

Anabolic Androgenic Steroid (AAS) Related Deaths: Autoptic, Histopathological and Toxicological Findings

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Abstract: Anabolic androgenic steroids (AASs) represent a large group of synthetic derivatives of testosterone, produced to maximize anabolic effects and minimize the androgenic ones. AAS can be administered orally, parenterally by intramuscular injection and transdermally. Androgens act by binding to the nuclear androgen receptor (AR) in the cytoplasm and then translocate into the nucleus. This binding results in sequential conformational changes of the receptor affecting the interaction between receptor and protein, and receptor and DNA.

Skeletal muscle can be considered as the main target tissue for the anabolic effects of AAS, which are mediated by ARs which after exposure to AASs are up-regulated and their number increases with body building. Therefore, AASs determine an increase in muscle size as a consequence of a dose-dependent hypertrophy resulting in an increase of the cross-sectional areas of both type I and type II muscle fibers and myonuclear domains. Moreover, it has been reported that AASs can increase tolerance to exercise by making the muscles more capable to overload therefore shielding them from muscle fiber damage and improving the level of protein synthesis during recovery.

Despite some therapeutic use of AASs, there is also wide abuse among athletes especially bodybuilders in order to improve their performances and to increase muscle growth and lean body mass, taking into account the significant anabolic effects of these drugs.

The prolonged misuse and abuse of AASs can determine several adverse effects, some of which may be even fatal especially on the cardiovascular system because they may increase the risk of sudden cardiac death (SCD), myocardial infarction, altered serum lipoproteins, and cardiac hypertrophy.

The aim of this review is to focus on deaths related to AAS abuse, trying to evaluate the autoptic, histopathological and toxicological findings in order to investigate the pathophysiological mechanism that underlines this type of death, which is still obscure in several aspects. The review of the literature allowed us to identify 19 fatal cases between 1990 and 2012, in which the autopsy excluded in all cases, extracardiac causes of death.

Keywords: Anabolic Androgenic Steroids (AAS), cardiovascular effects, sudden cardiac death, toxicity.

INTRODUCTION

Anabolic androgenic steroids (AASs) represent a large group of synthetic derivatives of testosterone, produced to maximize anabolic effects and minimize the androgenic ones [1, 2].

AASs can be administered orally, parenterally by intramuscular injection and transdermally. The most common oral and injectable AASs with their chemical structures are reported in Table 1. Androgens act by binding to the nuclear androgen receptor (AR) in the cytoplasm and then translocate into the nucleus. This binding results in sequential conformational changes of the receptor affecting the interaction between receptor and protein, and receptor and DNA [3].

The basic structure of all steroids is a perhydrocyclopentano phenanthrene ring system that can be modified in order to obtain several designed chemical modifications [3]. The most important chemical modification, in which the basic structure can undergo, is reported in Fig. 1.

The name “anabolic androgenic steroids” already suggests their “anabolic” (from Greek ἀναβολή “throw upward”) and “androgenic” (Greek ἀνδρικός “of a man” + -γενής “born”) properties.

Anabolism is defined by Kuhn [4] as “any state in which nitrogen is differentially retained in lean body mass, either through stimulation of protein synthesis and/or decreased breakdown of protein anywhere in the body”. Skeletal muscle can be considered as the main target tissue for the anabolic effects of AAS, which are mediated by ARs which after exposure to AASs are up-regulated and their number increases with body building [5]. Therefore, AASs determine an increase in muscle size as a consequence of a dose-dependent hypertrophy resulting in an increase of the cross-sectional areas of both type I and type II muscle fibers and

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Table 1. AASs most commonly abused (oral and injectable formulations).

Oral Agents <i>17α-alkyl derivatives</i>		Injectable Agents <i>17β-ester derivatives</i>	
Methandrostenolone		Testosterone	
Methyltestosterone		Nandrolone	
Oxandrolone		Boldenone	
Oxymetholone		Methenolone	
Stanozolol		Trenbolone	
Ethylestrenol		Stanozolol	
Fluoxymesterone		Dromostanolone	
Danazol			

