinophilic cross-bands formed by seg-ments of hypercontracted sarcomeres with extremely thickened Z lines, as shown ultrastructurally [115,133] (Fig. **1**).

Clarke M. [134] and McNeil [135] demonstrated the release of acidic and basic fibroblast growth factors (>FGF and AFGF, respectively) from the cytosol of cardiac

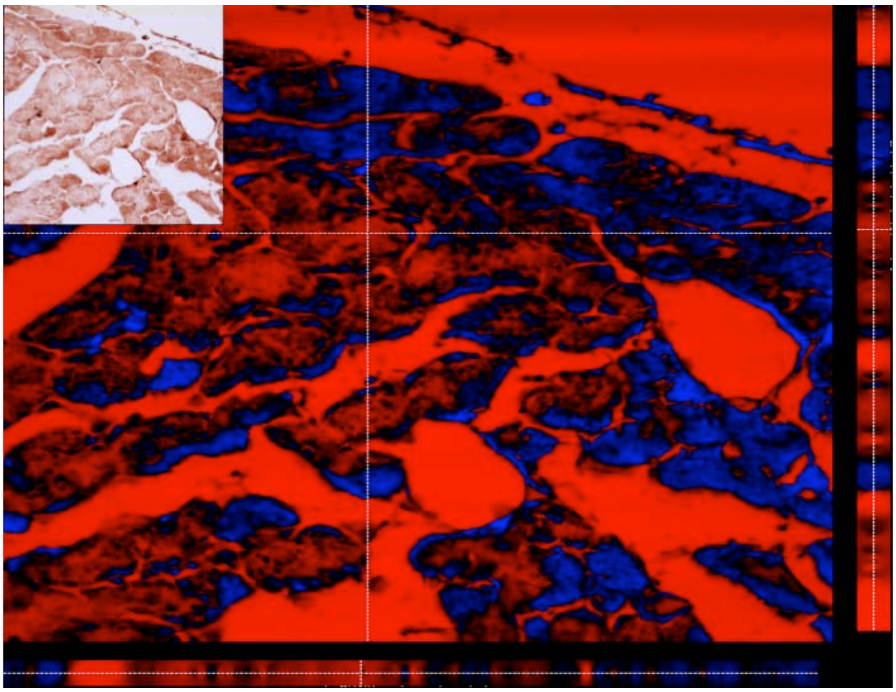

Fig. (1). Confocal laser scanning microscope. High degree of depletion of troponin C in group C mice.

myocytes in response to membrane damage caused by an increase in the rate and force of cardiac contraction [123]. So, the membrane damage, subsequent to an increate rate and force of cardiac contraction during endurance exercise, may provide a mechanism by which cytosolic troponin is released into the circulation. High-intensity exercise also results in an increase production of oxygen free radicals that may lead to membrane disruption and, hence, cTnT release [123].

The heart may be vulnerable to peroxidative damage due to oxidative stress [136] because it is both highly aerobic [137] whose metabolic process produce ROS at rest and during exercise [138], and it has reduced antioxidant enzyme activity compared with other tissues [136].

There is only a human study where no link was observed [139]. However in this paper the ROS markers were assayed in the systemic circulation and no localized the action or damage in the myocardium. Serum cTnT was elevated in all animal post-prolonged exercise and the same temporal pattern occurred for MDA (marker of lipid peroxidation in cell membranes subsequent to reactions with ROS). Therefore myocardial anti-oxidant capacity is not elevated in the face of an exercise stress that is known to increase ROS.

Venditti has found that prolonged aerobic exercise (210min) produces a 22% decrease in overall antioxidant capacity of rat heart while exhaustive exercise (444min) produces a 50% decrease [110,119,140]. This finding suggests that a key role in the myocardial damage could be played by the duration of exercise.

In another study Hollander *et al*. demonstrated that an acute bout of exercise increased binding of the transcription factors NF-kB and AP-1 in skeletal muscle. These factors stimulated Mn–SOD mRNA transcription and increased CuZn–SOD protein levels upon exercise [94,141]. On the same floor are the results of Ji *et al.* that showed that exercise induces the activation of NF-kB signalling cascade in a redox-sensitive manner during muscular contraction and this would be associated with increased production of free radicals [142].

However, at present there is little information on the myocardial response to free radical insults induced by exercises of different duration as well as on the response of the heart of exercised animals to physio- pathological conditions that cause free radical-induced heart dysfunction.

Although the mechanisms by which DECA promote the deleterious effects in rat heart are not known, the main outcome would be that DECA treated animals do not show the adaptive response of the exercise-induced increase of antioxidant enzymes activities establishing a chronic oxidative stress condition, which would explain the cardiac injuries frequently found in AAS users [3,7].

In Chaves study sedentary and swim-exercised rats treated with nandrolone laurate showed and increase susceptibility of hearts to I/R injuries [94]. These data are in agreement with Du Toit *et al.* [7] however the sedentary treated animals showed a significant reduction in the rate pressure product after reperfusion compared to non-treated sedentary animals. This could be explained by differences in the AAS used (laurate instead of decanoate) or in the rat strain (Long-Evans instead of Wistar).

Chaves *et al.* for the first time proposed a mechanistic explanation for the observed impairment of exercise-induced cardioprotection promoted by an AAS. They suggest that the cascade of events that lead to cardioprotection is impaired by

DECA at least the one involved in cellular ability to detoxify ROS. The study evaluated the influence of high doses of AAS in treadmill exercised rat heart tolerance to I/R injuries and the involvement of antioxidant enzymes in the lack of cardioprotection observed in exercised AAS treated rats [94].

The molecular mechanisms involved in these adaptive responses include increased expression of heat shock proteins [101,102] induction of nitric oxide synthase [108], protein kinase C activation [106] as well as increased antioxidant enzyme activities [100,102,104,105,109].

In our study we found a numerous and sparse foci of positive immunoistochemical reaction for HSP-70 in groups A (1.875 mg ND per kilogram twice per week, free movement) and B (1.875mg per kilogram twice per week, running on a tapìs ròulant); an intense and massive positive reaction was found in perivasal areas of the deep layers of myocardium and in the subendocardial layer in group C (5mg per kilogram twice per week, running on a tapìs ròulant) [31].

It's known that exercise is also a type of stressor that increases the content of HSPs in mammalian cardiac muscle, and some evidence indicates that exercise-induced HSPs play a protective role in mammalian heart against stresses [56]. The increase in the induction of cardiac expression of HSP-72, a member of the stress-induced HSP-70 protein family, may reflect the high intensity of the intracellular stress generated by the forced treadmill running regime.

Lunz *et al*. investigated the effects of administering nandrolone decanoate (weekly intramuscular injection of 6.5 mg kg^{-1} body weight of nandrolone decanoate to sedentary or chronically exercised rats: a progressive exercise training program on a treadmill, 5 days/week, for 8 weeks) on the induction of cardiac HSP-72 expression [56].

The exercised animals exhibited a higher (two-way ANOVA, $P < 0.05$) HW/BW compared with sedentary animals (mean ± SEM; 4.65 ± 0.10 *vs* 4.20 ± 0.11 mg/g, respectively), independently of receiving nandrolone. This indicates that the training regime induced cardiac hypertrophy, a physiological adaptation also reported by others [143]. Likewise, the nandrolone-treated rats showed a higher ($P < 0.05$) HW/BW than untreated animals (4.68 ± 0.10 *vs* 4.18 ± 0.11 mg/g, respectively), independent of the exercise training. They demonstrated that exercised animals exhibited a greater (two-way ANOVA, $P < 0.05$) accumulation of HSP-72 in the cardiac muscle compared to sedentary animals (mean ± SEM; 677.16 ± 129.14 *vs* 246.24 ± 46.30 relative unit, respectively), independently of the nandrolone administration. They found that exercise-induced HSP-72 expression was not affected by nandrolone. Nandrolone dose used (6.5 mg/kg body weight) was not sufficient to cause any deleterious effects on the myocardium of these animals in 8 weeks. These levels of HSP-72 expression in response to nandrolone administration suggest either a low intracellular stress or a possible less protection to the myocardium.

Such lack of HSPs accumulation could explain, at least in part, the deleterious effects of nandrolone administration on myocardium observed by some Authors [56,144] inasmuch as protection of cardiac cells by HSPs may not have occurred [56].

INTRACELLULAR MECHANISMS: GENOMIC AND NON-GENOMIC EFFECTS

Numerous studies have been conducted to understand the pathogenesis of ventricular remodelling, cardiomyopathy, and sudden cardiac death associated with AAS abuse. It is known that heart is a target organ for AASs [145] and AAS receptors were previously identified in cardiomyocytes of monkeys and rats. Cardiac myocytes contain intracellular androgen receptors [146], which regulate the expression of several genes [147,148].

Although steroids primarily modulate nuclear transcription by intracellular steroid-binding proteins, nongenomic effects of steroids such as the anesthetic and analgesic effects of progesterone are well established [149]. These effects are mediated by membrane-receptor-second messenger cascades and are likely to be involved in the induction and commitment of apoptotic cell death in cardiomyocytes.

Altamirano *et al*. propose that cell growth produced by anabolic steroid hormones requires both androgen receptor (AR) activity and translation control trough mTOR signalling pathway by a coordinated mechanism, where mTOR regulated translation and the AR regulates gene expression [36]. They showed that testosterone action has been explained not only by activation of the intracellular androgen receptors (canonical genomic mechanism), but also by activation of mTORC1 pathway (mammalian target of rapamycin complex 1), a multifunctional protein complex involved in survival, proliferation, differentiation and growth [150], in cardiomyocytes thorough inositol 1,4,5-trisphosphate (IP3)-mediated Ca^{2+} release and MEK/ERK1/2 (extracellular – regulated kinase) to induced hypertrophy [36]. It stimulates protein translation and ribosome biosynthesis, playing an important role during the shift from normal to hypertrophied cardiomyocytes, as demonstrated *in vitro* [151-156] and *in vivo* [157-161].

Physical exercise might activate mTORC1 through P13K/Akt [156,161], while testosterone activates mTORC1/S6K1 axis through MEK/ERK1/2 and IP3/Ca2+ signalling as phenylephrine on development of cardiac hypertrophy.

These effects are mediated by membrane receptor-second messenger cascades that increase intracellular Ca^{2+} influx and Ca^{2+} mobilization from the sarcoplasmic reticulum [162]. It's described that AASs increase polyamines in cardiomyocytes, which are known to mediate uncontrolled transmembrane Ca^{2+} flux in the Ca^{2+} paradox [163]. Moreover, steroids were reported to couple to pertussis-sensitive guanine nucleotide-binding proteins, which activate phosphoinositide-phospholipase C, thereby increasing intracellularCa^{2+} *via* Ca^{2+} influx and Ca^{2+} mobilization from sarcoplasmic reticulum [162].

