

Hepatotoxicity associated with illicit use of anabolic androgenic steroids in doping

R. SOLIMINI, M.C. ROTOLO, L. MASTROBATTISTA, C. MORTALI,
A. MINUTILLO, S. PICHINI, R. PACIFICI, I. PALMI

Department of Therapeutic Research and Medicines Evaluation, Drug Abuse and Doping Unit,
Istituto Superiore di Sanità, Rome, Italy

Abstract. – Anabolic Androgenic Steroids (AAS) abuse and misuse is nowadays a harmful habit involving both professional or recreational athletes, as well as general population. AAS are also frequently present in over-the-counter dietary supplements without being declared in the list of ingredients, leaving consumers unaware of the risks of adverse effects. Indeed, health risks of AAS consumption in pharmaceutical preparations or dietary complements seem still underestimated and under-reported. The variety of complications due to AAS misuse involves cardiovascular, central nervous, musculoskeletal and genitourinary systems of both males and females; psychiatric and behavioral effects, damages to metabolic system, skin and mainly liver. For instance, relevant concern has been raised by the AAS hepatotoxicity including adenoma, hepatocellular carcinoma, cholestasis, and peliosis hepatis.

The present review reports the information available on the hepatotoxic effects of AAS use in professional and amateur athletes.

Key Words:

Anabolic androgenic steroids, Doping, Hepatotoxicity, Illicit use.

Introduction

Testosterone is the male sex hormone and the typical endogenous anabolic steroid with specific properties of stimulating muscle mass growth (anabolic aspect) and enhancing male sexual characteristics (androgenic aspect)¹⁻⁵.

Testosterone was first isolated from bull testes in 1935 by David and co-workers^{1,2,6} and in the same year, it was chemically synthesized by Butenandt in Göttingen (Germany) and Ruzicka in Basel (Switzerland)⁷. In 1940s testosterone was supposedly used by Germans in war contexts^{4,6}, and it was prescribed in psychiatry for depression and andropause until the introduction of new treatments in 1950s⁶.

Since then, several synthetic analogs of testosterone, known as Anabolic Androgenic Steroids (AAS) or designer steroids (e.g. methyltestosterone in 1935, nandrolone in 1950 and 1953, testosterone esters from mid-1950s, stanozolol and oxymetholone in 1959, oxandrolone in 1962) have been produced in the attempt to minimize the androgenic effects and improve the anabolic ones^{1,2,7-9}. However, a successful complete separation of the anabolic and the androgenic properties has never been realized in the synthetic derivatives of testosterone^{1,2,10,11}. Among those, nandrolone was the first compound which showed a partial dissociation between anabolic and androgenic properties and therefore, it started to be used in the medical practice^{2,12}.

After the Second World War, the use of AAS for performance improvement started to be widely spread in the sports circles and progressively turned into a business since almost all sports showed an economic importance strictly linked to the image of the power of the countries⁹.

The introduction of AAS in medical practice induced elite athletes to use them “off-label” with the aim of increasing muscle mass and enhancing sports performance⁶, but with a dosage usually much higher than the therapeutic one, with consequent serious health risks^{2,9,13,14}.

In the 1950s, the use of AAS by Soviet and USA weightlifters was reported^{2,4}. During the 1960s, the Council of Europe introduced the concept of doping in sports after the deaths of a couple of competitive athletes and the spread of harmful drugs use^{2,15}.

The International Olympic Committee (IOC) started doping control tests in the Olympics of 1968 and subsequently AAS were introduced in the list of prohibited substances in 1976^{4,6,10}.

In the 1970s competitive athletes and body builders were largely using AAS^{4,6} and in 1988

the sprinter Ben Johnson was prosecuted for being found positive for anabolic steroid stanozolol at Seoul Olympics and his gold medal was withdrawn by IOC⁴. In the 1980s the exclusive use confined to elite sport up to then dangerously shifted to amateur athletes and even to non-athletes (mostly adolescents and young men) and recreational drug users^{1,4,6,8,9}.

Since 1991, in the USA, AAS have been DEA (Drug Enforcement Administration) Schedule III substances under Controlled Substances Act^{2,4,6,13,16}.