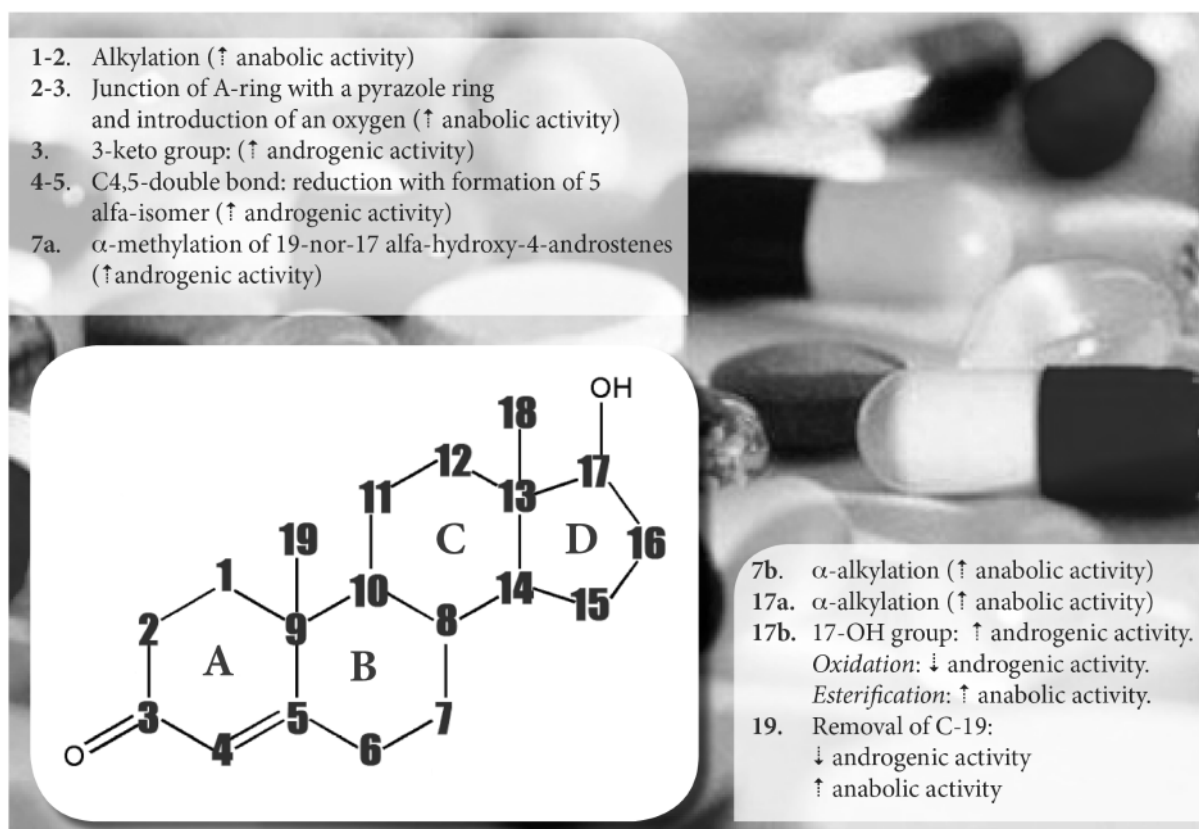


Fig. (1). The basic structure of steroids can be changed in order to obtain several designed chemical modifications.

myonucleardomains [6]. Moreover, it has been reported that AASs can increase tolerance to exercise by making the muscles more capable to overload therefore shielding them from muscle fiber damage and improving the level of protein synthesis during recovery [7].

Despite some therapeutic use of AASs (severe burns, primary or secondary hypogonadism, short stature, HIV wasting syndrome etc.) there is also wide abuse among athletes especially bodybuilders in order to improve their performances and to increase muscle growth and lean body mass, taking into account the significant anabolic effects above reported. Not by chance, these substances fall within the vast group of “performance-enhancing drugs”, which also include: stimulants, painkillers, sedatives and anxiolytics, diuretics, blood boosters and masking drugs. A high-dose regimen is “stacked” by combining numerous oral and injectable AASs, which are self-administered in drug “cycles” which last from 4 to 12 weeks [7-9].

Furthermore, AAS users frequently associate other substances to AASs, the so called “steroid-accessory drugs”, such as ephedrine, growth hormone, insulin, diuretics, GHB etc [10-15] for several reasons.

The prolonged misuse and abuse of AASs can determine several adverse effects, some of which may be even fatal especially on the cardiovascular system because they may increase the risk of sudden cardiac death (SCD), myocardial

infarction, altered serum lipoproteins, and cardiac hypertrophy [7]. The most frequent cardiovascular adverse effects due to AASs are summarized in Fig. 2.

The aim of this review is to focus on deaths related to AAS abuse, trying to evaluate the autptic, histopathological and toxicological findings in order to investigate the pathophysiological mechanism that underlines this type of death, which is still obscure in several aspects.

MATERIALS AND METHODS

Some databases, from 1975 to June 2014, were searched: Medline, Cochrane Central, Scopus, Web of Science, Science Direct, EMBASE and Google Scholar, using the following keywords: Anabolic Androgenic Steroid, death, cardiovascular effects, toxicity, side/adverse effects. The main key word “Anabolic Androgenic Steroid” was individually searched in association to each of the others. The 189 sources found after the initial screening in order to exclude duplicate sources and retrospective studies, were selected according to the “inclusion criteria”, which allowed the identification of 10 sources. A comprehensive flow diagram with inclusion criteria is reported in Fig. 3.

RESULTS

The review of the literature using the flow diagram reported in Fig. 3 allowed us to identify 19 fatal cases between 1990 and 2012. The most important autptic,

