Side Effects of AAS Abuse: An Overview

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Abstract: Anabolic – androgenic steroids (AAS) were originally developed to promote growth of skeletal muscle. AAS abuse is commonly associated with bodybuilders, weightlifters, and other athletes. The issue of AAS toxicity is not yet completely understood since the adverse effects outline a varied scenario with side effects reported affecting many organs and systems in humans. The true incidence of AAS related medical problems is not known, due to several drawbacks in human studies. The entity of side effects depends on the sex, the dose, the duration of treatment, whether they are taken during exercise training or under sedentary conditions, and the susceptibility of the individuals themselves to androgen exposure partly depending on genetic factors. Both the acute and the chronic effects can lead to toxicity, but generally the serious and even fatal effects depend on the time and the duration of AAS administration. A limitation of human studies is represented by the fact that information about the intake of steroids are, generally, self reported and it is hardly possible to assess the exact dosage. AAS are often used in combination with other dugs or substances, so it is difficult to separate their toxic effects from those caused by the other drugs abused. Hence experimental studies conducted on animal models are mandatory to investigate the mechanisms underlying to AAS toxicity and the organ alterations due to these substances. Finally, clinicians should be aware of the complex and varied pattern of toxicity so as to be able to perform correct diagnoses and treatments.

Keywords: Abuse, anabolic androgenic steroids, mechanisms, side effects, toxicity.

INTRODUCTION

Anabolic – androgenic steroids (AAS), or anabolic steroids as they are commonly known, were originally developed in the late 1930s to promote growth of skeletal muscle and to develop male sexual characteristics. These drugs were seen as offering great potential for their protein – building properties, but their clinical use has been quite limited. The potential of AAS as therapeutic agents to increase weight, lean body mass and strength has been widely revisited [1, 2]. Actually AAS may play a significant role in clinical situations as HIV-related muscle wasting, severe burn injury, trauma following major surgery, neuromuscular disorders, malnutrition due to alcoholic cirrhosis, Duchenne's or Becker's muscular dystrophy, and sarcopenia. [1]. Finally, testosterone preparations are used for hormone replacement therapy.

Apart from these limited clinical applications, AAS use is more commonly associated with bodybuilders, weightlifters, and other male and female athletes. They have, in fact, been used by competitive athletes since 1950s; starting from 1980s these drugs spread from elite athletics to general population [3, 4] and to individuals who want to look leaner and more muscular [5-10]. The use of anabolic steroids for cosmetic benefits among both adults and adolescents in society may be incorrectly regarded as a comparatively harmless pharmacological manipulation that can aid the development of bulging muscles and a well-toned figure. Contrarily, for drug control in sport, anabolic steroids are regarded (correctly) as performance enhancers, as well as harmful to health [2].

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AAS TOXICITY

AAS are synthetic derivatives of the male sex hormone, testosterone, and have androgenic (development and sustainment of secondary sex characteristics), anabolic (tissue-building), and hedonic effects [11]. The anabolic effects could not be separated entirely from the androgenic ones even if many efforts have been made to alter the relative anabolic androgenic potency, slow the rate of inactivation, and change the pattern of metabolism through structural modifications of testosterone molecule which is the most important androgen secreted in eugonadal man (Fig. **1**).

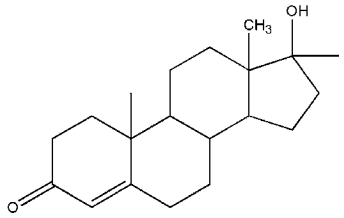

Fig. (1). Testosterone formula.

The basic structure of all steroids is a perhydrocyclopentano phenanthrene ring system which comprises of three fused six- membered rings (A, B, and C) and one five – membered ring (D) (Fig. **2**); they may be modified in order to obtain designed chemical modifications (Fig. **2**). Alkylation at 17-alpha position with methyl or ethyl group

1389-5575/11 $58.00+.00

creates orally active compounds because it slows the degradation of the drug by the liver while esterification at the 17-beta position makes the molecule more soluble in lipid vesicles used for injection and hence slows the release of the injected steroid into the circulation. Besides these chemical modifications, alterations of the ring structure were applied for both oral and parenteral agents to seeking to obtain different anabolic to androgenic effect ratios [12]. Several modification of ring A (junction with a pyrazol ring, introduction of an oxygen atom at position C-2, introduction of alkyl substituents into ring A at position C-1 or at position C-2, in combination or not with a double bond at position C-1, -2) result in an increase of the anabolic activity; the removal of the C-19 methyl group greatly reduces androgenic properties and dissociates, partially, the androgenic and anabolic activities of a molecule. Also 17α-alkylation is a structural feature of steroids which contributes to the prolongation of the anabolic effect. Other structural modifications, such as the presence of a17-OH group in the D-ring, of a C-4, -5 double bond, of a 3-keto group in the A-ring are all characteristics necessary for the androgen effects. As previously underlined, the removal of the C-19 methyl group greatly reduces androgenic properties [12, 13] (Fig. **3**).

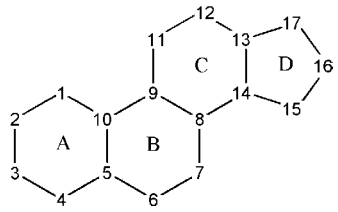

Fig. (2). Basic structure of all steroids.

AAS are believed to exert their actions by several different mechanisms which include modulating androgen receptor expression as a consequence of (i) intracellular metabolism and by (ii) directly affecting the topology of the androgen receptor and thus subsequent interaction with co – activators and transcriptional activity, and ultimately increasing a wide variety of structural, enzymatic, and receptors proteins. The various clinical effects are determined by the type and concentrations of androgen receptors and enzymes controlling steroid metabolism in a given organ [14]. Other mechanisms include an anticatabolic effect by interfering with glucocorticoid receptor expression [2]. Conclusively, the classical pathway of androgen action involves steroid binding to the androgen receptors (ARs), a ligand-activated transcription factor, and single copy member of the nuclear receptor superfamily, acting on the genome (Fig. **4**). The genomic action of ARs is modulated by a large variety of coregulators, which are proteins that target gene expression by enhancing (coactivator) or restraining (corepressor) transcription [15]. Finally, AAS may also have direct rewarding or hedonic properties, mediated not so much by their genomic effects (although these may well contribute) but more directly by the effects of AAS and their metabolites on plasma membranes [16]. Rapid, nongenomic effects of steroid androgens are distinguished from genomic ones by 1) rapid onset (seconds to minutes) that is faster than genomic mechanisms, 2) insensitivity to inhibition of RNA and protein synthesis, 3) effects produced by steroids unable to access the nucleus (either covalently linked to membrane impermeable macromolecules or in cells lacking a nucleus), and 4) not usually blocked by classical antagonists due to different steroidal specificity from classical cognate nuclear receptors. As for other steroids, nongenomic androgen effects characteristically involve the rapid induction of conventional second messenger signal transduction cascades, including increases in cytosolic calcium and activation of protein kinase A, protein kinase C, and MAPK (mitogen-activated protein kinase), leading to diverse cellular effects