The increase in myocardial intracellular calcium release could explain proarrhythmic effects of nandrolone since

intracellular calcium overload has been associated with arrhythmogenesis during myocardial ischemia [164].

It has been found that exposure to testosterone rapidly (1-7 min) led to an increase of intracellular Ca^{2+} in cardiac myocytes, an effect that persisted in the absence of external Ca^{2+}. The role of elevated intracellular calcium concentrations in the induction of apoptosis is supported by many studies. In fact, elevated cytosolic calcium concentrations alter the permeability of mitochondrial membranes, which results in the release of pro-apoptotic factors including holocytochrome c, apoptosis-inducing factor and caspase-9 from damaged mitochondria [165,166].

Vicencio *et al*. report the early effects of testosterone on intracellular Ca^{2+} in cultured cardiac myocytes, demonstrating the first link between Ca^{2+} and AAS in cardiac myocytes and suggesting that this nongenomic effect of AAS can contribute to the documented androgen receptor mediated cardiotoxicity observed in AAS abuse. They found, in cultured cardiac myocytes, that testosterone induces a rapid and nongenomic intracellular Ca^{2+} release through activation of a plasma membrane androgen receptor associated with the PTX-sensitive G protein-PLC/IP3 signaling pathway [167].

Different hormones and growth factors stimulate cardiac myocyte hypertrophy through Ca^{2+}-dependent signalling pathways [168]. Calmodulin-activated phosphatase calcineurin, activated by increases in calcium, mediates the hypertrophic response through its downstream nuclear factor of activated T cells [169].

This is confirmed also by Phillis *et al*. [25] that observed Nandrolone has been shown to cause the release of intracellular calcium in rat primary myotubes, in a manner which is independent of intracellular androgen receptor, but dependent on inositol trisphosphate and the extracellular signal-regulated kinase pathway [170].

Melchert [171] and Welder [172] presented evidence for a direct toxic effect of testosterone cypionate, testosterone enanthate, testosterone proprionate and oxymetholone on primary neonatal rat myocardial cell culture. The data clearly show that 100μM concentration of testosterone cypionate, testosterone enanthate, testosterone proprionate and oxymetholone will cause myocardial cell toxicity after 4 hr of exposure. Based on the beating rate, MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) formazan production and intracellular calcium data, an alteration in the cell's ability to maintain normal high-energy phosphate may lead to a loss of plasma membrane integrity and eventual cell death. Cellular toxicity was determined by increase LDH (lactate dehydrogenase) leak-age, decrease neutral red retention and decrease MTT formazan production in myocardial cell culture exposed to 100μM testosterone cypionate for 4hr.

Several enzyme systems are involved by different AAS. Chainy and Kanungo found that testosterone increased the activity of pyruvate kinase in the heart of castrated male rats [173]. These results suggest that specific enzymes systems within the heart may be affected by testosterone administration. Koenig [174] reported that testosterone stimulated calcium fluxes and membrane transport process in rat ventricular myocytes *via* stimulation of ornithine decarboxilase where polyamines acted as intracellular messengers.

Literature data clearly show that AAS induce apoptotic cell death in a dose-dependent manner [37]. Zaugg *et al*. [37] described in their study that adult cardiomyocytes exposed to AASs (stanozolol: STZ, testosterone enanthate: TE and testosterone: T, 0.1 mmol/L, 1 mmol/L, 10 mmol/L, and 100 mmol/L for 20 h) undergo concentration-dependent apoptotic cell death, providing evidence that AASs at higher concentrations induce apoptosis in adult cardiomyocytes. Morphological and biochemical effects of testosterone in neonatal rat myocardial cultures was described [172], AASs exert primarily growth-promoting effects in cardiac tissue [163], STZ induced apoptotic cell death in skeletal muscle cells [175], and increased gene expression of the proto-oncogenes c-fos and c-myc [176] that was reported in hypertrophic cardiomyopathy [177], and precedes apoptotic cell death [178].

Animal studies show that abused AAS such as nandrolone at appropriately high doses may reverse vasodilator response and lead to growth-promoting effects on cardiac tissue, as seen in hypertrophic cardiomyopathy, followed by apoptotic cell death [37,177,179].

The loss of cell viability caused by AS toxicity may in some part be the result of alterations of intracellular ion concentrations after decreased plasma membrane integrity and the inability to synthesize high-energy phosphates [171,172].

In studies on cardiomyocyte ultrastructures after AAS applications in rododent, mitocondria and myofibrils showed aberrations that were similar to those in early heart failure [76].

The long-term effect of treating female rats with methandrostenolone on heart tissue was determined with transmission electron microscopy [75]. Rat hearts from the methandrostenolone-treated group had an increase in intermediate sized, nonmyofibrillar filaments in muscle cells of the left ventricle [76]. Heart cells from rats treated with methandrostenolone exhibited swollen and elongated mitocondria and disintegration of myofibrils [76].

An increase in sarcoplasmic reticulum volume and mitocondria was observed [69]. Behrendt and others have found that, after exposure to methandrostenolone (Dianabol; Novartis, East Hanover, NJ), the mitochondria within the rat left ventricle enlarge, become rounded with the appearance of membranous defects and an electronlucent matrix, and then elongate, leaving only a sparse matrix material and a few cristae [75]. Electron microscopy of the contractile apparatus within myocardium similarly treated shows completely destroyed sarcomeres, regional disappearance of ribosomes and polysomes, thickening and stretching of the Iband, and noncontractile globular networks of disrupted fragments of both thick and thin filaments [76].

These results suggest that this non-genomic effect of AAS can contribute to the documented androgen receptor mediated cardiotoxicity observed in AAS abuse [167].

Fanton *et al*. [165], Zaugg *et al.* [37] and Medei *et al*. [1] showed a reduction of total nuclei in the DECA – treated group suggests a toxic effect of DECA that may involve a pro – apoptotic mechanism with caspase-3 activity significantly increased in the heart samples.

These data were confirmed by immunohistochemical experimental studies. In our work we describe a significant randomly sparse apoptotic process in the damaged myocardium and the enhanced effect of TNF-α, HSP-70 and IL-1β production [31,180]. Myocytes nuclei labeled by TUNEL assay showed an intense, wide, positive reaction in the high dose AAS administration and physical training group, in which we also found an intense and massive positive immunoistochemical reaction for SMAC/DIABLO (second mitochondria-derived activator of caspases)/direct inhibitor of apoptosis (IAP)-binding protein) a mitochondrial protein that is release along with cytochrome c during apoptosis and activates the cytochrome c/Apaf-1/caspase-9pathway and BID (a BH3 domain-containing proapoptotic Bcl-2 family member) in the deep layers of the myocardium and in the subendocardial layer [181-185] (Fig. **2**).

TNF-α showed a wider positive expression in groups A and B and an intense positive reaction in group C, where in this group we observed a more extensive myocardial damage.

Again, our data would suggest that TNF-α plays a role in determining the severity of myocardial injury in our mice model. TNF-α expression was prominent in anabolic steroid-treated animals. Chronic use of supraphysiological concentrations of anabolic steroids, whether taken during exercise training programme or under sedentary conditions, increases myocardial susceptibility to nandrolone decanoate. Injury may be related to anabolic steroid-induced increases in TNF-α concentration in these hearts [7].

Apoptosis free oxygen radicals may regulate TNF-α production and act as the upstream initiators of AAS-induced apoptosis irrespective to the modulation of β-adrenoceptors [186]. These findings have suggested that a part of myocardial damage induced by AAS may be independent of their interaction with the β-adrenoceptor signal transduction system.

Recent studies have shown that circulating cytokines such as TNF-α may play a role in cardiac remodelling and that anabolic steroids strongly stimulate leukocyte TNF-α production [7].

In agreement with literature, the expression of inhibitor of apoptosis HSP-70 and inflammatory cytokine IL-1β, increased in the three groups. The rise of cardioinhibitory cytokines may be interpreted as the adaptive response of jeopardized myocardium with respect to the cardiac dysfunction resulting from nandrolone decanoate injection [180,187].

ANABOLIC ANDROGEN STEROID ABUSE AND SYMPATHETIC OVER ACTIVATION

It has been described that the combined effect of exercise and anabolic steroids causes an overstimulation followed by a transient functional and structural destabilization of sympathetic axon terminals; the transient destabilization of sympathetic axon terminals could be suggested as a reason for increased vulnerability to ventricular fibrillation [188].

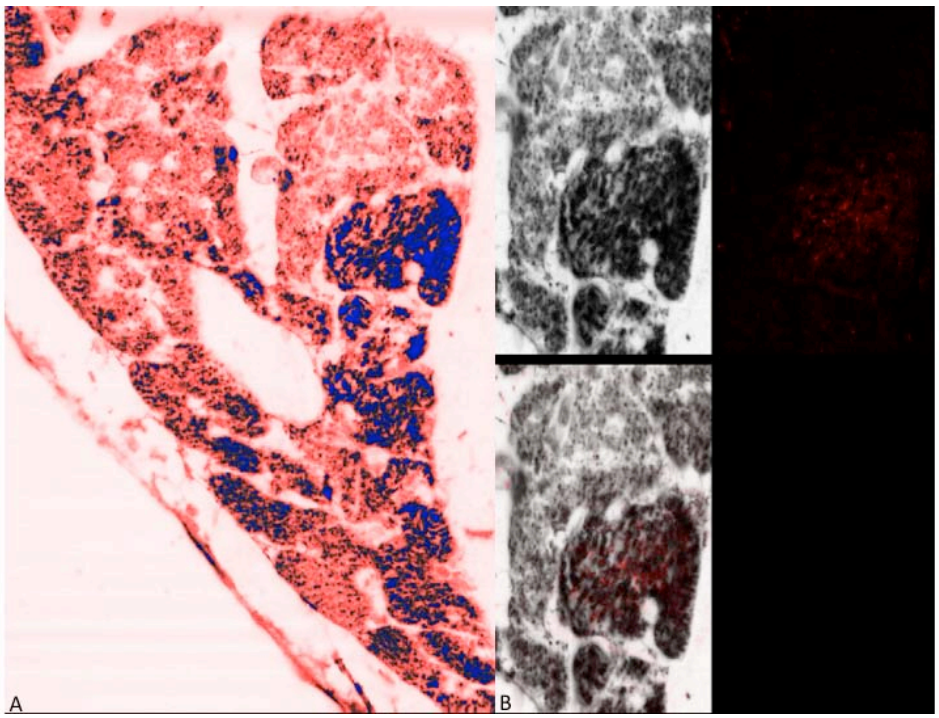

Fig. (2). Confocal laser scanning microscope. **A**: intense BID positive reaction (blue reactions) in Group C mice in deep layers of the myocardium. **B**: Massive expression (red reactions) of BID in deep layers of the myocardium (same image as in A with different laser beam field and higher magnification).