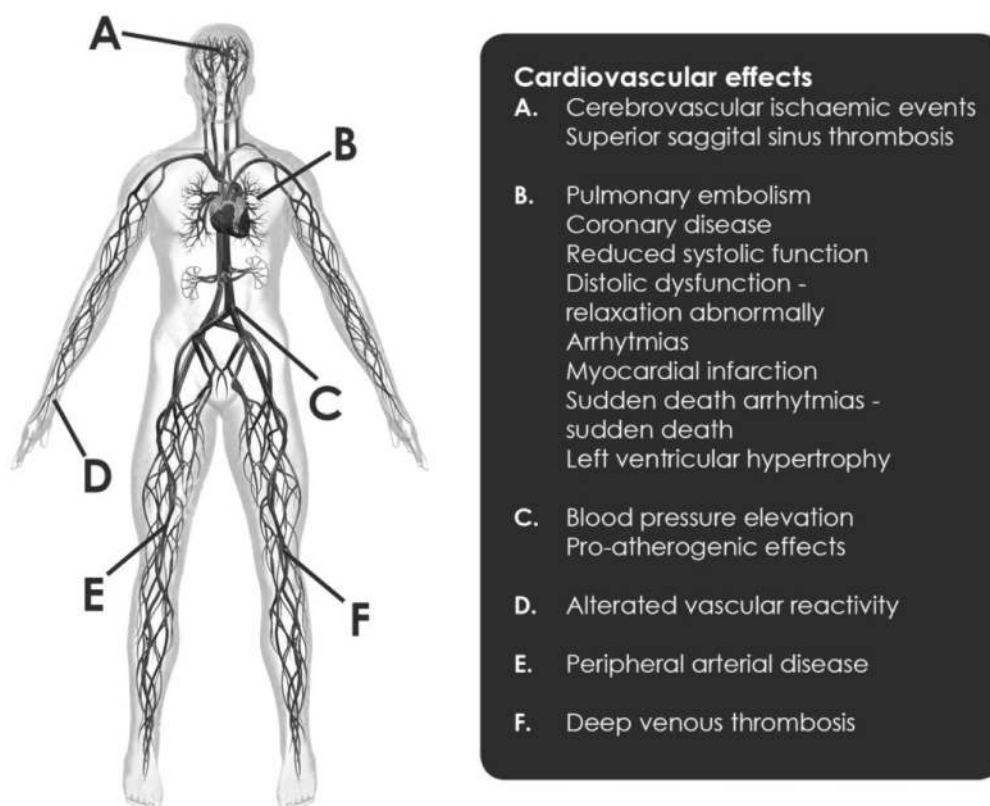


Fig. (2). Cardiovascular adverse effects due to a prolonged use of AASs.

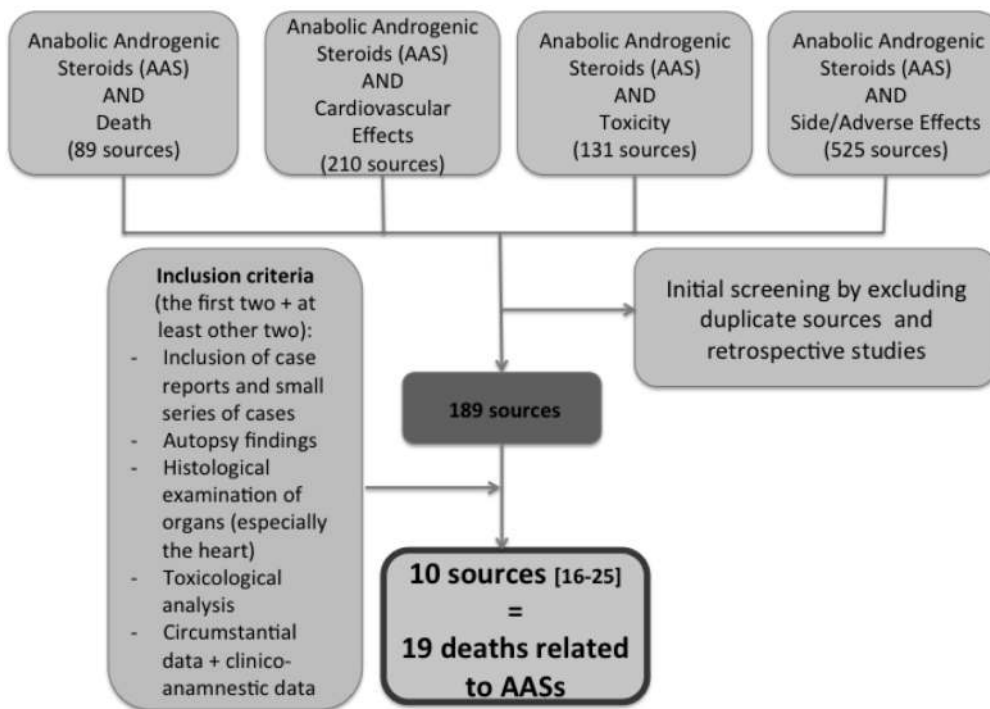


Fig. (3). Flow diagram with inclusion criteria for the selection of sources for the purpose of the review.

histopathological findings and circumstantial data are reported in Table 2, while the toxicological findings are reported in Table 3.

Of the 19 cases, 17 (89.5%) were males whereas only 2 (10.5%) were females. The age ranged from 18 to 37 years (mean age: 28 ± 4.4). Among the 19 fatal cases, in 14 bodies

Table 2. Autoptic, macroscopic and histological findings in 19 AAS related deaths.

Study	Number of Cases	Age (yrs), Sex, Height and Weight	Heart Dimensions Wall Thickness (mm)				Cardiac Macroscopic and Histopathological Findings	Other Findings
			Weight(g)	LV	RV	IS		
Luke <i>et al</i> (1990) [16]	1	27 M 96.6kg	530	16	4	--	Marked cardiac hypertrophy with regional myocardial fibrosis. Contraction band necrosis, lymphocytic infiltration	Renal hypertrophy, hepatosplenomegaly
Ferenchick G.S. (1991) [17]	1	22 M	---	---	---	---	Occlusive thrombus in left coronary artery	
Kennedy <i>et al</i> (1993) [18]	2	18 M	410	13	4	17	Hypertrophic cardiomyopathy, myocarditis, disarray, focal fibrosis.	
		24 M	440	17	6	12	Focal fibrosis, lymphocytic infiltration	
Dickerman <i>et al</i> (1996) [19]	1	26 M 182cm 136kg	440	----	----	---	Left ventricular hypertrophy	Bilateral pulmonary embolism from deep venous thrombus of lower extremities
Hausmann <i>et al</i> (1998) [20]	1	23 M	500	----	----	---	Right ventricle dilatation, focal endocardial induration. Enlargement and nuclear polymorphism of left ventricle muscle fibres; disseminated focal necrosis with loss of nuclear staining, dehiscence of intercalated discs and interstitial fibrosis	Liver parenchyma soft and fragile, cerebral edema, acute vascular congestion in liver spleen and kidney. Capillary hyperemia, platelet aggregation, several fibrinous clots in lungs, liver and kidneys. Nuclear fat free vacuoles, pielosis hepatis.
Fineschi <i>et al</i> (2001) [21]	2	32 M 189cm 90kg	450	14	4	14	One grayish zone in internal half of the anterior-lateral wall of the left ventricle which corresponded histologically to a typical infarct necrosis, dead hyperdistended myocardial cells with sarcomeres in registered order. Occasional foci of contraction band necrosis and few fibrotic microfoci in internal portion of left ventricle and interventricular septum.	
		29 M 166cm 72kg	390	19	6	13	Occasional isolated myocardial cells with contraction bands and segmentation of myocardial cells	