The focus on AAS misuse in sports increasingly raised to the point that today the antidoping authorities monitor and control the phenomenon both on elite and recreational athletes^{16,17}.

AAS use prevalence in the general population worldwide is 6.4% for males and 1.6% for females^{18,19}. Abuse and misuse of AAS to boost muscles and sculpt the body also involves adolescents and young adults affected by body image disturbance or eating disorders, who use them for cosmetic purposes and showing increased masculinity^{2,6,18,20}.

AAS are illegally (meaning without a medical prescription) sold through the black market, websites, gyms, body building competitions, teammates, coaches, trainers and by inappropriate prescription or theft^{1,13,16}. Street names for illegally sold anabolic steroids include Gear, Winny, Deca, EQ, Tren-A, Fina, Arnolds, Juice, Pumpers, Roids, Stackers, Weight Gainers^{13,14,21}.

Overall AAS include testosterone, its synthetic derivatives, and precursors also known as prohormones (weaker formulations of AAS) like dehydroepiandrosterone (DHEA), dihydrotestosterone (DHT), 1-testosterone, 19-norandrostenedione, prostazonol and androstenedione, which are usually present in dietary supplements^{2,3,21}. Their administration can be oral, injectable (oil-based and water-based) and transdermal (testosterone cream)²⁻⁴, buccal and sublingual (i.e. tetrahydrogestrinone)².

Several side effects related to AAS abuse involve mostly cardiovascular, reproductive, central nervous, and musculoskeletal systems, skin and liver². The most relevant one is undoubtedly hepatotoxicity since it has been showed that primarily the oral forms of AAS may damage liver function.

This article reviews the mechanism of action and potential hepatotoxicity of AAS abuse and misuse as a doping offense.

AAS Mechanism of Action

Synthetic AAS are compounds derived from the natural testosterone from which they differentiate because of several modifications of the basic testosterone structure². Endogenous androgens as testosterone have both skeletal muscle-building (anabolic) and masculinizing (androgenic) effects, while, as above reported, most of the synthetic AAS have been developed with the aim to separate the anabolic from the androgenic properties, preferring synthetic androgens that have preferential anabolic activity and no androgenic one. Testosterone acts as an androgen either directly by binding to the androgen receptor or indirectly by conversion to 5alpha-dihydrotestosterone (DHT). The structural modification of AAS alters the relative anabolic or androgenic activity, the binding affinity for the androgen receptor and metabolic clearance⁴.

Testosterone and AAS pass into the blood system, through the target cell membrane, linking with intra-cytoplasmic receptors. This complex hormone-receptor is later transported into the nucleus of the cell, where it links up with DNA. It results in the production of RNA, DNA and the subsequent enhancement of protein synthesis (including increased amounts of actin and myosin in skeletal muscles)^{2,9}.

The main chemical substitutions occurring to testosterone are the 17-beta-esterification and the 17alpha-alkylation. Whereas testosterone is metabolized rapidly in the body, the esterification of the 17-beta-hydroxyl group makes the molecule more hydrophobic. When these esters of testosterone (i.e. testosterone enanthate, cypionate, and decanoate) are administered in an oily suspension, they are released very slowly into the aqueous plasma because of their hydrophobicity. This extends their duration of action⁴.

Indeed, an important metabolic pathway of testosterone and its synthetic derivative is oxidation of the 17-beta-hydroxy group with the formation of the 17-keto metabolites. These polar metabolites are biologically inactive. The 17-alpha-alkylation induces the inhibition of metabolic deactivation by oxidation of the 17-beta hydroxy group in the liver, thus 17alpha-alkylated androgens can be effectively administered orally. Often the alkylation of the C-17 position of testosterone alters the relative anabolic potency about the masculinizing effects (androgenic steroids).

The mostly used oral anabolic steroids are ethylestrenol, fluoxymesterone, mesterolone, methandienone, methenolone, methandrostenolone,

methyltestosterone, methenolone acetate, mibolone, norethandrolone, oxandrolone, oxymetholone, stanozolol^{3,4,22}. The oral forms are resistant to immediate degradation and hepatotoxic^{3,4,8}, but their parenteral administration also leads to hepatic dysfunction⁸.