An imbalance of ANS (autonomic nervous system) activity has been associated with increased cardiovascular mortality (ventricular arrhythmia and sudden cardiac death) [189].

Pereira PP *et al*. demonstrated cardiac autonomic impairment with marked reductions in parasympathetic activity in rats after 8 weeks of treatment with DECA [190]. They evaluated by power spectral analysis of HRV (heart rate variability), the effects of chronic treatment with supraphysiological doses of DECA on tonic cardiac autonomic regulation in sedentary rats. In the group of rats treated with AAS, as in ventricular arrhythmia and sudden cardiac death, they found a marked impairment of parasympathetic cardiac modulation with decreased HF power of HRV compared to the control group. Thus, it seems plausible that autonomic dysfunction constitutes an early sign of AAS-induced cardiac disease, which, per se, represents a pro-arrhythmogenic factor that could occur independently, or preceding functional and/or structural abnormalities.

McNutt and Kennedy and Lawrence [191,48] suggested that AAS chronic administration may induce a profile of increased responsiveness to catecholamine. The HRV power spectral analysis results indicated a trend to sympathetic overactivation, as the sympathetic modulation index LF/HF [192] was increased in the DECA group compared to the control group. One possible explanation to AAS-induced autonomic imbalance raises from its effect on central nervous system. It's described by numerous authors that DECA treatment influences several neurotransmitter systems, including dopaminergic, serotonergic and adrenergic [89,193-197].

In particular Tamaki *et al*. [197] demonstrated that nandrolone enhances both norepinephrine and its metabolite 4-hydroxy-3-methoxyphenylglycol levels in hypothalamus.

Chronic administration of high doses of AAS leads to dysfunction in tonic cardiac autonomic regulation suggesting an alternative mechanism for anabolic steroid-induced arrhythmia and sudden cardiac death [190].

The electrical and histological remodelling mediated by DECA taken together with the autonomic unbalance [190] strongly suggests that DECA treatment creates a substrate to induce electrical disturbance [1].

Steroid hormones, chemically related to nandrolone, can acutely inhibit the reuptake of chatecolamines into extraneuronal tissue [198], which could in turn increase catecholamine concentrations at receptor sites. Although

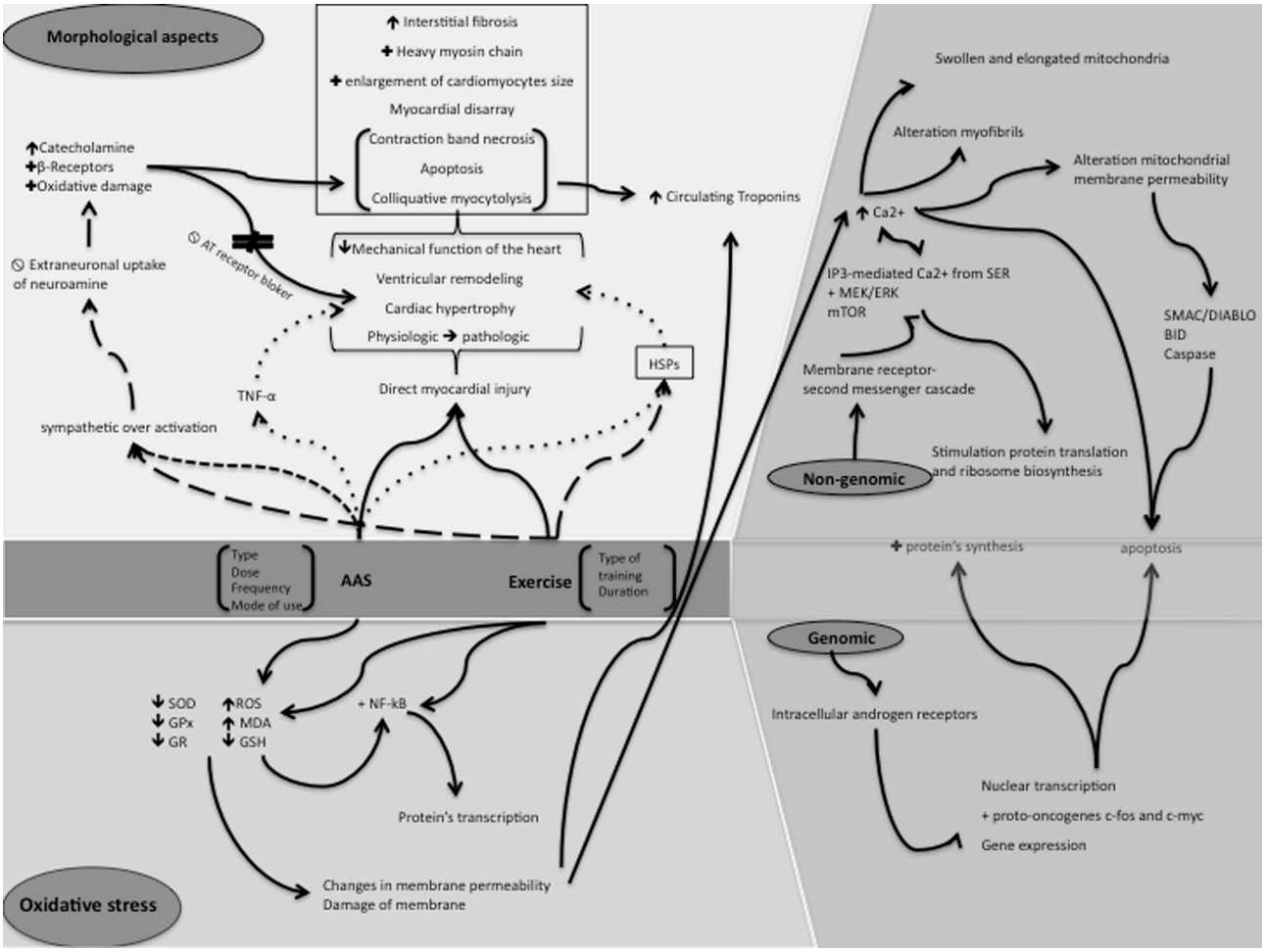

Fig. (3). Schematic illustration of cardiotoxicity's mechanisms.

normally responsible for reuptake of noradrenaline, during ischemia the neuronal catecholamine transporter has been shown to be responsible for nonexocytotic release of noradrenaline from sympathetic nerve terminals. Increase release of noradrenaline has been implicated in ischemia-induced arrhythmia [199,200-204].

Testosterone is a potent and selective inhibitor of extraneuronal norepinephrine uptake in the rat heart [198]. Administered with exercise, testosterone induced degenerative changes within the intracardiac sympathetic neurons of the mouse at 1 and 3 weeks, with adaptive regeneration at 6 weeks [27]. These changes appear to be the direct result of AS. However, this is another instance in which the indirect vascular response is potentially more cardiotoxic [28,47]. Both testosterone and methyltestosterone have been shown to enhance vascular reactivity to norepinephrine and subsequently generate hypertension [26,205].

CONCLUSION

In conclusion, data on experimental animal studies support the hypothesis that the combined effects of vigorous weight training, anabolic steroids abuse and stimulation of the sympathetic nervous system, may predispose to myocardial injury (myocardial disarray, contraction band necrosis, interstitial fibrosis, apoptosis) and subsequent cardiac failure (colliquative myocytolysis) [44,67,206] mediated by oxidative stress. These cardiovascular effects of AAS are mediated by genomic (intracellular androgen receptors – nuclear transcription – gene expression) and non-genomic mechanisms.

Cardiac hypertrophy is a leading predictor of progressive heart disease which often leads to heart failure and to a loss of cardiac contractile performance associated with profound alterations in intracellular calcium handling (Fig. **3**).

REFERENCES

[1] Medei, E.; Marocolo, M.; de Carvalho Rodrigues, D.; Arantes, P.C.; Takiya, C.M.; Silva, J.; Rondinelli, E.; Coeli dos Santos Goldenberg, R.; de Carvalho, A.C.C.; Nascimento, J.H.M. Chronic treatment with anabolic steroids induces ventricular repolarization disturbances: Cellular, ionic and molecular mechanism. *J. Mol. Cell. Cardiol.* **2010**, *49*(2), 165-175.

[2] Sullivan, M.L.; Martinez, C.M.; Gennis, P.; Gallagher, E.J. The cardiac toxicity of anabolic steroids. *Prog. Cardiovasc. Dis*. **1998**, *41*(1), 1-15.

[3] Committee on Sports Medicine and Fitness. Adolescents and anabolic steroids: A subject review. *Pediatrics*, **1997**, *99*(6), 904-908.

[4] Parkinson, A.B.; Evans, N.A. Anabolic androgenic steroids: A survey of 500 users. *Med. Sci. Sports Exerc.* **2006**, *38*(4), 644-651.

[5] Committee on sport medicine. Anabolic steroids and the adolescent athlete. *Pediatrics*, **1989**, *83*(1), 127-128.

[6] Woodiwiss, A.J.; Trifunovic, G.; Philippides, M.; Norton, G.R. Effect of an androgenic steroid on exercise-induced cardiac remodeling in rats. *J. Appl. Physiol*., **2000**, *88*(2), 409-415.

[7] Du Toit, E.F.; Rossouw, E.; Van Rooyen, J.; Lochner, A. Proposed mechanisms for the anabolic steroid-induced increase in myocardial susceptibility to ischaemia/reperfusion injury. *Cardiovasc. J. S. Afr*., **2005**, *16*(1), 21-28.

[8] Kutscher, E.C.; Lund, B.C.; Perry, P.J. Anabolic steroids: a review for the clinician. *Sports Med*., **2002**, *32*(5), 285-296.

[9] Parssinen, M.; Seppala, T. Steroid use and long termhealth risks informer athletes. *Sports Med.*, **2002**, *32*(2), 83-94.

[10] Sader, M.A.; Griffiths, K.A.; Skilton, M.R.; Wishart, S.M.; Handelsman, D.J.; Celermajer, D.S. Physiological testosterone replacement and arterial endothelial function in men. *Clin. Endocrinol. (Oxf).* **2003,** *59*(1), 62-67.

[11] Ammar, E.M.; Said, S.A.; Hassan, M.S. Enhanced vasoconstriction and reduced vasorelaxation induced by testosterone and nandrolona in hypercholesterolemic rabbits. *Pharmacol. Res.* **2004,** *50*(3), 253-259.

[12] Gonzales, R.J.; Krause, D.N.; Duckles, S.P. Testosterone suppresses endothelium dependent dilation of rat middle cerebral arteries. *Am. J. Physiol. Heart Circ. Physiol.* **2004,** *286*(2), H552-560.

[13] Cunha, T.S.; Moura, M.J.; Bernardes, C.F.; Tanno, A.P.; Marcondes, F.K. Vascular sensitivity to phenylephrine in rats submitted to anaerobic training and nandrolone treatment. *Hypertension*, **2005**, *46*(4), 1010-1015.