Table 2. continued

Study	Number of Cases	Age (yrs), Sex, Height and Weight	Heart Dimensions Wall Thickness (mm)				Cardiac Macroscopic and Histopathological Findings	Other Findings
			Weight(g)	LV	RV	IS		
Di Paolo <i>et al</i> (2007) [22]	4	29 M 190cm 127kg	490	13	----	---	Small vessels disease, severe interstitial and epicardial fibrosis, hypotrophic myocytes in fibrosis areas, hypertrophy in non fibrosis areas, focal fatty substitution in of the anterior LV wall, mild focal intimal hyperplasia in coronary arteries, myocardial bridge	
		27 M 190cm 100kg	360	11	----	---	Small vessels disease, focal interstitial and epicardial fibrosis, hypotrophic myocytes in fibrosis areas, mild focal intimal hyperplasia in coronary arteries	
		37 F 162cm 71kg	310	10	----	---	Focal interstitial and epicardial fibrosis, hypotrophic myocytes in fibrosis areas, unique granulation tissue focus, normal coronary arteries.	
		31 M 175cm 79kg	400	12	----	---	Small vessels disease, focal interstitial fibrosis and moderate/severe epicardial fibrosis, myocytes hypertrophy, occasional basophilic degeneration, mild intimal hyperplasia in coronary arteries	
Fineschi <i>et al</i> (2007) [23]	2	29 M 166 cm 72kg	380	----	----	---	Foci of contract band necrosis, two microfoci of fibrosis (subendocardial anterior left ventricle, interventricular septum) segmentation of myocardial cells, widening of intercalated discs, bundles of contracted myocardium alternating with bundles of distended myocardium with granular disruption of myocytes.	Cholestasis pielosis hepatis
		30 M 178 cm 90kg	400	----	----	---	Coronary scattered fatty streaks, focal myocardial fibrosis	
Thiblin <i>et al</i> (2009) [24]	1	29 F 172 cm 76kg	331	----	----	---	Isolated flat area of fatty thickening 0.5 x 0.3 cm in proximal part of left anterior descending coronary artery (LAD), few small foci of granulation tissue, lymphocytic infiltration around several middle size and small intramural vessels	Adrenal diminished of cortex and medulla. Uterus slightly larger and ovaries slightly smaller. Internal organ abnormally heavy (liver 2298g; kidneys 394g; lungs 1500g) Lung congested with multiples area of erythrocytes-containing alveoli

Table 2. continued

Study	Number of Cases	Age (yrs), Sex, Height and Weight	Heart Dimensions Wall Thickness (mm)				Cardiac Macroscopic and Histopathological Findings	Other Findings
			Weight(g)	LV	RV	IS		
Montisci <i>et al</i> (2012) [25]	4	32 M 180cm 110kg	450	15	4	16	Concentric left ventricular hypertrophy. Focal disarray with interstitial and replacement type fibrosis foci of lymphoplasmacellular infiltrates (CD3+) edema and patchy necrosis.	Multiorgan congestion, liver steatosis
		31 M 172cm 120kg	900	15	5	20	Biventricular eccentric hypertrophy and mild atrial dilatation, mural endocardial thrombosis anterior left ventricular wall. Hypertrophic myocytes, interstitial and replacement fibrosis colliquative myocytolysis.	Multiorgan congestion, acute pulmonary edema, liver steatosis (2700 g), testicular atrophy
		32 M 178cm 94 kg	580	16	5	18	Hypertrophy biventricular dilatation. Hypertrophied myocytes with bizarre nuclei, foci of myocytolysis with a mild inflammatory reaction and active fibrosis, fibrofatty replacement LAD stenosis 50%.	Multiorgan congestion, lung hemorrhagic infarction, acute hepatic congestion
		25 M 185cm 125kg	390	13	3.5	16	Diffuse interstitial edema, multiple foci of polymorphous inflammatory infiltrates with eosinophils.	Multiorgan congestion

(12 males and 2 females) the data available allowed to calculate the body mass index (BMI) and the results are reported in Table 4. In none of the cases the BMI was lower than 24.9, which is considered the upper limit of the normal healthy weight.

For all cases the autopsy excluded extracardiac causes of death, only in one case a bilateral pulmonary embolism from deep venous thrombus of lower extremities was found (Table 2).

Toxicological investigation performed mainly on urine samples but also in blood and hair samples, by using several screening tests and analytical methods revealed in 12 cases [16, 18, 20-25] the presence of AASs and/or their metabolites in urine specimens; in one case [23] nandrolone was detected in blood, whereas in another case [25] stanozolol was found in hair. In the remaining 6 cases in which the toxicological analysis was negative, circumstantial data and evidences reported by relatives and friends of the deceased highlighted a previous prolonged use of AASs.

DISCUSSION AND CONCLUSIONS

The chronic use of AASs can cause various pathological alterations, which are related to dose, frequency and patterns of use. Taking into account that numerous organs and apparatus are the target of AASs, several adverse effects can involve the liver, cardiovascular, reproductive, musculoskeletal, endocrine, renal, immunologic and hematological systems as

well as some psychological effects; a schematic representation is reported in Fig. 4.