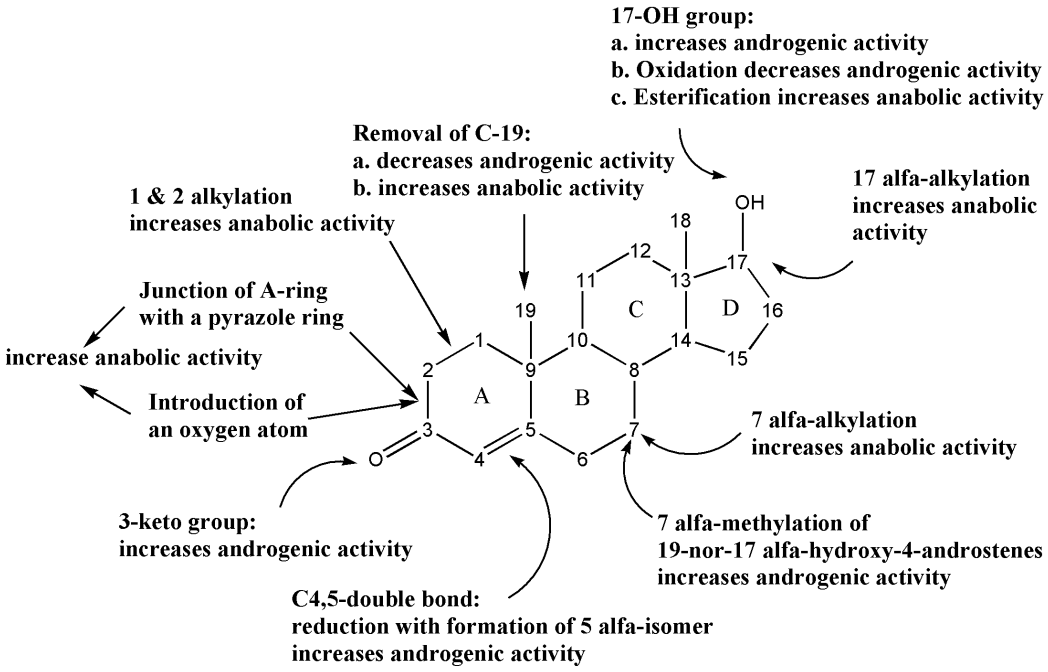

Fig. (3). Structural features influencing the expression of the androgenic and anabolic activities.

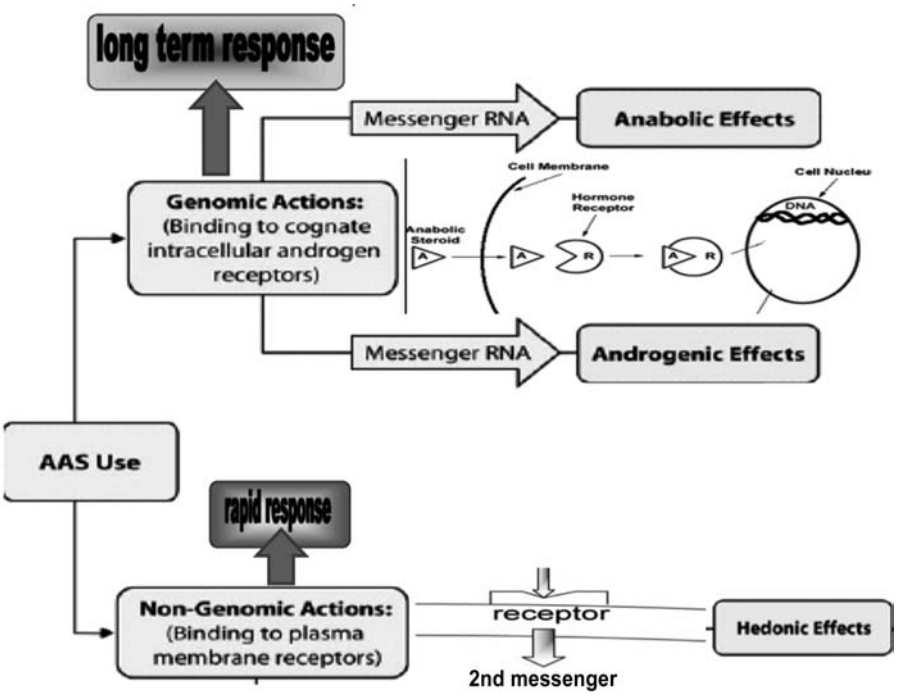

Fig. (4). Genomic and non – genomic mechanisms of action of AAS [11]. Modified from Kanayama *et al.* 2010.

including smooth muscle relaxation, neuromuscular and junctional signal transmission and neuronal plasticity [15-17]. Most nongenomic effects involve a membrane receptor, and putative binding sites are described for androgens [17].

The issue of AAS toxicity is not yet completely understood since the adverse effects of AAS outline a varied scenario with a lot of side effects reported affecting several organs and systems in humans.

Several drawbacks are, in fact, still present when we are dealing with the AAS adverse effects. First of all side effects associated with AAS use (i.e. under medical supervision) have to be differentiated from those caused by abuse (i.e. consumption of many drugs at high doses; any nonmedical use of substance) [18, 19]. The matter is even more complex due to the fact that many athletes consume multiple drugs in addition to anabolic steroids; polydrug use makes it difficult to attribute the observed effects to a single drug [14, 18, 20, 21]. AAS users are likely to self administer other drugs of abuse such as alcohol, opioids, cocaine, marijuana, and gamma hydroxybutyrate some of which can interact adversely with AAS [19]. In the last years the polydrug use has become more and more evident [22-26].

Moreover the health negative consequences of AAS are also dependent on the sex, the dose and the duration of administration, since many of the side effects are reversible. Many individuals who use only a few cycles of AAS in their careers, in fact, often report few adverse medical or psychological effects from AAS since most of the more severe side effects appear to develop during prolonged use [27-30].

Acute adverse effects of steroids use have been described: headaches, fluid retention (especially in the extremities), gastrointestinal irritation, diarrhea, stomach pain, oily skin, jaundice, menstrual abnormalities, hypertension, alteration of libido, sleeplessness, increased irritability, increased appetite, enhanced transpiration, increased feeling of well – being, depressive mood states, loss of head hair and gynecomastia [30].

Also the chronic effects of AAS intake, besides the neuropsychiatric and behavioural effects, include a great variability of somatic consequences on the cardiovascular, urogenital, reproductive, central nervous, and musculoskeletal systems. Liver is also affected in AAS abusers. Since a great number of organs and systems is the target of AAS action, the adverse effects of AAS include reproductive, hepatic, musculoskeletal, endocrine, renal, immunologic, infectious, and, finally, cardiovascular, cerebrovascular and hematological effects (Table **1**).

A number of clinical and experimental studies have investigated the somatic and psychic consequences associated with these drugs and have provided strong evidence of their risks on human health. The current paper is aimed to investigate the specific organ pathology related to AAS abuse which have a specific forensic interest and which, in particular, can explain many cases of sudden death observed in AAS abusers.