[14] Lane, H.A.; Grace, F.; Smith, J.C.; Morris, K.; Cockcroft, J.; Scanlon, M.F.; Davies, J.S. Impaired vasoreactivity in bodybuilders using androgenic anabolic steroids. *Eur. J. Clin. Invest.* **2006,** *36*(7), 483-488.

[15] Dorsett-Martin, W.A.; Hester, R.L. Sex hormones and aortic wall remodeling in an arteriovenous fistula. *Gend. Med.*, **2007**, *4*(2), 157-169.

[16] El-Mas, M.M.; Afify, E.A.; El-Din, M.M.M.; Omar, A.G.; Sharabi, F.M. Testosterone facilitates the baroreceptor control of reflex bradycardia: role of cardiac sympathetic and parasympathetic components. *J. Cardiovasc. Pharmacol*., **2001**, *38*(5), 754-763.

[17] El-Mas, M.M.; Afify, E.A.; Omar, A.G.; Sharabi, F.M. Cyclosporine adversely affects baroreflexes *via* inhibition of testosterone modulation of cardiac vagal control. *J. Pharmacol. Exp. Ther.*, **2002**, *301*(1), 346-354.

[18] Beutel, A.; Bergamaschi, C.T.; Campo, R.R. Effects of chronic anabolic steroid treatment on tonic and reflex cardiovascular control in male rats. *J. Steroid. Biochem. Mol. Biol*., **2005**, *93*(1), 43-48.

[19] Ward, G.R.; Abdel-Rahman, A.A. Effect of testosterone replacement or duration of castration on baroreflex bradycardia in conscious rats. *BMC Pharmacol*., **2005**, *5*, 9.

[20] Ward, G.R.; Abdel-Rahman, A.A. Orchirctomy or androgen receptor blockade attenuates baroreflex-mediated bradycardia in conscious rats. *BMC Pharmacol.*, **2006**, *6*, 2.

[21] Bissoli, N.S.; Medeiros, A.R.S.; Santosa, M.C.S.; Busato, V.C.W.; Jarske, R.D.; Abreu, G.R.; Moysés, M.R.; de Andrade, T.U. Long-term treatment with supraphysiological doses of nandrolone decanoate reduces the sensitivity of Bezold-Jarisch reflex control of heart rate and blood pressure. *Pharmacol. Res.*, **2009**, *59*(6), 379-384.

[22] Phillis, B.D.; Irvine, R.J.; Kennedy, J.A. Combined cardiac effects of cocaine and the anabolic steroid, nandrolone, in the rat. *Eur. J. Pharmacol*., **2000**, *398*(2), 263-272.

[23] Tseng, Y.T.; Rockhold, R.W.; Hoskins, B.; Ho, I.K. Cardiovascular toxicities of nandrolone and cocaine in spontaneously hypertensive rats. *Fundam. Appl. Toxicol*., **1994**, *22*(1), 113-121.

[24] Trifunovic, B.; Norton, G.R.; Duffield, M.J.; Avraam, P.; Woodiwiss, A.J. An androgenic steroid decreases left ventricular compliance in rats. *Am. J. Physiol*., **1995**, *268*(3 Pt 2), H1096-H1105.

[25] Phillis, B.D.; Abeywardena, M.Y.; Adams, M.J.; Kennedy, J.A.; Irvine, R.J. Nandrolone potentiates arrhythmogenic effects of cardiac ischemia in the rat. *Toxicol. Sci*., **2007**, *99*(2), 605-611.

[26] Baker, P.J.; Ramey, E.R.; Ramwell, P.W. Androgen-mediated sex differences of cardiovascular responses in rats. *Am. J. Physiol*., **1978**, *235*(2), H242-H246.

[27] Hartmann, G.; Addicks, K.; Donike, M.; Schanzer, W. Testosterone application influences sympathetic activity of intracardiac nerves in non-trained and trained mice. *J. Auton. Nerv. Syst*., **1986**, *17*(2), 85-100.

[28] Melchert, R.B.; Welder, A.A. Cardiovascular effects of androgenic-anabolic steroids. *Med. Sci. Sports Exerc*., **1995**, *27*(9), 1252-1262.

[29] Fineschi, V.; Baroldi, G.; Monciotti, F.; Paglicci Reattelli, L.; Turillazzi, E. Anabolic steroid abuse and cardiac sudden death: a pathologic study. *Arch. Pathol. Lab. Med*., **2001**, *125*(2), 253-255.

[30] Achar, S.; Rostamian, A.; Narayan, S.M. Cardiac and metabolic effects of anabolic-androgenic steroid abuse on lipids, blood

pressure, left ventricular dimensions, and rhythm. *Am. J. Cardiol*., **2010,** *106*(6), 893-901.

[31] Riezzo, I.; Di Paolo, M.; Neri, M.; Bello, S.; Cantatore, S.; D'Errico, S.; Dinucci, D.; Parente, R.; Pomara, C.; Rabozzi, R.; Turillazzi, E.; Fineschi, V. Anabolic steroid - and exercise- induced cardio-depressant cytokines and myocardial β1-receptor expression in CD1 Mice**.** *Curr. Pharm. Biotechnol*., **2011**, *12*(2):275-84.

[32] Marsh, J.D.; Lehmann, M.H.; Ritchie, R.H.; Gwathmey, J.K.; Green, G.E.; Schiebinger, R.J. Androgen receptors mediate hypertrophy in cardiac myocytes. *Circulation*., **1998,** *98*(3), 256-261.

[33] Cabral, A.M.; Vasquez, E.C.; Moyses, M.R.; Antonio, A. Sex hormone modulation of ventricular hypertrophy in sinoaortic denervated rats. *Hypertension*., **1988,** *11*(2 Pt 2), I93-I97.

[34] Malhotra, A.; Buttrick, P.; Scheuer, J. Effects of sex hormones on development of physiological and pathological cardiac hypertrophy in male and female rats. *Am. J. Physiol*., **1990**, *259*(3 Pt 2), H866-H871.

[35] Nahrendorf, M.; Frantz, S.; Hu, K.; von zur Muhlen, C.; Tomaszewski, M.; Scheuermann, H.; Kaiser, R.; Jazbutyte, V.; Beer, S.; Bauera, W.; Neubauerb, S.; Ertla,G.; Allolioa,B.; Callies, F. Effect of testosterone on post-myocardial infarction remodeling and function. *Cardiovasc. Res*., **2003**, *57*(2), 370-378.

[36] Altamirano, F.; Oyarce, C.; Silva, P.; Toyos, M.; Wilson, C.; Lavandero, S.; Uhlén, P.; Estrada, M. Testosterone induces cardiomyocyte hypertrophy through mammalian target of rapamycin complex 1 pathway. *J. Endocrinol*., **2009**, *202*(2), 299-307.

[37] Zaugg, M.; Jamali, N.Z.; Lucchinetti, E.; Xu, W.; Alam, M.; Shafiq, S.A.; Siddiqui, M.A. Anabolic- androgenic steroids induce apoptotic cell death in adult rat ventricular myocytes. *J. Cell. Physiol*., **2001**, *187*(1), 90-95.

[38] Tagarakis, C.V.; Bloch, W.; Hartmann, G.; Hollmann, W.; Addicks, K. Anabolic steroids impair the exercise induced growth of the cardiac capillary bed. *Int. J. Sports Med.*, **2000**, *21*(6), 412-418.

[39] Karhunen, M.K.; Ramo, M.P.; Kettunen, R.; Hirvonen, L. Anabolic steroids alter the hemodynamic effects of endurance training and deconditioning in rats. *Acta. Physiol. Scand*., **1988,** *133*(3), 297-306.

[40] Liang, M.T.C.; Paulson, D.J.; Kopp, S.J.; Glonek, T.; Meneses, P.; Gierke, L.W.; Schwartz, F.N. Effect of anabolic steroids and endurance exercise on cardiac performance. *Int. J. Sports Med*., **1993**, *14*(6), 324-329.

[41]Paulson, D.J.; Tahiliani, A.G. Cardiovascular abnormalities associated with human and rodent obesity. *Life Sci.*, **1992**, *51*(20), 1557-1569.

[42] Luke, J.L.; Farb, A.; Virmani, R.; Sample, R.H. Sudden cardiac death during exercise in a weight lifter using anabolic androgenic steroids: pathological and toxicological findings. *J. Forensic Sci*., **1990**, *35*(6), 1441-1447.

[43] Hausmann, R.; Hammer, S.; Betz, P. Performance enhancing drugs (doping agents) and sudden death — a case report and review of the literature. *Int. J. Leg. Med*., **1998**, *111*(5), 261-264.

[44] Di Paolo, M.; Agozzino, M.; Toni, C.; Luciani, A.B.; Molendini, L.; Scaglione, M.; Inzani, F.; Pasotti, M.; Buzzi, F.; Arbustini, E. Sudden anabolic steroid abuse-related death in athletes. *Int. J. Cardiol*., **2007**, *114*(1), 114-117.

[45] Takala, T.E.S.; Ramo, P.; Kiviluoma, K.; Vihko, V.; Kainulainen, H.; Kettunen, R. Effects of training and anabolic steroids on collagen synthesis in dog heart. *Eur. J. Appl. Physiol*., **1991**, *62*(1), 1-6.

[46] Fleck, S. J.; Pattany, P.M.; Stone, M.H.; Kraemer, W.J.; Thrush, J.; Wong, K. Magnetic resonance imaging determination of left ventricular mass: junior olympic weightlifters. *Med. Sci. Sports Exerc*., **1993**, *25* (4), 522-527.

[47] Rockhold, R.W. Cardiovascular toxicity of anabolic steroids. *Annu. Rev. Pharmacol. Toxicol*., **1993**, *33*, 497-520.

[48] Kennedy, M.C.; Lawrence, C. Anabolic steroid abuse and cardiac death. *Med. J. Aust*., **1993**, *158*(5), 346-348.

[49] Sachtleben, T.R.; Berg, K.E.; Elias, B.A.; Cheatham, J.P.; Felix, G.L.; Hofschire, P.J. The effects of anabolic steroids on myocardial structure and cardiovascular fitness. *Med. Sci. Sports Exerc*., **1993**, *25*(11), 1240-1245.

[50] Yokoyama, T.; Nakano, M.; Bednarczyk, J.L.; McIntyre, B.W.; Entman, M.; Mann, D.L. Tumor necrosis factor-alpha provokes hypertrophic growth response in adult cardiac myocytes. *Circulation*, **1997**, *95*(5), 1247-1252.

[51] Hughes, T.K.; Fulep, E.; Juelich, T.; Smith, E.M., Stanton, G.J. Modulation of immune responses by anabolic androgenic steroids. *Int. J. Immunopharmacol*., **1995**, *17*(11), 857-863.

[52] Kiang, J.G.; Tsokos, G.C. Heat shock protein 70 kDa: molecular biology, biochemistry, and physiology. *Pharmacol. Ther*., **1998**, *80*(2), 183-201.