Here 19 fatal cases are reported; although only single case report or small series of cases were included, whereas retrospective studies and other papers that did not fulfill the inclusion criteria were not taken into account, some consideration can be formulated; in all cases the autopsy findings together with the histological examination have highlighted cardiac causes of death. Only in one case [19] a mechanical cardiovascular cause of death was found (a bilateral pulmonary embolism from deep venous thrombus of lower extremities). In numerous cases [16, 18, 19, 22, 25], a common finding was a left ventricular hypertrophy, frequently associated with fibrosis and myocytolysis. A myocardial hypertrophy was not found in the 4 cases reported by Fineschi *et al* in two different reports [21, 23].

What is the significance that could be attributed to the myocardial hypertrophy? A vigorous training in young athletes can determine a left ventricular hypertrophy, independently of the use of AASs (the so called “athlete’s heart”) [25-28].

Melchert and Welder [29] categorized the effects of AAS on the cardiovascular system into four groups of activities: vasospastic, atherogenic, thrombotic and direct myocardial injuries. AAS can induce adverse cardiovascular effects such as left ventricular hypertrophy (LVH), hypertension, impaired diastolic filling, arrhythmia, erythrocytosis, thrombosis and

Table 3. Toxicological findings and circumstantial data in 19 AAS related deaths.

Study	Number of Cases (n=19)	Age (yrs), Sex, Height and Weight	Circumstances	History of Abuse and Route of Administration	Method of Detection	Toxicological Findings
Luke <i>et al</i> (1990) [16]	1	27 M 96.6kg	Collapse during a bench press workout	He had taken anabolic androgenic steroids parenterally for several months previously.		URINE Nandrolone (19-nor-testosterone) and metabolites
Ferrenchick G.S. (1991) [17]	1	22 M				
Kennedy <i>et al</i> (1993) [18]	2	18 M 24 M	cardiac arrests during training sessions			URINE Oxymesterone URINE Oxymesterone
Dickerman <i>et al</i> (1996) [19]	1	26 M 182cm 136kg	Collapse while moving furniture	History of anabolic steroid use		
Hausmann <i>et al</i> (1998) [20]	1	23 M 192cm 94kg	Found dead at home	He had taken anabolic steroid for 9 months. In his apartment were found: -Testosterone cyclopentilpropionate 250 i.m. -Methenolone enantate 100 mg i.m. -Liothyronin hydrochloride 100 ug tablets -Spironolactone 100 mg tablets -Clomifen 25 mg capsules -Clenbuterol hydrochloride 0.02 mg tablets	EIA and GC-MS after derivatisation	URINE Mesterolone, Methandienone Testosterone, Nandrolone and Clenbuterol T/E ratio 64:1
Fineschi <i>et al</i> (2001) [21]	2	32 M 189cm 90kg 29 M 166cm 72kg	Sudden death during a weight lifting workout at the gymnasium Found unconscious in bed	For several months he had been taking testosterone propionate (700mg/wk) and nandrolone (200 mg/wk) parenterally and stanozolol (70 mg/wk) per os He had used anabolic steroids parenterally (nandrolone 250mg/wk and stanazolol 350 mg/wk) for several months	Urine screening, GC-MS Urine screening, GC-MS	URINE 19-nor-androsterone; 19-nor-etiocholanolone, nor-epiandrosterone (metabolites of Nandrolone) 3-idrossi-stanozolol 3-idrossi-17-epistazonozolol (metabolites of Stanozolol) URINE 19-nor-androsterone; 19-nor-etiocholanolone, nor-epiandrosterone (metabolites of nandrolone) 3-idrossi-stanozolol 3-idrossi-17-epistazonozolol (metabolites of stanozolol)

Table 3. continued

Study	Number of Cases (n=19)	Age (yrs), Sex, Height and Weight	Circumstances	History of Abuse and Route of Administration	Method of Detection	Toxicological Findings
Di Paolo <i>et al</i> (2007) [22]	4	29 M 190cm 127kg	Loss of consciousness during spin bike lesson	History of AAS abuse	Testosterone GC-MS Stanozolol ELISA HPLC/MS	URINE Negative
		27 M 190cm 100kg	Sudden death while in a night club	History of AAS abuse	Testosterone GC-MS Stanozolol ELISA HPLC/MS	URINE Stanozolol Testosterone
		37 F 162cm 71kg	Found dead in her car	History of AAS abuse	Testosterone GC-MS Stanozolol ELISA HPLC/MS	URINE Negative
		31 M 175cm 79kg	Found dead in his bedroom	History of AAS abuse	Testosterone GC-MS Stanozolol ELISA HPLC/MS	URINE Stanozolol
Fineschi <i>et al</i> (2007) [23]	2	29 M 166 cm 72kg	Collapsed and died after dinner in his apartment	He had been taking testosterone, nandrolone e stanozolol parenterally for several years	Screening, GC-MS-MS	URINE Nandrolone (non misurable) Stanozolol 43 µg/l T/E ratio= 28.7
		30 M 78 cm 90kg	Collapsed and died at home	Had taken AAS 6 months before. In a ashtray near the body a 2-ml of nandrolone decanoate was found. The apartment contained a veritable arsenal of drug	Screening, GC-MS-MS	BLOOD Nandrolone 19 ng/ml URINE Norandrosterone 208.4 ng/ml T/E ratio=42
Thiblin <i>et al</i> (2009) [24]	1	29 F 172 cm 76kg	Found dead at home	She had used nine different AASs in various combinations during the previous 8 months. Moreover, ephedrine, tadalafil metandienon, mestanolon stanozolol pills and clenbuterol tablets were found	Immunological screening, GC-MS	BLOOD Ephedrine 0.4 µg/l and Norephedrine 0.1 µg per g blood URINE Testosterone 31.4 µg/ml; OH-stanozolol 29.3 ng/ml; 16βOH-stanozolol 16.5 ng/ml; Boldenon 2109 ng/ml T/E ratio=28.3
Montisci <i>et al</i> (2012) [25]	4	32 M 180cm 110kg	Found dead in his bed	He had been taking AAS parenterally for 7 years	Screening with EMIT-ELISA LC-MS/MS	Negative
		31 M 172cm 120kg	Collapsed during training. Heart failure during hospitalization	Consumption of AAS (boldenone dromostanolone metanolone enantato stanazolol tranbolone) for several years	Screening with EMIT-ELISA LC-MS/MS	HAIR Stanozolol 5.0 pg/ng Negative
		32 M 178cm 94 kg	Death after a dentistry visit	He had been taking AAS for several years	Screening with EMIT-ELISA LC-MS/MS	URINE Testosterone 94 ng/ml Epitestosterone 9 ng/ml
		25 M 185cm 125kg	Death while sleeping	Several drugs (AAS and anorectics) were found in the apartment	Screening with EMIT-ELISA LC-MS/MS	Nortestosterone 3500 ng/ml