EFFECTS ON CARDIOVASCULAR SYSTEM

Although in the last years a strong body of evidence had outlined the cardiovascular toxicity of AAS, cardiac effects of AAS still remain quite unclear. Since the study of Melchert and Welder [32], several hypothetical mechanisms have been suggested to explain AAS induced cardiovascular effects [33, 34] which can be divided into direct effect on the myocardium and the vasculature and indirect effects due to

Table 1. Adverse Effects of AAS

ORGAN/APPARATUS	ADVERSE EFFECTS OF ANABOLIC STEROIDS
Reproductive	**Male:**
	decreased reproductive hormones
	testicular atrophy
	oligospermia/azoospermia
	impotence
	prostatic hypertrophy
	prostatic carcinoma
	gynecomastia
	priapism
	Female:
	menstrual irregolarities
	clitoral hypertrophy
	uterine atrophy
	breast atrophy
	teratogenicity
Liver	hepatocellular damage
	cholestatis
	peliosis hepatis
	hepatoadenoma
	hepatocarcinoma
Cardiovascular and hematologic effects	increased cholesterol
	decreased HDL cholesterol
	hypertension
	thrombosis
	pro-atherogenic effects
	left ventricular hypertrophy
Musculoskeletal	early epiphyseal closure in children
	increased rate of muscle strains/ruptures
	increased risk of muskulotendinous
Endocrine (other than reproductive)	decreased glucose tolerance
Larynx	deepening of the voice
Integument	acne
	alopecia
	hirsutism
	male pattern baldness
	edema
Urinary	elevated BUN (blood urea nitrogen), creatinine
	acute renal failure
	focal segmental glomerulosclerosis
	membranoproliferative glomerulonephritis
	Wilm's tumor
Immunologic and infectious effects	decreased IgA levels
	hepatitis B or C; HIV infection
Psychologic	mood swings
	aggressive behavior
	depression
	psychosis
	addiction
	withdrawal and dependency disorders

[1][31] Modified from Landry and Primos 1990.

alterations in lipids and hemorrheologic properties of the blood [1].

Animal model studies have been conducted to evaluate the impact of AAS supraphysiological doses on the cardiovascular system and myocardial injury which may be of paramount importance in understanding the pathogenesis of ventricular remodeling and disfunction, cardiomyopathy, ventricular arrhythmias, and sudden cardiac death associated with AAS abuse since many of the reported case histories are anecdotal and lack in demonstrating the causal relationship between AAS abuse and cardiovascular diseases.

An atherogenic model involving the effects of AAS on lipoprotein concentration was proposed by Melchert and Welder in 1995 [32]. Since several Authors reported an association between AAS abuse and coronary atherosclerosis [35-37] a direct link between steroid abuse and the development of atherosclerotic disease was clearly observed in the small pilot study by Santora *et al.*, [38] who measured coronary artery calcification in body builders using AAS over an extended period of time, founding increased coronary calcium in these subjects. Hence there is evidence that abuse of AAS has a proatherogenic effect and contributes directly to the early development of coronary atherosclerosis. Whether the pathogenesis is indirectly due to the unfavorable effect upon lipid profile, or a direct toxic or inflammatory effect of the steroids on the endothelium, remains unknown [33].

Surely, AAS cause changes in lipid metabolism, including reductions in highdensity lipoprotein (HDL) levels by 20 to 70% and elevations in lowdensity lipoprotein (LDL) levels by > 20% [34, 39]. The decline of high – density lipoprotein is significantly less with use of aromatizable androgens such testosterone [40].

More generally, lipoproteins disorders are associated with atherogenesis; the alterations in lipid profiles AAS induced increase the risk for coronay artery disease by three – to sixfold [41, 42]. However, the AAS induced lipid alterations seem to be reversible and may normalize after some weeks to 3–5 months [43]. Studies on animal models (transgenic mice with a human lipaemic phenotype) [44] showed that treatment with mesterolone increased total cholesterol, triglyceride, low-density lipoprotein cholesterol (LDL-c) and very LDL-c (VLDL-c) plasma levels and that exercise blunted some of these deleterious effects by increasing high-density lipoprotein cholesterol and decreasing LDL-c, VLDL-c and triglycerides. Several autoptical case reports showed more or less evident atherogenic alterations in body builders who had used anabolic steroids [45, 46]. Conversely, many reports refer to sudden cardiac death in AAS abusers without atherosclerosis. To summarize, there is a comprehensive body of evidence documenting the various alterations of lipid metabolism induced by AAS. The most prominent changes are concomitant elevations of LDL and decreases of HDL, the combined effects of which are deleterious [33].

Another supposed mechanism of AAS induced cardiotoxicity is a thrombosis model involving clotting factors and platelets aggregation. This appears to be a direct effect on the coagulation/fibrinolytic system, which increases levels of coagulation factors in both the intrinsic and extrinsic pathway. Experimental studies showed alterations at different stages of the hemostatic and fibrinolytic system by AAS administration, as well as effects on platelet function [47]. Androgen-mediated enhancement of platelet aggregability and augmenting of thrombosis have been shown in experimental animal models of thrombosis, in studies done in the 1970s and 1980s [48-52]. In addition, there is increased production of thromboxane A2 (TXA2) and decreased production of prostaglandins that may cause a hypercoagulable state and promote platelet production and aggregation [39]. It has been reported that testosterone can directly modulate the number of functional TXA2 receptors on platelet and vascular cells [53-55]. Some of these effects seem to be reversible; Urhausen *et al.* [43] observed that the red blood count and thrombocytes in ex-abusers had returned to normal after at least 1 year. Conclusively, AAS have well known thrombogenic side effects [56] and their ability to modify platelet and vascular reactivity could play an important role in the increasing risk of precipitating the unfavourable myocardial and vascular accidents ascribed to this drug. Thromboembolic phenomena, including systemic arterial and intracardiac thrombosis, are reported in the literature [57-65]. Finally, deep venous thrombosis and pulmonary embolism as an adverse effect of AAS has been recently reported in a previously healthy man who had been given intramuscular injections of testosterone and the anabolic-androgenic steroid nandrolone [66]. Since testosterone is converted by aromatization to estradiol, it may be prothrombotic by the same mechanisms as estrogen-based therapies.

A vasospasm model perhaps superimposed on or triggered by pro-atherogenic state was also proposed by Melchert and Welder [32] in order to explain some adverse vascular effects of supraphysiologic doses of AAS which have been associated with coronary artery vasospasm and myocardial infarction in the absence of both atherosclerosis and thrombosis [67]. However the effect of AAS on vasculature is still contradictory and not completely understood.