[53] Atalay, M.; Oksala, N.K.; Laaksonen, D.E.; Khanna, S.; Nakao, C.; Lappalainen, J.; Roy, S. Hänninen, O. Sen, C.K. Exercise training modulates heat shock protein response in diabetic rats. *J. Appl. Physiol*., **2004**, *97*(2), 605-611.

[54] Noble, E.G.; Moraska, A.; Mazzeo, R.S.; Roth, D.A.; Olsson, M.C.; Moore, R.L.; Fleshner, M. Differential expression of stress proteins in rat myocardium after free wheel or treadmill run training. *J. Appl. Physiol*., **1999**, *86*(5), 1696-1701.

[55] Chicco, A.J.; Schneider, C.M.; Hayward, R. Voluntary exercise protects against acute doxorubicin cardiotoxicity in the isolated perfused rat heart. *Am. J. Physiol. Regul. Integr. Comp. Physiol*., **2005**, *289*(2), R424- R431.

[56] Lunz, W.; Oliveira, E.C.; Neves, M.T.D.; Fontes, E.P.B.; Dias, C.M.G.C.; Natali, A.J. Anabolic steroid- and exercise-induced cardiac stress protein (HSP72) in the rat. *Braz. J. Med. Biol. Res*., **2006**, *39*(7), 889-893.

[57] Ferrans, V.J.; Roberts, W.C. Intermyofibrillar and nucleomyofibrillar connections in human and canine myocardium: An ultrastructural study. *J. Mol. Cell. Cardiol*., **1973**, *5*(3), 247-257.

[58] Morano, I.; Gerstner, J.; Ruegg, J.C.; Ganten, U.; Ganten, D.; Vosberg, H.P. Regulation of myosin heavy chain expression in the hearts of hypertensive rats by testosterone. *Circ. Res*., **1990**, *66*(6), 1585-1590.

[59] Moore, L.G.; McCurty, I.F.; Reeves, J.T. Effects of sex hormones on cardiovascular and hematologic responses to chronic hypoxia in rats. *Proc. Soc. Exp. Biol. Med*., **1978**, *158*(4), 658-662.

[60] Lengsfeld, M.; Morano, I.; Ganten, U.; Rüegg, J.C. Gonadectomy and hormonal replacement changes systolic blood pressure and ventricular myosin isoenzyme pattern of spontaneously hypertensive rats. *Circ. Res*., **1988**, *63*(6), 1090-1094.

[61] Takala, T.E.S.; Ramo, P.; Kiviluoma, K.; Vihko, V.; Kainulainen, H.; Kettunen, R. Effects of training and anabolic steroids on collagen synthesis in dog heart. *Eur. J. Appl. Physiol*., **1991**, *62*(1), 1-6.

[62] Ramo, P. Anabolic steroids alter the haemodynamic responses of the canine left ventricle. *Acta Physiol. Scand*., **1987**, *130*(2), 209-217.

[63] Pesola, M.K. Reversibility of the haemodynamic effects of anabolic steroids in rats. *Eur. J. Appl. Physiol. Occup. Physiol*., **1988**, *58*(1-2), 125-131.

[64] Hall, C.E.; Hall, O. Methylandrostenediol hypertension induced without salt excess, observations on organ changes and serum composition. *Am. J. Pathol*., **1969**, *54*(3), 489-505.

[65] Brown, B.S.; Pilch, A.H. The effects of exercise and Dianabol upon selected performances and physiological parameters in the male rat. *Med. Sci. Sports*, **1972**, *4*(3), 159-165.

[66] Urhausen, A.; Holpes, R.; Kindermann, W. One- and two-dimensional echocardiography in bodybuilders using anabolic steroids. *Eur. J. Appl. Physiol. Occup. Physiol*., **1989**, *58*(6), 633-640.

[67] Dickerman, R. D.; Schaller, F.; Prather, I.; McConathy, W. J. Sudden cardiac death in a 20-year-old bodybuilder using anabolic steroids. *Cardiology*, **1995**, *86*(2), 172-173.

[68] Rocha, F. L.; Carmo, E. C.; Roque, F. R.; Hashimoto, N. Y.; Rossoni, L. V.; Frimm, C.; Anéas, I.; Negrão, C. E.; Krieger, J. E.; Oliveira, E. M. Anabolic steroids induce cardiac renin-angiotensin system and impair the beneficial effects of aerobic training in rats. *Am. J. Physiol. Heart Circ. Physiol*., **2007,** *293*(6), H3575-H3583.

[69] Fontana, K.; Oliveira, H.C.; Leonardo, M.B.; Mandarim-de-Lacerda, C.A.; da Cruz-Höfling, M.A. Adverse effect of the anabolic-androgenic steroid mesterolone on cardiac remodelling and lipoprotein profile is attenuated by aerobicz exercise training. *Int. J. Exp. Pathol*., **2008**, *89*(5), 358-366.

[70] Tagarakis, C.V.; Bloch, W.; Hartmann, G.; Hollmann, W.; Addicks, K. Testosterone-propionate impairs the response of the

cardiac capillary bed to exercise. *Med. Sci Sports Exerc*., **2000**, *32*(5), 946- 953.

[71] LeGros, T.; McConnell, D.; Murry, T.; Edavettal, M.; Racey-Burns, L.A.; Shephard, R.E.; Burns, A.H. The effect of 17 alpha-methyltestosterone on myocardial function *in vitro*. *Med. Sci Sports Exer*., **2000**, *32*(5), 897-903.

[72] Takala, T.E.S.; Ramo, P.; Kiviluoma, K.; Vihko, V.; Kainulainen, H.; Kettunen, R. Effects of training and anabolic steroids on collagen synthesis in dog heart. *Eur. J. Appl. Physiol. Occup. Physiol*., **1991**, *62*(1), 1-6.

[73] Campbell, S.E.; Farb, A.; Weber, K.T. Pathologic remodelling of the myocardium in a weightlifter taking anabolic steroids. *Blood Press*., **1993**, *2*(3), 213-216.

[74] Appell, H. J.; Heller-Umpfenbach, B.; Feraudi, M.; Weicker, H. Ultrastructural and morphological investigations on the effects of training and administration of anabolic steroids on the myocardium of guinea pigs. *Int. J. Sports Med*., **1983**, *4*(4), 268-274.

[75] Behrendt, H. Effect of anabolic steroids on rat heart muscle cells. I. Intermediate filaments. *Cell Tissue Res*., **1977**, *180*(3), 303-315.

[76] Behrendt, H.; Boffin, H. Myocardial cell lesions caused by an anabolic hormone. *Cell Tissue Res*., **1977**, *181*(3), 423-426.

[77] Sethi, R.; Saini, H.K.; Guo, X.; Wang, X.; Elimban, V.; Dhalla, N.S. Dependence of changes in β-adrenoceptor signal transduction on type and stage of cardiac hypertrophy. *J. Appl. Physiol*., **2007**, *102*(3), 978-984.

[78] Hannan, R. D.; Jenkins, A.; Jenkins, A. K.; Brandenburger, Y. Cardiac hypertrophy: a matter of translation. *Clin. Exp. Pharmacol. Physiol*., **2003**, *30*(8), 517-527.

[79] Ganguly, P.K.; Lee, S.L.; Beamish, R.E.; Dhalla, N.S. Altered sympathetic system and adrenoceptors during the development of cardiac hypertrophy. *Am. Heart J*., **1989**, *118*(3), 520-525.

[80] Karliner, J.S.; Barnes, P.; Brown, M.; Dollery, C. Chronic heart failure in the guinea pig increases cardiac α1- and β-adrenoceptors. *Eur. J. Pharmacol.*, **1980**, *67*(1), 115-118.

[81] Adameova, A.; Abdellatif, Y.; Dhalla, N.S. Role of the excessive amounts of circulating catecholamines and glucocorticoids in stress-induced heart disease. *Can. J. Physiol. Pharmacol*., **2009**, *87*(7), 493-514.

[82] D'Errico, S.; Neri, M.; Nieddu, A.; Mazzeo, E.; Riezzo, I.; Turillazzi, E.; Fineschi, V. Cardiac β1-adrenoceptor expression in two stress-induced cardiomyopathy-related deaths. *Forensic. Sci. Int*., **2010**, In press.

[83] Bisognano, J.D.; Weinberger, H.D.; Bohmeyer, T.J.; Pende, A.; Raynolds, M.V.; Sastravaha, A.; Roden, R.; Asano, K.; Blaxall, B.C.; Wu, S.C.; Communal, C.; Singh, K.; Colucci, W.; Bristow, M.R.; Ginsburg, R.; Minobe, W.; Cubicciotti, R.S.; Sageman, W.S.; Lurie, K.; Billingham, M.E.; Harrison, D.C.; Stinson, E.B. Decreased catecholamine sensitivity and beta-adrenergic-receptor density in failing human hearts. *N. Engl. J. Med*., **1982**, *307*(4), 205-211.

[84] Penna, C.; Abbadessa, G.; Mancardi, D.; Spaccamiglio, A.; Racca, S.; Pagliaro, P. Nandrolone-pretreatment enhances cardiac β(2)-adrenoceptor expression and reverses heart contractile down-regulation in the post-stress period of acute-stressed rats. *J. Steroid Biochem. Mol. Biol*., **2007**, *107*(1-2), 106-113.

[85] Benjamin, I.J.; Jalil, J.E.; Tan, L.B.; Cho, K.; Weber, K.T.; Clark, W.A. Isoproterenol-induced myocardial fibrosis in relation to myocyte necrosis. *Circ. Res*., **1989**, *65*(3), 657-670.

[86] Bunemann, M.; Lee, K.B.; Pals-Rylaarsdam, R.; Roseberry, A.G., Hosey, M.M. Desensitization of G-protein-coupled receptors in the cardiovascular system. *Annu Rev. Physiol*., **1999**, *61*, 169-192.

[87] Chang, D.H.; Einstein, R. Changes in cardiovascular responsiveness to dopexamine and β1- and β2-adrenoceptor function after the chronic treatment of β-adrenoceptor antagonists and agonists in anaesthetized dogs. *J. Auton. Pharmacol*., **1996**, *16*(5), 269-279.

[88] Schlussman, S.D.; Zhou, Y.; Johansson, P.; Kiuru, A., Ho, A., Nyberg, F., Kreekm, M.J. Effects of the androgenic anabolic steroid, nandrolone decanoate, on adrenocorticotropin hormone, corticosterone and proopiomelanocortin, corticotropin releasing factor (CRF) and CRF receptor1 mRNA levels in the hypothalamus, pituitary and amygdala of the rat. *Neurosci. Lett*., **2000**, *284*(3), 190-194.