Table 4. Body Mass Index (BMI) in 14 fatal cases. In none of the cases the BMI was lower than 24.9, which is considered the upper limit of the normal healthy weight.

Authors	Age (yrs)	Sex	Height (cm)	Weight (kg)	BMI
Dickerman <i>et al</i> (1996) [19]	26	M	182	136	41.1
Fineschi <i>et al</i> (2001) [21]	32	M	189	90	25.2
	29	M	166	72	26.1
Di Paolo <i>et al</i> (2007) [22]	29	M	190	127	35.2
	27	M	190	100	27.7
	37	F	162	71	27.1
	31	M	175	79	25.8
Fineschi <i>et al</i> (2007) [23]	29	M	166	72	26.1
	30	M	178	90	28.4
Thiblin <i>et al</i> (2009) [24]	29	F	172	76	25.7
Montisci <i>et al</i> (2012) [25]	32	M	180	110	33.9
	31	M	172	120	40.6
	32	M	178	94	29.7
	25	M	185	125	36.5

BMI = 18.5-24.9, Normal
 BMI = 25.0-29.9, Overweight
 BMI = 30.0-34.9, Moderately Obese
 BMI = 35.0-39.9, Severely Obese
 BMI = ≥ 40 , Very Severely Obese

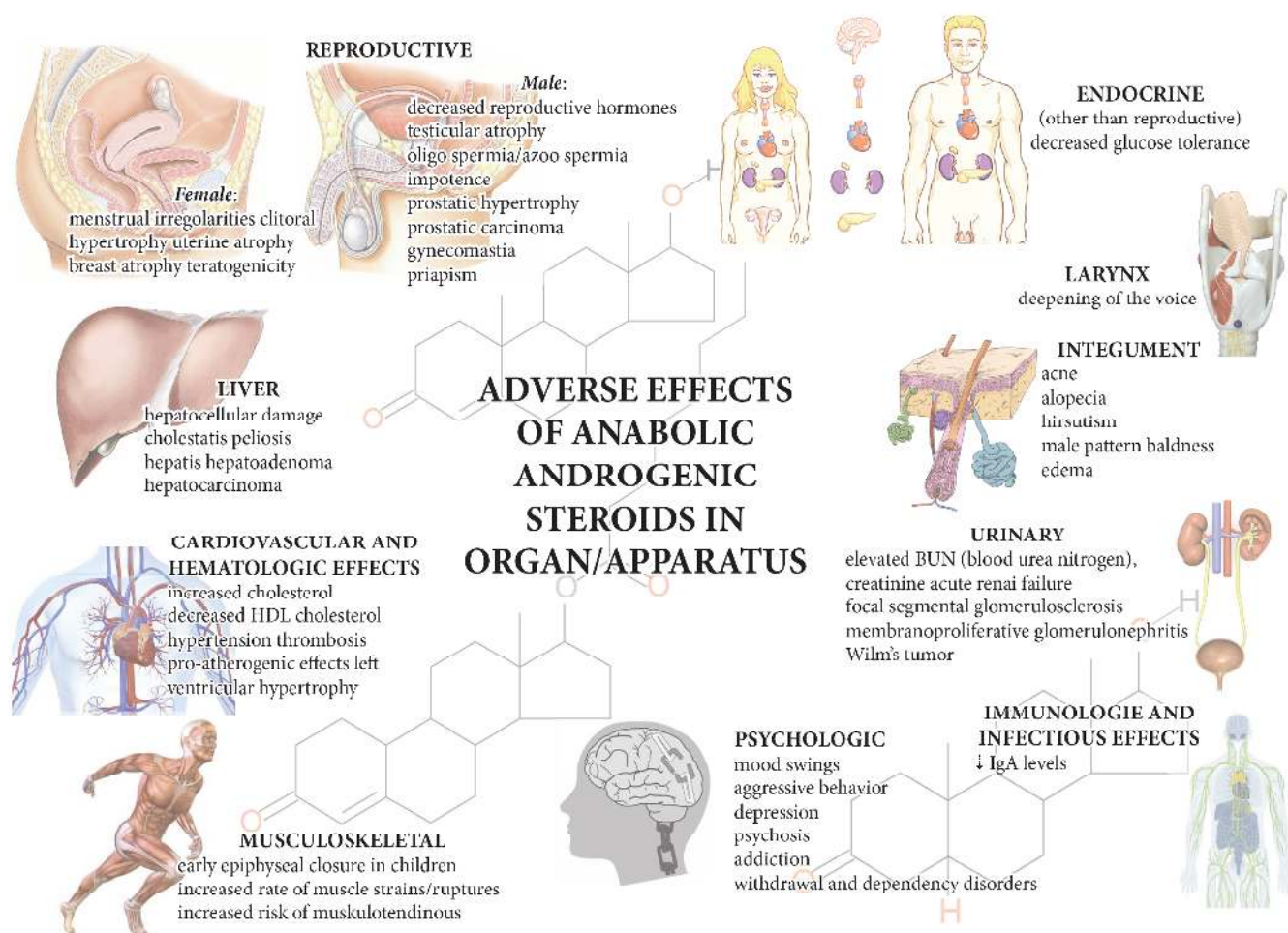


Fig. (4). Adverse effects due to AASs which can affect numerous organs and apparatus.

altered lipoprotein profiles [30]. Abnormalities in cardiovascular reflex control of the cardiovascular system [31-35] and in vascular reactivity [36-40] have also been reported.

Studies on isolated hearts from rats treated chronically with nandrolonedecanoate (ND) have also shown a raise in myocardial susceptibility to ischemia/reperfusion injuries [41, 42].

Nandrolone abuse combined with vigorous exercise training may lead to impaired diastolic function and concentric hypertrophy of the left ventricular (LV) wall [43]. Vigorous weight lifting itself would also cause LV wall mass and thickness increase but cardiac function would not be affected. However, when combined with AAS abuse pathological cardiac hypertrophy could be caused [44]. In another study, rats were treated with ND for 6 weeks (total dose 30 mg/kg). ND stimulated cardiomegaly that reversed after the end of treatment [45].

Rocha *et al.* [46] studied the effects on cardiac function in rats undergoing swimming training and those not undergoing it. They investigated that swimming training combined with high doses of nandrolone (5 mg/kg per injection, equal to 10 mg/kg per week) sharpens cardiac hypertrophy with interstitial fibrosis. Without a doubt these findings explain the high propensity to the onset and continuance of malignant cardiac arrhythmias. An explanation might be the change of the sympathetic autonomic activity modulated by the renin-angiotensin-system (RAS). Experiments have shown that RAS plays a significant role in the development of LVH and myocardial fibrosis.

Angiotensin II type 1 receptor's (AT1R's) stimulation is associated with the regulation of cell growth and proliferation of vascular smooth muscle cells, cardiomyocytes and endothelial cells involved in endothelial dysfunction, atherosclerotic vascular phenomena, congestive heart failure and myocardial infarction.