AAS bind to androgen receptors in the major arteries and physiologic levels may have a beneficial effects on coronary arteries *via* endothelial release of nitric oxid and inhibition of vascular smooth muscle tone [34]. There is, in fact, a strong body of evidence that testosterone therapy is associated with improvements in myocardial ischaemia in men with coronary artery disease [68-71] and that testosterone replacement therapy in men with chronic heart failure improves exercise duration, heart failure symptoms and quality of life, effects which are independent of an anabolic effect upon the skeletal muscle [72]. Testosterone and other androgens induce direct vasodilatation in a variety of vascular beds (large and smaller arteries), an action which is mediated *via* a non-genomic pathway, independent of the classical nuclear androgen receptor [73, 74]. The key mechanism underlying testosterone -induced smooth muscle ion channel function, particularly the inactivation of L-type voltage-operated Ca^{2+} channels and/or the activation of voltage-operated and Ca^{2+}-activated K^{+} channels [75]. Many experimental studies on animals confirmed this acute non – genomic vasorelaxation induced by testosterone and other AAS [71, 76-82].

Conclusively, acutely administered testosterone results in arterial vasodilatation in both human and animal studies,

through an endothelium-independent mechanism, involving ATP sensitive potassium channels on smooth muscle cells; however lower physiological concentrations of testosterone appear to induce vasodilatation *via* a, at least in part, endothelium dependent pathway [75].

On the other hand, at physiological concentrations of testosterone, vasoconstrictor responses have been observed [83, 84], including one study in which the higher, supraphysiological testosterone concentrations produced vasodilatation [84]. Vasoconstrictor responses have long been known in tissues obtained from androgen-treated animals [85-89], but the effective tissue androgen concentrations involved are not clear.

Supraphysiologic doses of the anabolic steroid nandrolone in an animal model resulted in impairment of both endothelium-dependent and endothelium-independent dilatation (of rabbit aortic rings) [89].

The flow-mediated dilatation (FMD), a non invasive test for arterial endothelial function, has allowed new insight into androgen effects on the human vasculature. Both endogenous and exogenous testosterone impair vascular reactivity in men. Vascular reactivity is enhanced by castration in older men with advanced prostate cancer compared with age-matched and cancer controls [90], so suggesting a deleterious effect of physiological levels of androgens. In a large well-controlled study of 36 newly diagnosed, nonsmoking hypogonadal men, androgen deficiency was associated with markedly increased FMD compared with age-matched eugonadal controls [91]. A recent study assessing the effects of long-term androgenic anabolic steroid use in competition bodybuilders, however, did not reveal any significant difference in arterial reactivity compared to bodybuilders who had never used anabolic steroids [92]. Interestingly, both bodybuilding groups had significantly impaired arterial reactivity compared to sedentary controls, suggesting the exercise undertaken may have had a deleterious effects.

In summary, there is conflicting evidence regarding vasoreactivity in users of AAS. AAS abusers demonstrate unfavorable measurements, whether it is involving endothelial-dependent or endothelial-independent vasodilatation; however a degree of reversibility exists. The inconsistent results are likely to arise from the obvious study limitations which are unavoidable in such populations of abusers of AAS. There is a diversity of substances abused, often in association with other drugs, and a variety of duration and route of administration. Anyway, there is evidence of a deleterious effect of AAS abuse on vascular function [33].

Finally, a direct myocardial injury AAS related is supposed on the basis of demonstration that anabolic steroids are associated with marked hypertrophy in myocardial cells, extensive regional fibrosis and necrosis, cellular modifications that include changes in the contractile apparatus (i.e. the sarcomere), disintegration and Z – band distortion or dissolution, and disturbance in the energy unit of the cell (i.e. mitochondria), as shown by swelling damage.

Some observations in animal model suggest the presence of a quite specific pattern of AAS induce myocardial injury. Zaugg *et al*. [93] observed dose-dependent apoptotic cell death in adult rat ventricular myocytes *in vitro* exposed to stanozolol, testosterone enanthate and testosterone. Similar results were reported by Fanton *et al.* [94] who observed the presence of apoptotic lesions in the myocytes of norethandrolone-treated rabbits. These lesions consisted of sparse foci of myocardial cells with very dense hyperchromatic nuclei, or irregularly interspersed chromatin within a vesiculous cytoplasm without striations. The same cytonuclear features were observed in some intramyocardial vessels, which strongly supports the induction of apoptotic lesions in these rabbits. These effects are likely mediated by membrane receptor–second messenger cascades that increase intracellular Ca^{2+} influx and Ca^{2+} mobilization from the sarcoplasmic reticulum. Increases in Ca^{2+} affect mitochondrial permeability, leading to the release of apoptogenic factors such as holocytochrome c, apoptosis-inducing factor, and caspase-9. These results suggest that this non-genomic effect of AAS can contribute to the documented androgen receptor mediated cardiotoxicity observed in AAS abuse [95]. 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 [94]. 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 [96].

In the mouse myocardial hypertrophy has been associated with inadequate vascularisation of the hypertrophic myocardium, and in isolated rat ventricular myocytes it has been linked to increased apoptosis. 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; Du Toit *et al.* [97] found that supraphysiological doses of anabolic steroids, whether taken during exercise training or under sedentary conditions increased myocardial susceptibility to ischaemia/reperfusion injury in an animal models. The Authors suggested that this increased susceptibility could be related to steroid-induced increases in the pre-ischaemic myocardial cAMP concentrations and/or increases in both pre-ischaemic and reperfusion TNF-α concentrations. Hassan *et al.* [98] studied the morphological changes in cardiac muscle fibers of albino adult rats following the administration of sustanon and found showed focal areas of degeneration with loss of striations and vacuolation in the experimental group. Ultrastructural examination showed disturbance of the banding pattern of the cardiac muscle fiber with disintegration, loss of striations, dehiscent intercalated disc, and interrupted Z-bands. In according to other studies the ultrastructural changes in the rat myocytes observed by Hassan *et al.* were similar to those observed in early heart failure. Similar findings were observed also by Behrendt [99] who found that myofibrils showed either disintegration, widened and twisted Z-bands or a complete dissolution of the sarcomeric units in male rats taking supraphysiological doses of AAS.

Recently our group [100] studied the mechanisms of AAS toxicity on mouse heart by administering nandrolone decanoate to strength-trained male CD1 mice, studying plasma lipid analysis, cardiac histopathological features,

cardiac β_1-adrenergic receptor expression, and the effects of myocardial expression of inflammatory mediators (IL-1β, TNF-α) on the induction of cardiomyocytes apoptosis (HSP 70, TUNEL), using proteomic and immunohistochemical analysis. Our experimental study showed that recurring high dose AAS administration and physical training in mice produce moderate increase of heart weight, morphologically extensive cardiac hypertrophy and wide colliquative myocytolysis which could result in significant heart failure, in the high dose AAS administration and physical training group in agreement with previous studies on cadiomyocyte ultrastructures after AAS exposition in rodents, where mitochondria and myofibrils showed pathological findings similar to those in early heart failure [99].