[89] Lindblom, J.; Kindlundh, A.M.S.; Nyberg, F.; Bergstrom, L., Wikberg, J.E.S. Anabolic androgenic steroid nandrolone decanoate reduces hypothalamic propiomelanocortin mRNA levels. *Brain Res*., **2003**, *986*(1-2), 139-147.

[90] Bristow, M. R.; Port, D. J. Myocardial-directed overexpression of the human β(1)-adrenergic receptor in transgenic mice. *J. Mol. Cell. Cardiol*., **2000**, *32*(5), 817-830.

[91] Lamba, S.; Abraham, W.T. Alterations in adrenergic receptor signaling in heart failure. *Heart Fail. Rev*., **2000**, *5*(1), 7-16.

[92] Ahmed, A. Myocardial β-1 adrenoceptor down-regulation in aging and heart failure: implications for β-blocker use in older adults with heart failure. *Eur. J. Heart Fail*., **2003**, *5*(6), 709-715.

[93] Turillazzi, E.; Baroldi, G.; Silver, M. D.; Parolini, M.; Pomara, C.; Fineschi, V. A systematic study of a myocardial lesion: colliquative myocytolysis. *Int. J. Cardiol*., **2005**, *104*(2), 152-157.

[94] Chaves, E.A.; Pereira-Junior, P.P.; Fortunato, R.S.; Masuda, M.O.; de Carvalho, A.C.; de Carvalho, D.P.; Oliveira, M.F.; Nascimento, J.H. Nandrolone decanoate impairs exercise-induced cardioprotection: Role of antioxidant enzymes. *J. Steroid Biochem. Mol. Biol*., **2006**, *99*(4-5), 223-230.

[95] Bowles, D.K.; Farrar, R.P.; Starnes, J.W. Exercise training improves cardiac function after ischaemia in the isolated, working rat heart. *Am. J. Physiol*., **1992**, *263*(3 Pt 2), H804-H809.

[96] Libonati, J.R.; Gaughan, J.P.; Hefner, C.A.; Gow, A.; Paolone, A.M.; Houser, S.R. Reduced ischaemia and reperfusion injury following exercise training. *Med. Sci. Sports Exerc*., **1997,** *29*(4), 509-516.

[97] Marganato, V.; Milano, G.; Allibardi, S.; Merati G, de Jonge R, Samaja M. Swim training improves myocardial resistance to ischaemia in rats. *Int. J. Sports Med*., **2000**, *21*(3), 163-167.

[98] Ji, L.L.; Fu, R.G.; Mitchell, E.W.; Griffiths, M.; Waldorp, T.G.; Swartz, H.M. Cardiac hypertrophy alters myocardial response to ischaemia and reperfusion *in vivo*. *Acta Physiol. Scand*., **1994**, *151*(3), 279-290.

[99] Bowles, D.K.; Farrar, R.P.; Starnes, J.W. Exercise training improves cardiac function after ischemia in the isolated working rat heart. *Am. J. Physiol*., **1992**, *263*(3 Pt 2), H804-H809.

[100] Demirel, H.A.; Powers, S.K.; Zergeroglu, M.A.; Shanely, R.A.; Hamilton, K.; Coombes, J.; Naito, H. Short-term exercise improves myocardial tolerance to *in vivo* ischemia-reperfusion in the rat. *J. Appl. Physiol*., **2001**, *91*(5), 2205-2212.

[101] Hamilton, K.L.; Staib, J.L.; Phillips, T.; Hess, A.; Lennon, S.L.; Powers, S.K. Exercise, antioxidants, and HSP72: protection against myocardial ischemia/reperfusion. *Free Rad. Biol. Med*., **2003,** *34*(7), 800-809.

[102] Powers, S.K.; Demirel, H.A.; Vincent, H.K.; Coombes, J.S.; Naito, H.; Hamilton, K.L.; Shanely, R.A.; Jessup, J. Exercise training improves myocardial tolerance to *in vivo* ischemia-reperfusion in the rat. *Am. J. Physiol*., **1998**, *275*(5 Pt 2), R1468-R1477.

[103] Powers, S.K.; Locke, M.; Demirel, H.A. Exercise, heat shock proteins, and myocardial protection from I-R injury. *Med. Sci. Sports Exer*., **2001,** *33*(3), 386-392.

[104] Quindry, J.; French, J.; Hamilton, K.; Lee, Y.; Mehta, J.L.; Powers, S. Exercise training provides cardioprotection against ischemia-reperfusion induced apoptosis in young and old animals. *Exp. Gerontol*., **2005**, *40*(5), 416-425.

[105] Yamashita, N.; Hoshida, S.; Otsu, K.; Asahi, A.; Kuzuya, T.; Hori, M. Exercise provides direct biphasic cardioprotection *via* manganese superoxide dismutase activation. *J. Exp. Med*., **1999**, *189*(11), 1699-1706.

[106] Yamashita, N.; Baxter, G.F.; Yellon, D.M. Exercise directly enhances myocardial tolerance to ischemia-reperfusion injury in the rat through a protein kinase C mediated mechanism. Heart, **2001**, *85*(3), 331-336.

[107] Starnes, J.W.; Taylor, R.P.; Ciccolo, J.T. Habitual low-intensity exercise does not protect against myocardial dysfunction after ischemia in rats. *Eur. J. Cardiovasc. Prev. Rehabil*., **2005**, *12*(2), 169-174.

[108] Sessa, W.C.; Pritchard, K.; Seyedi, N.; Wang, J.; Hintze, T.H. Chronic exercise in dogs increases coronary vascular nitric oxide production and endothelial cell nitric oxide synthase gene expression. *Circ. Res*., **1994**, *74*(2), 349-353.

[109] Powers, S.K.; Criswell, D.; Lawler, D.; Martin, D.; Lieu, F.K.; Ji, L.L.; Herbet, R.A. Rigorous exercise training increases superoxide dismutase activity in ventricular myocardium. *Am. J. Physiol*., **1993**, *265*(6 Pt 2), H2094-H2098.

[110] Venditti, P.; Masullo, P.; Di Meo, S.; Agnisola, C. Effects of prolonged aerobic exercise on myocardial responses to ischaemia-reperfusion in the rat. *Exp. Physiol*., **2001,** *86*(3), 341-348.

[111] King, D.W.; Gollnick, P.D. Ultrastructure of rat heart and liver after exhaustive exercise. *Am. J. Physiol*., **1970**, *218*(4), 1150-1155.

[112] Pierce, G.N.; Kutryk, M.J.; Dhalla, K.S., Beamish, R.E., Dhalla, N.S. Biochemical alterations in heart after exhaustive swimming in rats. *J. Appl. Physiol*., **1984**, *57*(2), 326-331.

[113] Maher, J.T.; Goodman, A.L.; Francesconi, R.; Bowers. W.D.; Hartley, L.H.; Angelakos, E.T. Responses of rat myocardium to exhaustive exercise. *Am. J. Physiol*., **1972,** *222*(1), 207-212.

[114] Dhalla, N.S.; Elmoselhi, A.B.; Hata, T.; Makino, N. Status of myocardial antioxidants in ischemia-reperfusion injury. *Cardiovasc. Res*., **2000,** *47*(3), 446-456.

[115] Cerretani, D.; Riezzo, I.; Fiaschi, A.I.; Centini, F.; Giorgi, G.; D'Errico,S.; Fiore, C.; Karch, S.B.; Neri, M; Pomara C.; Turillazzi E.; Fineschi,V. Cardiac oxidative stress determination and myocardial morphology after a single ecstasy (MDMA) administration in a rat model. *Int. J. Legal Med*., **2008**, *122*(6), 461-469.

[116] Davies, K.J.; Quintanilha, A.T.; Brooks, G.A.; Packer, L. Free radicals and tissue damage produced by exercise. *Biochem. Biophys. Res. Commun*., **1982**, *107*(4), 1198-1205.

[117] Alessio, H.M.; Goldfarb, A.H. Lipid peroxidation and scavenger enzymes during exercise: adaptive response to training. *J. Appl. Physiol*., **1988**, *64*(4), 1333-1336.

[118] Goldfarb, A.H. Antioxidants: role of supplementation to prevent to exercise-induced oxidative stress. *Med. Sci. Sports Exerc*., **1993**, *25*(2), 232-236.

[119] Venditti, P.; Dimeo, S. Antioxidants, tissue damage, and endurance in trained and untrained young male rats. *Arch. Biochem. Biophys*., **1996**, *331*(1), 63-68.

[120] Kumar, C.T.; Reddy, V.K.; Prasad, M.; Thyagaraju, K.; Reddanna, P. Dietary supplementation of vitamin E protects heart tissue from exercise-induced oxidant stress. *Mol. Cell. Biochem*., **1992,** *111*(1-2), 109-115.

[121] Venditti, P.; Dimeo, S. Effects of training on antioxidant capacity, tissue damage, and endurance of adult male rats. *Int. J. Sports Med*., **1997,** *18*(7), 497-502.

[122] Leeuwenburgh, C.; Leichtweis, S.; Hollander, J.; Fiebig, R.; Gore, M.; Ji, LL. Effects of acute exercise on glutathione deficient heart. *Mol. Cell. Biochem*., **1996**, *156*(1), 17-24.

[123] Shave, R.; George, K.P.; Atkinson, G.; Hart, E.; Middleton, N.; Whyte, G.; Gaze, D.; Collinson, P.O. Exercise-induced cardiac Troponin T release: A meta-analysis. *Med. Sci. Sports Exerc*., **2007**, *39*(12), 2099-2106.

[124] Chen, Y.; Serfass, R.C.; Mackey-Bojack, S.M.; Kelly, K.L.; Titus, J.L.; Apple, F.S. Cardiac troponin T alterations in myocardium and serum of rats after stressful, prolonged intense exercise. *J. Appl. Physiol*., **2000,** *88*(5), 1749-1755.

[125] Nie, J.; Close, G.; George, K.P.; Tong, T.K.; Shi, Q. Temporal association of elevations in serum cardiac troponin T and myocardial oxidative stress after prolonged exercise in rats. *Eur. J. Appl. Physiol*., **2010,** *110*(6), 1299-1303.

[126] Starnes, J.W.; Bowles, D.K. Role of exercise in the cause and prevention of cardiac dysfunction. *Exerc. Sport Sci. Rev*., **1995**, *23*, 349-373.

[127] Hickman, P.E.; Potter, J.M., Aroney, C; Koerbin, G.; Southcott, E.; Wu, A.H.B.; Roberts, M.S. Cardiac troponin may be released by ischemia alone, without necrosis. *Clin. Chim. Acta*., **2010**, *411*(5-6), 318-323.

[128] Lippi, G.; Banfi, G. Exercise-related increase of cardiac troponin release in sports: an apparent paradox finally elucidated? *Clin. Chim. Acta*., **2010**, *411*(7-8), 610-611.