Marques Neto *et al.* [47] pointed out that the treatment with supraphysiological, chronic doses of AASs induce cardiac parasympathetic disturbances in ventricular depolarization in both exercised and sedentary rats. Unambiguously, it has been shown that the blockage of the RAS, and in particular of AT1R by losartan, obstructs QT prolongation.

Down-regulation of ion channel subunits, KChIP2, Kv1.4 and Kv4.3, could explain the autonomic dysfunction and cardiac repolarization disturbances caused by chronic treatment with supraphysiological doses of ND. Moreover, prolonged QT intervals and ventricular action potential could be explained by the reduced density of the transient outward potassium [48]. No augmentation in tissue collagen content or in the mRNA expression of types I and III collagens have been shown by histological analysis. However, Rocha *et al.* [46] found an increment in heart collagen content but not in mRNA expression. The previously mentioned unconformity could be attributed to the duration of treatment with ND and the age of rats used. Participation of the potassium (K) current in the generation of prolonged QT and potential action duration has been noticed. I_{to} is the transient outward K^+ current which is one of the main repolarizing currents in the mammalian myocardium and is generally believed to flow

through Kv1.4, Kv4.2 and Kv4.3 channels in rats [49, 50]. In heart hypertrophic cases I_{to} is down-regulated [51-53].

Low I_{to} density, Kv1.4 and Kv4.3 down-regulation in the left ventricle and prolonged action was noticed in the group treated with nandrolone compared to the control group. Homogenous distribution of Kv4.3 channel in the rat's ventricular wall, higher Kv4.2 in the epicardial and lower in the endocardial ventricular wall have been observed [49, 50]. These differences may partially explain the up-regulation of Kv4.2 and prolonged QTc interval and action potential. The expression of KChIP2 is considerably decreased in heart failure and hypertrophy [54, 55]. KChIP2 was found to be significant for I_{to} expression in the human heart and the correlation between KChIP2 absence and a total loss of I_{to} together with an increased susceptibility to ventricular arrhythmias in mice has been shown [56].

According to Riezzo *et al.* [57] the following effects were produced in physically trained mice intramuscularly treated with ND: moderate increase of heart weight, morphologically extensive cardiac hypertrophy and a wide colliquative myocytolysis which together could result in a significant heart failure. The increase of the heart weight suggested enhanced heart protein synthesis.

Medei *et al.* [58] found approximately 25% less nuclei and higher cardiomyocyte nuclei diameter in the ventricles of the group treated with ND. Lower nuclei suggests a toxic effect of ND which may involve a pro-apoptotic mechanism [59].

Tanno *et al.* [60] found that ND treatment whether combined with resistance training or not induced pathological concentric hypertrophy, re-expression of fetal genes, systolic and diastolic function impairment and an incremented myocardial collagen content leading to LVH.