The expression of inhibitor of apoptosis HSP 70 and inflammatory cytokine IL-1β, increased in all the treated groups in our study; TNF-α showed an intense positive reaction in the group with a more extensive myocardial damage. Moreover our findings of myocardial disarray associated with contraction band necrosis, present in all forms, from early to late healing stages, and of focal myocardial fibrosis, is highly significant. In fact, it suggests the need to reconsider the significance of myocardial disarray. Its focal presence in specific areas of normal heart and around myocardial scar, may be a structural expression of ''nodal junctions'' where myocardial muscles or bundles change their direction; a centre of force to help contraction. It may provide a substrate for the occurrence of potentially lethal arrhythmias and sudden, unexpected cardiac death [101].

Elevated testosterone concentrations induce cardiac hypertrophy *in vitro* [102] and *in vivo* [103-105], but the molecular mechanisms are still poorly understood. Protein synthesis is essential for both normal and hypertrophic growth of cardiomyocytes [106] and in cardiac cells, testosterone action has been explained by activation of the intracellular androgen receptors which stimulates cellular protein synthesis [102]. Recently, Altamirano *et al*. [107] showed that in addition to the classic genomic mechanism, testosterone activates the mammalian target of rapamycin complex 1 (mTORC1) pathway (a major regulator of cell growth) to induce cardiomyocyte hypertrophy and propose that cell growth produced by anabolic steroid hormones requires both androgen receptor activity and translation control through mTOR signaling pathway.

Another line of evidence concerns the arrhythmogenic effect of AAS. Pereira Junior *et al*. [108] showed that chronic administration of high doses of AAS leads to dysfunction in tonic cardiac autonomic regulation. One possible explanation to AAS-induced autonomic imbalance raises from their effect on central nervous system, since nandrolone decanoate treatment influences several neurotransmitter systems, including dopaminergic, serotonergic and adrenergic [109-114]. Moreover, in a recent work Hamson *et al*. [115] have shown the presence of androgen receptors in nucleus ambiguus, a brain center related to cardiac vagal control. This way, the existence of some kind of direct androgen-mediated vagal effect on cardiac autonomic regulation can also be hypothesized. Phillis *et al*. [116] administered to male rats an high dose of nandrolone acutely, and potentiated ischemia-induced arrhythmia and thereby decreased the proportion of rats surviving the ischemia. The Authors suggested that nandrolone may cause the release of intracellular calcium and thereby explain the proarrhythmic effects observed in the rats. In a recent experimental study Rocha *et al*. [117] treated rats with supraphysiologic doses of nandrolone decanoate in association with exercise and observed an increase in the heart collagen concentration associated with the activation of the cardiac renin–angiotensin system. These pathophysiologic changes undoubtedly provide a pathoanatomic substrate that may explain the increased propensity to the generation and continuation of malignant cardiac arrhythmias. Recently, Medei *et al*. [118] studied the cellular, ionic and molecular mechanism of ventricular repolarization disturbances in Male Wistar rats chronically treated with nandrolone decanoato (DECA). They found that supraphysiological doses of AAS induce morphological remodeling in both ventricles and that electrical remodeling is mainly observed in the left ventricle. These findings, in association with the autonomic unbalance observed in rats administered with AAS, could explain the electrical disturbance induced by supraphysiologic doses of AAS [118].

Taken together this experimental background can be of a great usefulness to give evidence and to clarify the underlying mechanism of the cardiovascular toxicity observed in AAS abusers which is widely reported in the literature and which is summarized in the figure adapted from Vanberg and Atar (Fig. **5**) [33].

Supraphysiologic doses of AAS led to both morphological and functional changes of the heart, including the development of pathological left ventricular hypertrophy [92, 119-133], a possible increase of heart chamber diameters, dyastolic dysfunction and isovolumteric relaxation time [122, 134-136], and a subclinically compromised left ventricular contractile function [134, 135] which are often demonstrated in athletes abusing AAS.

AAS can induce an unfavourable enlargement and thickening of the left ventricle, thereby losing diastolic properties with the mass increase [137].

Myocardial hypertrophy induced by AAS has to be distinguished from the physiologic adaptative cardiac hypertrophy seen in endurance and power athletes not taking drugs; it is quite reversible but the reduced compliance of the left ventricle and the decreased inotropic capacity of the myocardium is irreversible [58].

Finally, in several clinical reports, AAS intake was associated with cardiac arrhythmias and sudden cardiac death and [101, 126, 128, 129, 136-141]. The number of autopsy cases of sudden and unexpected death associated with AAS use is not sufficient to explain the mechanism of the sudden cardiac arrest. To complicate the issue, there is the widespread use of other drugs among abusers of AAS, both recreational substances as stimulants, marijuana, cocaine and alcohol, the concomitant use of masking agents such as diuretics, and the use of anti-estrogens, thyroid hormones and growth hormone [142]. Another important issue to bear in mind is that hard physical exercise combined with AAS and other drugs of various kinds can trigger a malignant arrhythmia in particularly predisposed individuals (i.e. subjects

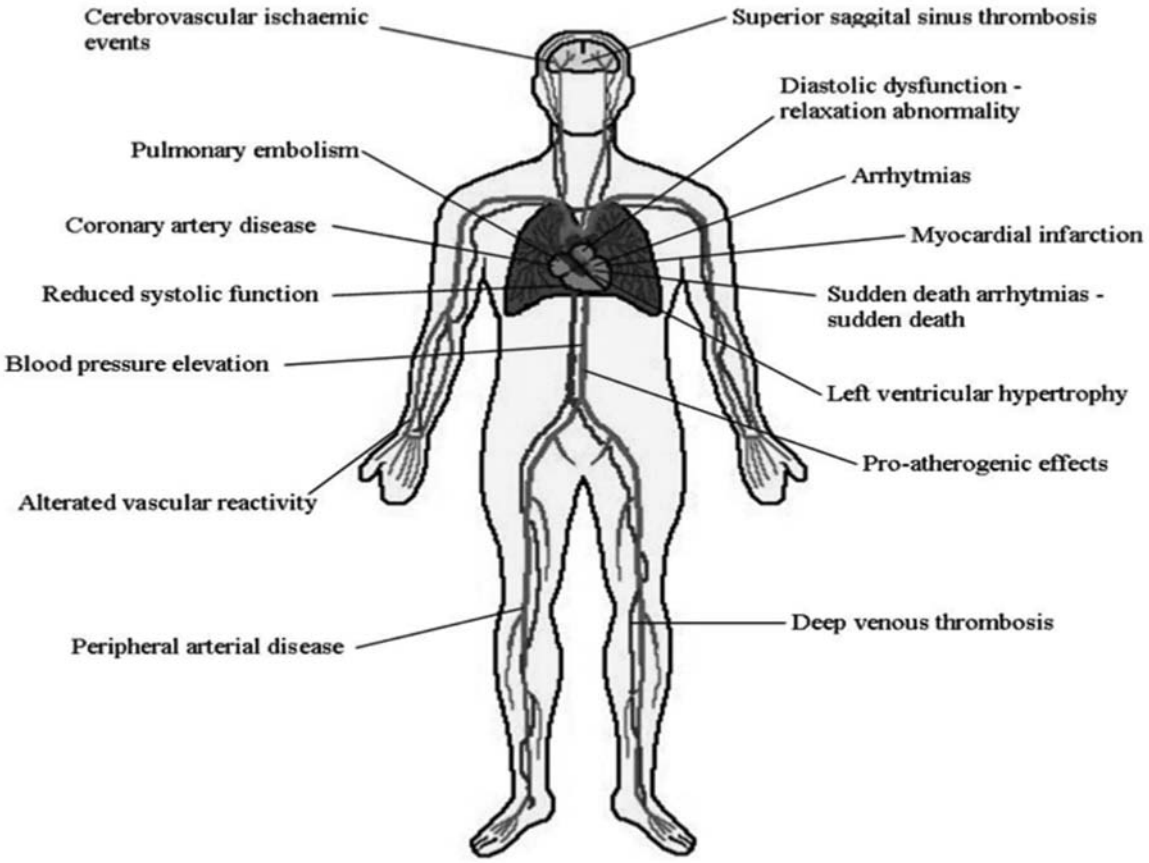

Fig. (5). Potential harmful cardiovascular effects of AAS.