[129] Neumayr, G.; Pfister, R.; Mitterbauer, G.; Maurer, A.; Gaenzer, H.; Sturm, W.; Hoertnagl, H. Effect of the ''Race Across The Alps'' in elite cyclists on plasma cardiac troponins I and T. *Am. J. Cardiol*., **2002**, *89*(4), 484-486.

[130] Sahlin, K.; Shabalina, I.G.; Mattsson, C.M.; Bakkman, L.; Fernstrom, M.; Rozhdestvenskaya, Z.; Enqvist, J.K.; Nedergaard, J.; Ekblom, B.T., Tonkonogi, M. Ultraendurance exercise increases the production of reactive oxygen species in isolated mitochondria from human skeletal muscle. *J. Appl. Physiol.*, **2010**, *108*(4), 780-787.

[131] Sen, C.K. Antioxidants in exercise nutrition. *Sports Med*., **2001**, *31*(13), 891-908.

[132] Ji, L.L. Exercise at old age: does it increase or alleviate oxidative stress? *Ann. N.Y. Acad. Sci*., **2001**, *928*, 236-247.

[133] Fineschi, V.; Baroldi, G.; Centini, F.; Cerretani, D.; Fiaschi, A.I.; Micheli, L.; Parolini, M.; Turillazzi, E.; Giorgi, G. Markers of cardiac oxidative stress and altered morphology after intraperitoneal cocaine injection in a rat model. *Int. J. Legal Med*., **2001**, *114*(6), 323-330.

[134] Clarke, S.F.; Caldwell, R.W.; Chiao, H.; Miyake, K.; McNeil, P.L. Contraction-induced cell wounding and release of fibroblast growth factor in heart. *Circ. Res*., **1995,** *76*(6), 927-934.

[135] McNeil, P.L; Steinhardt, R.A. Plasma membrane disruption: repair, prevention, adaptation. *Annu. Rev. Cell Dev. Biol*., **2003**, *19*, 697-731.

[136] Kakarla, P.; Vadluri, G.; Reddy, K.S.; Leeuwenburgh, C. Vulnerability of the mid aged rat myocardium to the age-induced oxidative stress: influence of exercise training on antioxidant defence system. *Free Radic. Res*., **2005**, *39*(11), 1211-1217.

[137] Chance, B.; Sies, H.; Boveries, A. Hydroperoxide metabolism in mammalian organs. *Physiol. Rev*., **1979,** *59*(3), 527-605.

[138] Di Meo, S.; Venditti, P. Mitochondria in exercise-induced oxidative stress. *Biol. Signals Recept.*, **2001**, *10*(1-2), 125-140.

[139] Whyte, G.; George, K.P.; Shave, R.; Dawson, E.; Stephenson, C.; Edwards, B.; Gaze, D.; Oxborough, D.; Forster, J.; Simpson, R. The impact of marathon running on cardiac structure and function in recreational runners. *Clin. Sci. (Lond)*., **2005**, *108*(1), 73-80.

[140] Venditti, P.; Piro, M.C.; Artiaco, G.; Di Meo, S. Effect of exercise on tissue anti-oxidant capacity and heart electrical properties in male and female rats. *Eur. J. Appl. Physiol. Occup. Physiol*., **1996**, *74*(4), 322-329.

[141] Hollander, J.; Fiebig, R.; Gore, M.; Ookawara, T.; Ohno, H.; Ji, L.L. Superoxide dismutase gene expression is activated by a single bout of exercise in rat skeletal muscle. *Pflugers Arch*., **2001,** *442*(3), 426- 434.

[142] Ji, L.L. Exercise-induced modulation of antioxidant defense. *Ann. N.Y. Acad. Sci*., **2002**, *959*, 82-92.

[143] Natali, A.J.; Wilson, L.A.; Peckham, M.; Turner, D.L.; Harrison, S.M.; White, E. Different regional effects of voluntary exercise on the mechanical and electrical properties of rat ventricular myocytes. *J. Physiol*., **2002**, *541*(Pt3), 863-875.

[144] González, B.; Hernando, R.; Manso, R. Anabolic steroid and gender-dependent modulation of cytosolic HSP70s in fast- and slow-twitch skeletal muscle. *J. Steroid Biochem. Mol. Biol*., **2000**, *74*(1-2), 63-71.

[145] McGill, H.C. Jr; Anselmo, V.C.; Buchanan, J.M.; Sheridan, P.J. The heart is a target organ for androgen. *Science*, **1980**, *207*(4432), 775-777.

[146] Ruizeveld de Winter, J.A.; Trapman, J.; Vermey, M.; Mulder, E.; Zegers, N.D.; van der Kwast, T.H. Androgen receptor expression in human tissues: an immunohistochemical study. *J. Histochem. Cytochem*., **1991**, *39*(7), 927-936.

[147] Golden, K.L.; Marsh, J.D.; Jiang, Y.; Moulden, J. Gonadectomy alters myosin heavy chain composition in isolated cardiac myocytes. *Endocrine*, **2004**, *24*(2), 137-140.

[148] Golden, K.L.; Marsh, J.D.; Jiang, Y. Testosterone regulates mRNA levels of calcium regulatory proteins in cardiac myocytes. *Horm. Metab. Res*., **2004**, *36*(4), 197-202.

[149] Wehling, M. Specific, nongenomic actions of steroid hormones. *Annu. Rev. Physiol*., **1997**, *59*, 365-393.

[150] Richardson, C.J.; Schalm, S.S.; Blenis, J. PI3-kinase and TOR: PIKTORing cell growth. *Semin. Cell. Dev. Biol*., **2004**, *15*(2), 147-159.

[151] Proud, C.G. Ras, PI3-kinase and mTOR signaling in cardiac hypertrophy. *Cardiovasc. Res*., **2004**, *63*(3), 403-413.

[152] Takano, H.; Komuro, I.; Zou, Y.; Kudoh, S.; Yamazaki, T.; Yazaki, Y. Activation of p70 S6 protein kinase is necessary for angiotensin II-induced hypertrophy in neonatal rat cardiac myocytes. *FEBS Letters*., **1996**, *379*(3), 255-259.

[153] Boluyt, M.O.; Zheng, J.S.; Younes, A.; Long, X.; O'Neill, L.; Silverman, H.; Lakatta, E.G.; Crow, M.T. Rapamycin inhibits α 1-adrenergic receptor-stimulated cardiac myocyte hypertrophy but not activation of hypertrophy-associated genes. Evidence for involvement of p70 S6 kinase. *Circ. Res*., **1997,** *81*(2), 176-186.

[154] Simm, A.; Schluter, K.; Diez, C.; Piper, H.M.; Hoppe, J. Activation of p70(S6) kinase by β-adrenoceptor agonists on adult cardiomyocytes. *J. Mol. Cell. Cardiol*., **1998**, *30*(10), 2059-2067.

[155] Rolfe, M.; McLeod, L.E.; Pratt, P.F.; Proud, C.G. Activation of protein synthesis in cardiomyocytes by the hypertrophic agent phenylephrine requires the activation of ERK and involves phosphorylation of tuberous sclerosis complex 2 (TSC2). *Biochem. J*., **2005**, *388*(Pt 3), 973-984.

[156] Kenessey, A.; Ojamaa, K. Thyroid hormone stimulates protein synthesis in the cardiomyocyte by activating the Akt-mTOR and p70S6K pathways. *J. Biol. Chem*., **2006**, *281*(30), 20666-20672.

[157] Shioi, T.; McMullen, J.R.; Tarnavski, O.; Converso, K.; Sherwood, M.C.; Manning, W.J.; Izumo, S. Rapamycin attenuates load-induced cardiac hypertrophy in mice. *Circulation*, **2003**, *107*(12), 1664-1670.

[158] McMullen, J.R.; Sherwood, M.C.; Tarnavski, O.; Zhang, L.; Dorfman, A.L.; Shioi, T.; Izumo, S. Inhibition of mTOR signaling with rapamycin regresses established cardiac hypertrophy induced by pressure overload. *Circulation*, **2004,** *109*(24), 3050-3055.

[159] Gao, X.M.; Wong, G.; Wang, B.; Kiriazis, H.; Moore, X.L.; Su, Y.D.; Dart, A.; Du, X.J. Inhibition of mTOR reduces chronic pressure-overload cardiac hypertrophy and fibrosis. *J. Hypertens*., **2006**, *24*(8), 1663-1670.

[160] Kuzman, J.A.; O'Connell, T.D.; Gerdes, A.M. Rapamycin prevents thyroid hormone-induced cardiac hypertrophy. *Endocrinology*, **2007**, *148*(7), 3477-3484.

[161] Kemi, O.J.; Ceci, M.; Wisloff, U.; Grimaldi, S.; Gallo, P.; Smith, G.L.; Condorelli, G.; Ellingsen, O. Activation or inactivation of cardiac Akt/mTOR signaling diverges physiological from pathological hypertrophy. *J. Cell. Physiol*., **2008**, *214*(2), 316-321.

[162] Lieberherr, M.; Grosse, B. Androgens increase intracellular calcium concentration and inositol 1,4,5-triphosphate and diacylglycerol formation *via* a pertussis toxin-sensitive G-protein. *J. Biol. Chem*., **1994**, *269*(10), 7217-7223.

[163] Koenig, H.; Goldstone, A.D.; Lu, C.Y.. Testosterone-mediated sexual dimorphism of the rodent heart. Ventricular lysosomes, mitochondria, and cell growth are modulated by androgens. *Circ. Res*., **1982**, *50*(6), 782-787.

[164] Clusin, W.T.; Bristow, M.R.; Karagueuzian, H.S.; Katzung, B.G.; Schroeder, J.S. Do calcium-dependent ionic currents mediate ischemic ventricular fibrillation? *Am. J. Cardiol*., **1982**, *49*(3), 606-612.

[165] Fanton, L.; Belhani, D.; Vaillant, F.; Tabib, A.; Gomez, L.; Descotes, J.; Dehina, L.; Bui-Xuan, B.; Malicier, D.; Timour, Q. Heart lesions associated with anabolic steroid abuse: comparison of post-mortem findings in athletes and norethandrolone-induced lesions in rabbits. *Exp. Toxicol. Pathol*., **2009**, *61*(4), 317-323.

[166] Kroemer, G.; Dallaporta, B.; Resche-Rigon, M. The mitochondrial death/life regulator in apoptosis and necrosis. *Annu. Rev. Physiol*., **1998**, *60*, 619-642.

[167] Vicencio, J.M.; Ibarra, C.; Estrada, M.; Chiong, M.; Soto, D.; Parra, V.; Diaz-Araya, G.; Jaimovich, E.; Lavandero, S. Testosterone induces an intracellular calcium increase by a nongenomic mechanism in cultured rat cardiac myocytes. *Endocrinology*, **2006**, *147*(3), 1386-1395.

[168] Frey, N.; McKinsey, T.A.; Olson, E.N. Decoding calcium signals involved in cardiac growth and function. *Nat. Med*., **2000**, *6*(11), 1221-1227.