Increased relative left ventricle wall thickness (RWT) was observed as a consequence of intensive physical training in rats treated with ND compared to the respective non-trained ones. In addition, the non-trained nandrolone treated group also produced higher RWT compared to the non-trained treated group. Increased interventricular septum thickness in the end-diastole (IVSDia) was noticed in both the non-trained nandrolone treated and trained vehicle-treated groups, compared to the non-trained vehicle treated rats.

A considerable lower ratio of maximum early to late transmitral flow velocity (E/A ratio) was observed in the trained groups, in comparison with non-trained groups. Nandrolone-treated groups (both trained and non-trained) showed lower E/A ratio in comparison with the respective vehicle-treated groups. Moreover, significant decrease in the expression of alpha-myosin heavy chain (α -MHC) mRNA and beta-myosin heavy chain (β -MHC) mRNA in the left ventricle was induced by nandrolone and resistance training respectively. Penna C. *et al.* [61] found that short-term ND treatment induces an overexpression of β_2 -adrenoceptors without cardiac hypertrophy.

Increment in cardiovascular mortality has been associated to an imbalance of (ANS) activity [54].

AASs can acutely inhibit the reuptake of catecholamines into extraneuronal tissue [62] and consequently the increment of catecholamine concentrations at receptor sites occurs. Although, the neuronal catecholamine transporter is normally responsible for the reuptake of noradrenaline, it has also been proved responsible for nonexocytotic release of noradrenaline from sympathetic nerve terminals during ischemia. An increased release of noradrenaline has been implicated in ischemia-induced arrhythmia [63, 64].

Tylicki [65] investigated the short-term effects of ND in rats' cardiac system. An increased activity of 6-phosphogluconate dehydrogenases and glucose-6-phosphate was observed in rat hearts, also ND activated isocitrate dehydrogenase and malic enzyme, which are other NADP-linked dehydrogenases. During the same study a significantly increased heart weight was also observed 10 days after nandrolone administration. It was shown that treatment with ND causes small QRS complex extension that might slightly reduce the spreading rate of the action potential through the heart ventricles, possibly because of the greater heart mass.

It is known that administration of doses higher than normal (supraphysiological) of ND impair exercise-induced cardioprotection in treadmill-exercised rats. Chaves *et al.* [66] were the first to say that enhanced levels of antioxidant enzyme levels produced after exercise are impaired with ND treatment (10mg/kg for 8 weeks), a fact which is well correlated to the cardiac injurious effects of the drug. It was observed that the hearts of animals treated with nandrolone and having undergone training (DT group) showed lower glutathione peroxidase (GPx), superoxide dismutase (SOD) and glutathione reductase (GR) activities compared with controls and trained groups of animals (CT). The latter observation indicates that nandrolone could act through blocking or down regulating the mechanisms implicated in the improvement of antioxidant defenses in DT animals, which might explain the lower percentage of left ventricular developed pressure and augmented infarct size in DT group.

It has been shown, in other researches on rats that exercise training improves myocardial resistance to reperfusion injury/ischemia [67-69] since physiological cardiac hypertrophy amends the sensitiveness of that heart making it more resistant to the previously mentioned disorders *in vivo* rat hearts [70]. Notwithstanding the strenuous research efforts, the molecular mechanism(s) involved in exercise-induced cardio protection is still debatable.

The numerous studies above reported in animal models, especially in rats, have called into question several pathophysiological mechanisms, which may explain some of the macroscopic and microscopic finding regarding the 19 cases here reported; however, we have to underline that these cases are single case report or small series of cases and not experimental studies. Moreover, the users of these substances frequently associate numerous steroids, in different forms, singularly and in several temporal combinations and cycles, and commonly, various steroid-accessory drugs are also used. Therefore, the interpretation of the postmortem findings is particularly difficult and no comprehensive conclusions can be done.

Finally, a brief remark must be placed regarding the BMI which was in all cases (12 males and 2 females) higher than 25. Although according to the BMI ranges from an "overweight" (9 cases) to a "very severely obese" (2 cases) were found, however, the BMI is not a direct measure of body fatness and BMI is calculated from an individual's weight which includes both muscle and fat. As a result, some subjects such as highly trained athletes may have a high BMI because of increased muscularity rather than increased body fatness; therefore, these results should be evaluated with caution.

The relationship between AAS abuse, vigorous exercise training, and cardiac death can be evaluated only by the application of an investigative protocol, which must include a rigorous methodology covering:

- A complete autopsy with a special regard to AAS target organs and apparatus (the cardiovascular system *in primis*).
- Histological and immunohistochemical analysis of AAS target organs.
- A broad toxicological investigation, preceded by a careful evaluation of clinico-anamnestic data, in order to confirm an AAS consumption (including type of AASs, concentration and interval of exposure) and the possible detection of other substances which could have contributed to the fatal outcome. For this purpose, different matrices can be used; urine is the most common, because it provides a prolonged detection time window, but also several other matrices such as: blood, serum, plasma, hair, oral fluid and nails can be used.

The comparison of the cases reported here, allows us to support the hypothesis that the combined effects of strong workout, the prolonged/chronic or previous abuse of AASs in different forms and combinations, have predisposed these subjects to develop different patterns of myocardial injuries and consequent sudden cardiac death [21, 23].

Therefore, the authors would strengthen the "warning" already expressed in previous reports [21, 23] against the use/abuse of these substances among professional and non-professional athletes. Moreover, only through a careful examination of all suspicious cases of AAS related deaths with the application of a rigorous investigative protocol, these cases can be identified and they could provide further information and data that may increase the knowledge of this type of deaths.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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