carrying inherited arrhythmogenic diseases, such as long QT syndrome and other ion channel diseases and arrhythmogenic right ventricular cardiomyopathy) [33].

Conclusively, whether these arrhythmias are directly triggered by AAS or whether they occur as indirect or secondary consequences of AAS, as for instance through left ventricular hypertrophy or cardiomyopathy, is unclear [33]. In most of those reported in medical literature, lesions at any level of the coronary system were absent, even in the presence of a myocardial infarction [139]. We have previously reported focal myocardial necrosis in AAS users [46, 139], coagulative myocytolysis (or contraction band necrosis), typical of catecholamine myocytotoxicity, linked with ventricular fibrillation and sudden death. The focal fibrous area described could be interpreted as the healing phase, with progressive collagenization ending in a fibrous scar of previously focal myocell necrosis. From these findings, it can be concluded that anabolic steroids and exercise lead to a stimulation of the sympathetic nervous system. 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 the sympathetic axon terminals; the transient destabilization of sympathetic axon terminals could be suggested as a reason for increased vulnerability to ventricular fibrillation [143]. The cases described by our group and by other Authors support the hypothesis that the combined effects of vigorous weight training, anabolic steroid use, and androgen sensitivity may have predisposed these young men to myocardial injury and subsequent SCD (sudden cardiac death) [126]. Vascular and structural changes within ventricular myocardium such as fibrosis, increase collagen growth are postulated as plausible mechanism that could account for arrhythmogenesis and sudden cardiac death in the population of AAS abusers [35, 126, 127, 144].

HEPATIC TOXICITY

The liver adverse effects are among the most common and serious associated with AAS use and are virtually always associated with the oral active 17 α – alkylated androgens such as methyltestosterone, methandrostenolone, oxandrolone, and stanozol. The alkylation of the 17 - α portion of these compounds, in fact, allows increased oral absorption and slower hepatic degradation and clearance, so resulting in greater hepatic toxicity [145]. Welder *et al*. [146] showed that 17α- alkylated anabolic steroid are directly toxic to rat hepatocytes with increase of liver enzymes levels whereas the nonalkylated steroids show no effects at similar doses.

Animal studies clearly shown liver alterations induced by AAS. Gragera *et al.* [147] observed ultrastructural alterations of hepatocytes, the most prominent changes being swelling of mitochondria and marked increase in the number of lysosomes. These changes were evident in both sedentary and trained treated rats, indicating that liver cell damage is

produced by anabolic-androgenic steroids despite the simultaneous realization of physical exercise. These findings are similar to those of Saborido *et al*. [148] and Molano *et al*. [149] who observed that treatment with stanozolol, either with or without concurrent exercise training, affects lysosomal hydrolases and mitochondrial respiratory chain electron transport in rat liver, without modifying classical serum indicators of hepatic function. Acute adaptative changes on the liver tissue (slight to moderate multifocal lobular inflammation with acidophilic degeneration and evident Kuppfer cells reactivity) were observed by Boada *et al.* [150] in rats administered with stanozol for a short time in association with minimal to mild variability in the size of cell nuclei and increased mitosis and binucleation. In the majority of the livers from long – term treatment the Authors observed cytoplasmic vacuolation, and lipidic degeneration; in addition, as in the case of acute ST-treated animals, they found increased mitosis and binucleation and variability in the size of cell nuclei. A nuclear response (i.e. modifications in status of chromatin condensation and exture, geometric parameters and Feulgen-DNA values in hepatocyte nuclei) to mesterolone and physical exercise action in liver cells of transgenic mice was demonstrated by Fontana *et al*. [44]. In addition to a direct toxic effect to hepatocytes, oxidative stress could play a role in AAS induced hepatotoxicity. Since prolonged administration of stanozolol provokes dysfunction of mitochondrial respiratory chain complexes and mono-oxygenase systems, it would be possible that these alterations were accompanied by an increased ROS generation. Although liver is endowed with high levels of enzymatic and non-enzymatic antioxidant defences, an enhancement in ROS production exceeding the antioxidant defences and repair capacity could lead to oxidative stress and cell damage. Texting this hypothesis, Pey *et al.* [151] experimentally demonstrated that prolonged 17 – α alkylated anabolic steroid treatment could cause an oxidative stress situation in rat liver as indicated by enhanced lipid peroxidation extent, so suggesting that ROS could been implicated in the pathogenesis of AAS liver injury as it happens in the hepatic damage induced by several toxic drugs.

In humans several liver diseases associated with AAS use, ranging from transient, mild and asymptomatic elevations of liver function tests (aminotransferases, creatinechinase, alkaline phosphates, conjugated bilirubin, γ – glutamyl – transferase, and plasma proteins) [152, 153] to various pathological alterations have been described (Fig. **6**). Anecdotally severe and prolonged jaundice leading to nephropathy have been reported [154]. Cholestasis is one of the more frequently reported AAS induced hepatic injury and, generally, it is a pure one (not associated with hepatocellular damage) even if the features of cholestasis (hepatocytes bearing brown granules in their cytoplasm and dilated canaliculi containing pigment material) may be associated with some degree of liver cell necrosis and inflammatory infiltration [20, 155-158]; liver biopsies of the reported cases show portal or periportal inflammation, canalicular cholestasis, and, fibrosis. Stimac *et al.* [158] described a pattern of predominant hepatocellular necrosis in the absence of cholestasis in a 26 year – old man bodybuilder who self – administered AAS; liver biopsy revealed portal inflammation with lymphocytes and plasmacytes in association with evident ballooning degeneration and necrosis of the hepatocytes in portal and periportal areas. Peliosis, a pathological entity characterized by multiple blood-filled cavities, mostly involving the liver (peliosis hepatis), is an uncommon serious complications associated with the use and the abuse of androgen-steroids [159-163]. Recently Vougiouklakis *et al*. [164] described the first case of fatal pulmonary peliosis without any other organ involvement in a young testosterone abusing male. AAS have been proved to be involved in the development of hepatic adenomas [165]. Finally, the possibility of fatty liver disease associated with AAS use has been recently reported [166, 167]; one possible explanation is the that most AAS, when used exogenously, inhibit the normal process of steroid biosynthesis. The first step in steroidal hormone production is hindered and cholesterol is not converted into pregnenolone, the product of the initial cholesterol side chain cleavage reaction. This can result in cholesterol storage and possibly the creation of a proper environment for fatty liver accumulation. Moreover, probably the reactive oxygen species (ROS) are involved in the pathogenesis of AAS induced fatty liver disease.