[169] Wilkins, B.J.; Molkentin, J.D. Calcium-calcineurin signaling in the regulation of cardiac hypertrophy. *Biochem. Biophys. Res. Commun*., **2004**, *322*(4), 1178-1191.

[170] Estrada, M.; Espinosa, A.; Muller, M.; Jaimovich, E. Testosterone stimulates intracellular calcium release and mitogen-activated protein kinases *via* a G protein-coupled receptor in skeletal muscle cells. *Endocrinology*, **2003**, *144*(8), 3586-3597.

[171] Melchert, R.B.; Herron, T.J.; Welder A.A. The effect of anabolic-androgenic steroids on primary myocardial cell cultures. *Med. Sci. Sports Exerc*., **1992**, *24*(2), 206-212.

[172] Welder, A.A.; Robertson, J.W.; Fugate, R.D.; Melchert, R.B. Anabolic-androgenic steroid-induced toxicity in primary neonatal rat myocardial cell cultures. *Toxicol. Appl. Pharmacol*., **1995,** *133*(2), 328-342.

[173] Chainy, G.B.; Kanungo, M.S. Effects of estradiol and testosterone on the activity of pyruvate kinase of the cardiac and skeletal muscles of rats as a function of age and sex. *Biochim. Biophys. Acta*., **1978**, *540*(1), 65-72.

[174] Koenig, H.; Fan, C.C.; Goldstone, A.D.; Lu, C.Y.; Trout, J.J. Polyamines mediate androgenic stimulation of calcium fluxes and membrane transport in rat heart myocytes. *Circ Res*., **1989**, *64*(3), 415-426.

[175] Abu-Shakra, S.; Alhalabi, M.S.; Nachtman, F.C.; Schemidt, R.A.; Brusilow, W.S.. Anabolic steroids induce injury and apoptosis of differentiated skeletal muscle. *J. Neurosci. Res*., **1997,** *47*(2), 186-197.

[176] Abu-Shakra, S.R.; Nachtman, F.C. Anabolic steroids induce skeletal muscle injury and immediate early gene expression through a receptor-independent mechanism. *Ann. N.Y. Acad. Sci*., **1995**, *761*, 395-399.

[177] Kai, H.; Muraishi, A.; Sugiu, Y.; Nishi, H.; Seki, Y.; Kuwahara, F.; Kimura, A.; Kato, H.; Imaizumi, T. Expression of proto-oncogenes and gene mutation of sarcomeric proteins in patients with hypertrophic cardiomyopathy. *Circ. Res*., **1998**, *83*(6), 594-601.

[178] Smeyne, R.J.; Vendrell, M.; Hayward, M.; Baker, S.J.; Miao, G.G.; Schilling, K.; Robertson, L.M.; Curran, T.; Morgan, J.I. Continuous c-fos expression precedes programmed cell death *in vivo*. *Nature*, **1993**, *363*(6425), 166-169.

[179] Ferrer, M.; Encabo, A.; Marin, J.; Balfagon, G. Chronic treatment with anabolic steroid, nandrolone, inhibits vasodilator responses in rabbit aorta. *Eur. J. Pharmacol*., **1994**, *252*(2), 233-241.

[180] Fedak, P.W.; Verma, S.; Weisel, R.D.; Li, R.K. Cardiac remodeling and failure: from molecules to man (Part I). *Cardiovasc. Pathol*., **2005**, *14*(1), 1-11.

[181] van Empel, V.P.; Bertrand, A.T.; Hofstra, L.; Crijns, H.J., Doevendans, P.A.; De Windt, L.J. Myocyte apoptosis in heart failure. *Cardiovasc. Res*., **2005**, *67*(1), 21 - 29.

[182] Jiang, B.; Xiao, W.; Shi, Y.; Liu, M.; Xiao, X. Role of Smac/DIABLO in hydrogen peroxide-induced apoptosis in C2C12 myogenic cells. *Free Radic. Biol. Med*., **2005**, *39*(5), 658-667.

[183] Clerk, A.; Cole, S.M.; Cullingford, T.E.; Harrison, J.G.; Jormakka, M.; Valks, D.M. Regulation of cardiac myocyte cell death. *Pharmacol. Ther*., **2003**; *97*(3), 223-261.

[184] Pawan, K.; Singala ; Neelam, K.; Vince, P.; Dinender, K. The role of oxidative stress in the genesis of heart disease. *Cardiovasc. Res*., **1998**, *40*(3), 426-432.

[185] Kumar, D.; Lou, H.; Singal, P.K. Oxidative Stress and Apoptosis in Heart Dysfunction. *Herz*, **2002**, *27*(7), 662-668.

[186] Neri, M.; Cerretani, D.; Fiaschi, A.I.; Laghi, P. F.; Lazzerini, P. E.; Maffione, A. B.; Micheli, L.; Bruni, G.; Nencini, C.; Giorgi, G.; D'Errico, S.; Fiore, C.; Pomara, C.; Riezzo, I.; Turillazzi, E.; Fineschi, V. Correlation between cardiac oxidative stress and myocardial pathology due to acute and chronic norepinephrine administration in rats. *J. Cell. Mol. Med*., **2007**, *11*(1), 156-170.

[187] Kosmala, W.; Przewlocka-Kosmala, M.; Mazurek, W. Proinflammatory cytokines and myocardial viability in patients after acute myocardial infarction. *Int. J. Cardiol*., **2005**, *101*(3), 449-456.

[188] Bloch, W. Anabolic steroids alter cardiac adaptation to exercise, in: *Biomedical Side Effects of Doping*; Peters, C.; Schulz, T.; Michna, H., Eds.; Verlag Sport und Buch Strauß: Köln, **2002**; pp.51-59.

[189] Schwartz, P.J.; La Rovere, M.T.; Vanoli, E. Autonomic nervous system and sudden cardiac death. Experimental basis and clinical observations for post-myocardial infarction risk stratification. *Circulation*, **1992**, *85*(1 Suppl), I77-91.

[190] Pereira, P.P.; Chaves, E.A., Costa-E-Sousa, R.H.; Masuda, M.O.; de Carvalho, A.C.; Nascimento, J.H. Cardiac autonomic dysfunction in rats chronically treated with anabolic steroid. *Eur. J. Appl. Physiol*., **2006**, *96*(5), 487-494.

[191] McNutt, R.A.; Ferenchick, G.S.; Kirlin, P.C.; Hamlin, N.J. Acute myocardial infarction in a 22-year-old world class weight lifter using anabolic steroids. *Am. J. Cardiol*., **1988**, *62*(1), 164.

[192] Sanyal, S.N.; Ono, K. Derangement of autonomic nerve control in rat with right ventricular failure. *Pathophysiology*., **2002**, *8*(3), 197-203.

[193] Thiblin, I.; Finn, A.; Ross, S.B.; Stenfors, C. Increased dopaminergic and 5-hydroxytryptaminergic activities in male rat brain following long-term treatment with anabolic androgenic steroids. *Br. J. Pharmacol*., **1999**, *126*(6), 1301-1306.

[194] Schlussman, S.D.; Zhou, Y.; Johansson, P.; Kiuru, A.; Ho, A.; Nyberg, F.; Kreek, M.J. Effects of the androgenic anabolic steroid,

nandrolone decanoate, on adrenocorticotropin hormone, corticosterone and proopiomelanocortin, corticotrophin releasing factos (CRF) and CRF receptor1 mRNA levels in the hypothalamus, pituitary and amygdala of the rat. *Neurosci. Lett*., **2000**, *284*(3), 190-194.

[195] Kindlundh, A.M.S.; Lindblom, J.; Bergstrom, L.; Nyberg, F. The anabolic-andogenic steroid nandrolone induces alteration in the density of serotonergic 5HT1B and 5HT2 receptors in the male rat brain. *Neuroscience*, **2003**, *119*(1), 113-120.

[196] Kindlundh, A.M.S.; Rahman, S.; Lindblom, J.; Nyberg, F. Increased dopamine transporter density in the male rat brain following chronic nandrolone decanoate administration. *Neurosci. Lett.*, **2004,** *356*(2), 131-134.

[197] Tamaki, T.; Shiraishi, T.; Takeda, H.; Matsumiya, T.; Roy, R.R.; Edgerton, V.R. Nandrolone decanoate enhances hypothalamic biogenic amines in rats. *Med. Sci. Sports Exerc*., **2003,** *35*(1), 32-38.

[198] Salt, P.J. Inhibition of noradrenaline uptake 2 in the isolated rat heart by steroids, clonidine and methoxylated phenylethylamines. *Eur. J. Pharmacol,*. **1972,** *20*(3), 329-340.

[199] Du, X.J.; Dart, A.M. Mechanisms of noradrenaline release in the anoxic heart of the rat. *Cardiovasc. Res*., **1993,** *27*(11), 2011-2015.

[200] Du, X. J.; Woodcock, E.A.; Little, P.J.; Esler, M.D.; Dart, A.M. Protection of neuronal uptake-1 inhibitors in ischemic and anoxic hearts by norepinephrine-dependent and -independent mechanisms. *J. Cardiovasc. Pharmacol*., **1998,** *32*(4), 621-628.

[201] Richardt, G.; Blessing, R.; Schomig, A. Cardiac noradrenaline release accelerates adenosine formation in the ischemic rat heart: Role of neuronal noradrenaline carrier and adrenergic receptors. *J. Mol. Cell Cardiol*., **1994**, *26*(10), 1321-1328.

[202] Schomig, A. Catecholamines in myocardial ischemia. Systemic and cardiac release. *Circulation*., **1990**, *82*(3 Suppl), II13-II22.

[203] Schomig, A.; Dart, A.M.; Dietz, R.; Mayer, E.; Kubler, W. Release of endogenous catecholamine in the ischaemic myocardium of the rat. Part A: Locally mediated release. *Circ. Res.*, **1984,** *55*(5), 689-701.

[204] Schomig, A.; Fischer, S.; Kurz, T.; Richardt, G.; Schomig, E. Nonexocytotic release of endogenous noradrenaline in the ischemic and anoxic rat heart: Mechanism and metabolic requirements. *Circ. Res*., **1987,** *60*(2), 194-205.

[205] Greenberg, S.; George, W.R.; Kadowitz, P.J.; Wilson, W.R. Androgen-induced enhancement of vascular reactivity. *Can. J. Physiol. Pharmacol*., **1974**, *52*(1), 14-22.

[206] Fineschi, V.; Riezzo, I.; Centini, F.; Silingardi, E.; Licata, M.; Beduschi, G.; Karch, S.B. Sudden cardiac death during anabolic steroid abuse: morphologic and toxicologic findings in two fatal cases of bodybuilders. *Int. J. Legal Med*., **2007**, *121*(1), 48-53.

Received: December 13, 2010 Revised: January 14, 2011 Accepted: February 07, 2011