RENAL TOXICITY

Evidence of side effects affecting kidney and the renal function are sporadically emerging from clinical reports of renal disorders among AASs users, especially with elevated and prolonged use [168-173], since AASs are commonly excreted in urine mainly as glucuronide conjugates, the formation of which is catalyzed by various uridine diphosphate-glucuronosyltransferase (UGT) enzymes. Glucuronidation of steroids and their phase I metabolites is an important detoxification and deactivation metabolic pathway of AAS which can, in part, explain the renal effects of AAS [174].

Many studies conducted on animal models reported that AAS administration induce renal hypertrophy: Mills *et al*. reported that testosterone administration to female mice for 25 days produced a 70% increase in total kidney protein [175]. These findings are coherent with those of Hoseini *et al.* [176] who observed a significant increase in the weight of the kidneys of mice treated with nandrolone; in particular they observed tubular hypertrophy in the treated animals which could be explained by the high expression of androgen receptors within the kidney in the proximal tubule cells as reported by Chang *et al*. [177] and by the fact that the main action of androgens is the stimulation of cell growth including hypertrophy and hyperplasia. In addition to this action on androgen-regulated genes expressed in proximal tubule cells, some regulatory effects on gene(s) expressed in the more distal segments of the renal tubule, where the mineralocorticoid receptor is specifically located [178] have been reported. Thus also distal convoluted tubules and collecting ducts could be influenced by androgens, so explaining the kidney hypertrophy reported in animal models by many Authors [176].

In humans, the clinical spectrum of renal dysfunction varies from mild transient elevation in serum creatinine, blood urine nitrogen, and uric acid which often return to normal once the drugs are discontinued [179, 180] to acute renal failure as complication of of rhabdomyolysis [181] (Fig. **7**). Recently, Herlitz [168] described renal injuries in a

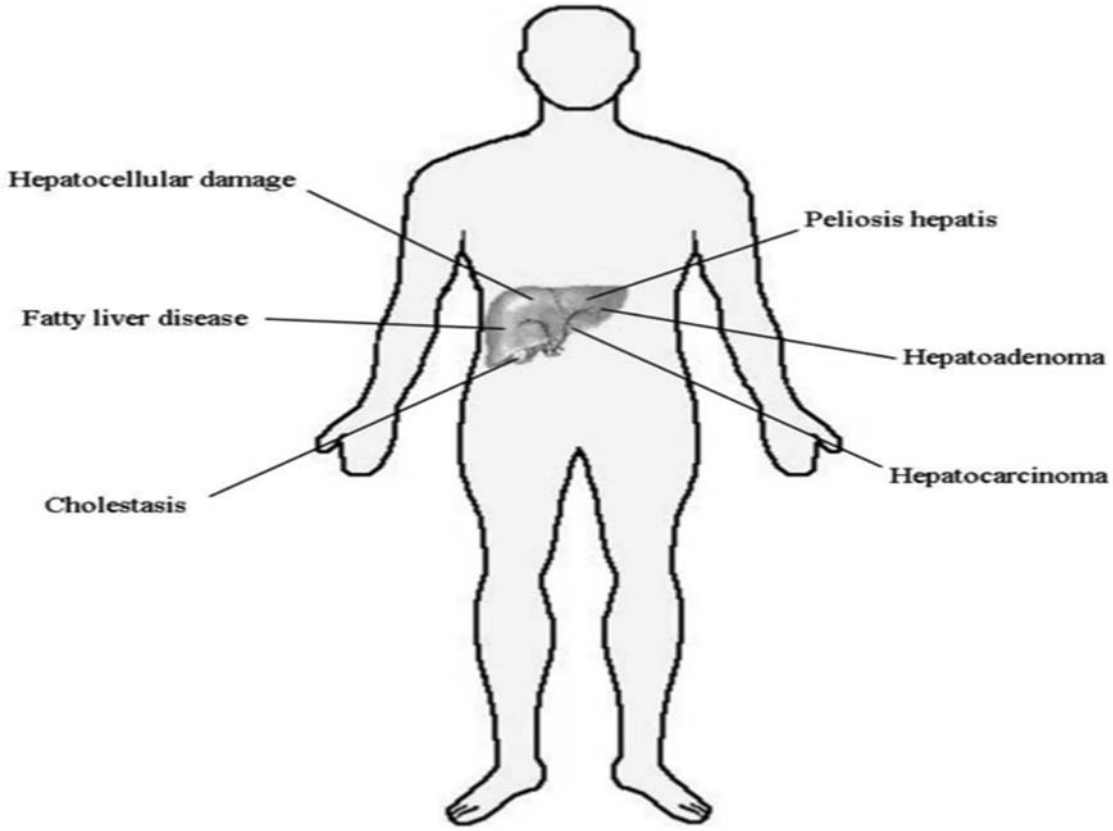

Fig. (6). Potential harmful hepatic effects of AAS [33]. Modified from Vanberg and Atar 2010.

small cohort of bodybuilders who developed a wild range of renal impairment from subnephrotic proteinuria or nephrotic-range proteinuria to full nephrotic syndrome after long – term intake of AAS. Renal biopsies showed focal segmental glomerulosclerosis in association with tubular atrophy and interstitial fibrosis. Several histomorphological lesions were observed: advanced fibrosis and glomerulosclerosis, hypertrophied glomerulus with perihilar segmental sclerosis that has collapsing features with overlying podocyte hyperplasia, segmental wrinkling and retraction of the glomerular basal membranes. This pattern of lesion is though to be mediated by elevated glomerular filtration rate (hyperfiltration), glomerular pressure and other adaptive structural-functional responses within the kidneys. Furthermore, many reports exist on adverse renal conditions such as acute kidney injury, nephropathy, diffuse type 1 membranoproliferative glomerulonephritis and also renal failure owing to long term use of AAS [169, 170]. Finally, androgens may have an important role in the pathogenesis of renal cancer in humans and experimental animals. Wilm's tumor, uncommon in adults, has, in fact, been reported in several athletes using anabolic steroids [182]. There is evidence suggesting that steroids are weak carcinogens that can initiate tumor growth or promote such growth in the presence of other carcinogens [183, 184].

CONCLUSIONS

This review deals with experimental and human pathological findings that are directly attributable to the known toxic actions of androgen anabolic steroids. According to literature, there is a wild scenario of AAS side effects which affect, mainly, the cardiovascular system, the liver, the kidney, the musculoskeletal and the endocrine systems. In the past years it has became more and more evident that anabolic steroids are very harmful to health. The mechanisms involved in the genesis of these toxic effects are not yet fully clarified. However it is understood that a genomic mechanism is involved in AAS toxicity: the effects of androgens are modulated at cellular level by the steroid-converting enzymes within the particular target tissue. Modulation of the effects of androgens may also occur at the molecular level due to differences in the distribution of androgen receptor coregulators in various tissues, these coregulators being proteins that affect the transcriptional activity of the androgen receptor [12-17]. A non genomic direct mechanism of action involves the bindings of AAS and their metabolities to plasma membranes receptors.

The true incidence of AAS related medical problems is not known, due to several drawbacks in human studies. The entity of AAS side effects, in fact, depends on the sex, the dose, the duration of treatment, whether they are taken during exercise training or under sedentary conditions, and the susceptibility of the individuals themselves to androgen exposure partly depending on genetic factors. Both the acute and the chronic effects can lead to toxicity, but generally the serious and even fatal effects depend on the time and the duration of AAS administration, the most serious being observed when AAS are used in high dose and over prolonged time and the milder and more frequently seen side effects disappearing upon discontinuation of use. A limitation of

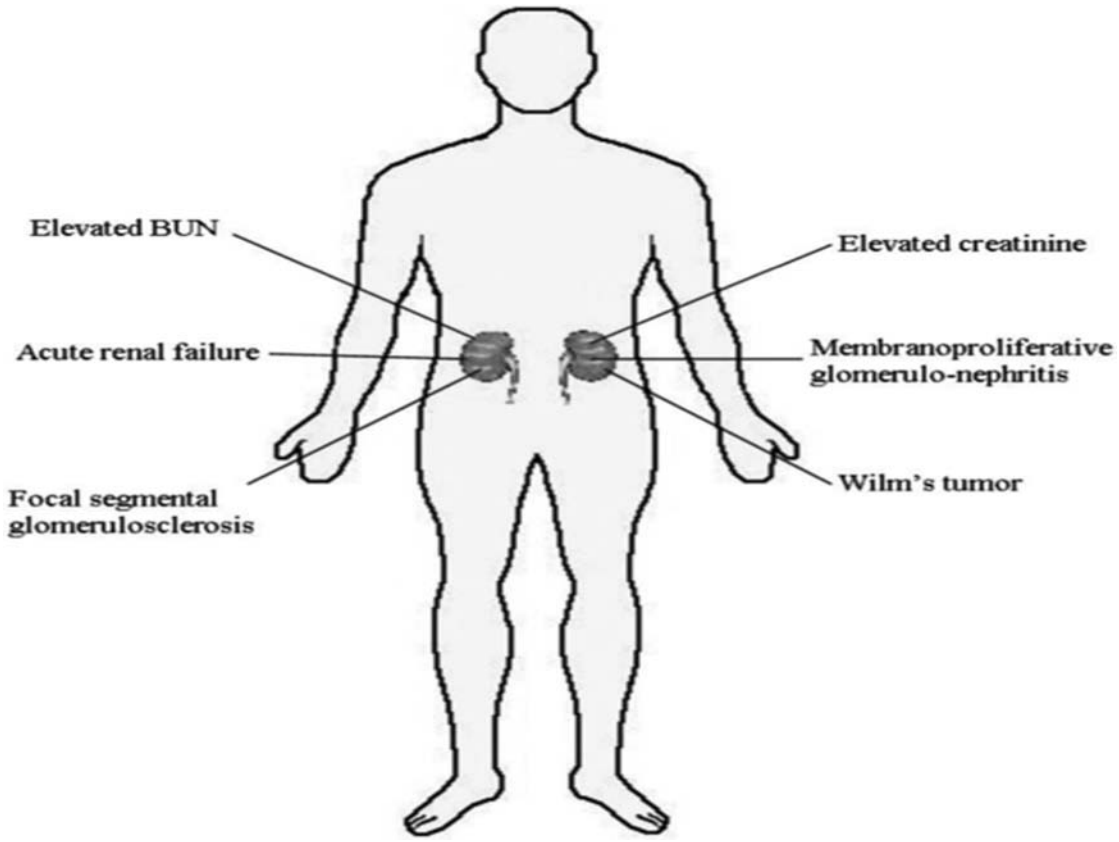

Fig. (7). Potential harmful renal effects of AAS [33]. Modified from Vanberg and Atar 2010.

human studies is represented by the fact that information about the intake of steroids are, generally, self reported and it is hardly possible to assess the exact dosage in an objective way. Furthermore AAS are often used in combination with other dugs or substances at high dosage, so it is extremely difficult to separate the toxic effects of AAS from those caused by the other drugs abused.

Taken together these matters explain the great difficulties existing in studying the human toxicity of AAS. Many anecdotal case reports on AAS related adverse effects and even on AAS related sudden deaths exist in the literature but they do not really investigate the causal relationship eventually existing between AAS intake and the serious or fatal diseases reported. Likewise, the existing cross – sectional studies (i.e. on the cardiovascular effects of AAS) [123, 124] are not designed to study the causal relationship since they only observe the morphological and functional differences between AAS abusers and controls. So these studies must be interpreted with caution. As Hartgens and Kuipers outlined some years ago most of the early studies assessed one drug given at therapeutic dose, while athletes and AAS addicts use to self- administer polydrug regimens that may be extremely higher than those investigated in the studies reported by the literature. Human studies mimicking the real entity of self – underground administration of AAS activity are infeasible since it would be unethical to administer the high dose regimens in controlled studies over prolonged periods of time to evaluate the risks to health [20]. Hence experimental studies conducted on animal models are mandatory to investigate the mechanisms underlying to AAS toxicity and the organ alterations due to these substances. The evidence coming from the great mess of experimental studied is that AAS produce severe toxic effects in animals which may be of great importance in understanding the health negative consequences observed in AAS abusers. In particular, the researches on animal and experimental models which outline the effects on cardiovascular system, although not yet conclusive, can explain severe side effects and sudden death in AAS abusers which represent the most dramatic unexpected events occurring in athletes and non athletes self administering AAS. In fact it is well known that athletes who had used AAS experienced an higher mortality compared with age – matched control population [185].

Some conclusions can be drawn. First of all it is time that the awareness of the deleterious effects of the AASs increases in the population of AAS users. Statements circulating in the underground world of AAS abusers have to be considered now definitively flawed since the deleterious effects of AAS have to be clearly emphasized. It is concluded that the axiom that 'the more you take, the more you grow' [186] should be accompanied with 'the more you may damage your health' [2]. Second, clinicians should be aware of the complex and varied pattern of toxicity so as to be able to perform correct diagnoses and treatments, so improving their knowledge on AAS detrimental effects and, of consequence, AAS users' trust in physicians' knowledge about AAS [187]. Currently, in fact, few AAS users seek medical

treatment and many are skeptical of physicians' knowledge about AAS side effects [11].

Finally, from a diagnostic standpoint, in all AAS related deaths, a complete post-mortem study should be performed with special concern to target organs.

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Received: December 15, 2010 Revised: January 22, 2011 Accepted: February 07, 2011