The injectable AAS are boldenone undecylate, clostebol, drostanolone propionate, methenolone enanthate, nandrolone decanoate, nandrolone phenpropionate, nandrolone undecanoate, testosterone cypionate, testosterone esters blends, testosterone enanthate, testosterone propionate, trenbolone, trenbolone acetate, trenbolone hexahydrobencylcarbonate, testosterone undecanoate, stanozolol^{2,22}. The injectable agents have undergone esterification of the 17-beta-hydroxy group to make them more soluble in lipids, leading to a slower release of the steroid into circulation³.

Hepatotoxicity of AAS

A relevant concern raised by the chronic administration of AAS is the toxic effect on liver⁹.

Since the 1950s, case reports about anabolic steroids occasional effects on the liver such as cholestasis, liver tumors, and peliosis hepatis have been observed and reported in the literature^{8,23}. Nevertheless, the prevalence of toxic effects following AAS administration could and cannot be defined due to underreporting and lack of specific studies²⁴.

However, it seems that in some cases AAS-induced hepatotoxicity might be overestimated because rhabdomyolysis caused by heavy workouts in athletes can increase transaminases, and this occurrence may be erroneously interpreted as abnormal liver function⁴.

Studies to evaluate hepatotoxicity, especially on a long time, on AAS abusers are difficult, usually for the unknown administered doses that are far beyond those used for therapeutic purposes^{2,17}. Moreover, in the case of using illicit samples of AAS, their real composition, and type of production or their concentrations are often not declared on the label, and the users do not know exactly the quality and the quantity of the substances they are assuming².

There are also ethical limitations to conducting a study since excessive and highly variable dosages cannot be proposed in clinical trials^{4,17}. The available scientific information mostly comes from AAS users population surveys or retrospective studies^{18,25-27}, cross-sectional surveys²⁸⁻³¹ and case reports³²⁻³⁶ as well as animal studies^{4,37,38}.

Hepatotoxicity of AAS may be correlated to individual susceptibility and genetic factors, and it is associated with an increase in infiltration of lymphocytes, neutrophils and eosinophils in liver tissue, subsequently to repeated exposure²⁴. Oxidative stress could represent a causal factor of liver toxicity due to AAS abuse^{19,24,39}. It has also been hypothesized that androgen receptor activation in hepatic cells may cause an increase in reactive oxygen species which leads to mitochondrial degeneration of hepatic cells, ultimately advancing to the clinical signs of hepatotoxicity observed with the intake of 17 α -alkylated steroids. In fact, some antioxidants have been found to exhibit a hepatoprotective effect against AAS-induced hepatotoxicity. Due to the limited data, the effects of antioxidants in AAS-induced hepatotoxicity need further research³⁹.

17-Alpha-Alkylated Steroids and Liver Damage

The 17 α -alkylated (17 α) steroids like methyltestosterone, methandrostenolone, oxymetholone, danazol, fluoxymesterone, stanozolol, norethandrolone, oxandrolone, dehydrochloromethyl-testosterone, formebolone, methandienone, testosterone (cypionate, enanthate, propionate)^{5,19}, can be medically used for increasing weight and muscle growth in catabolic states, for treatment of aplastic anemia and bone marrow failure, as well as off-label for doping purposes. All these types of steroids have been associated with cases of liver damage such as cholestatic jaundice, peliosis hepatis, nodular regeneration, hepatic adenoma and hepatocellular carcinoma^{1,5,17}. Oxymetholone and methyltestosterone, instead, have frequently been linked to hepatocellular carcinoma¹. Stanozolol, either following intramuscular or oral administration, causes liver damage, in particular, inflammatory or degenerative lesions in centrilobular hepatocytes, ultrastructural alterations in the canaliculi and degenerative changes in mitochondrial and lysosomes^{17,24}.

On the other side, cholestasis induced by esterified testosterone and 19-nortestosterone derivatives is quite rare, although their prolonged use may increase the risk of hepatic tumors and nodular transformation, seemingly at a lower rate than the 17-alkylated testosterones^{5,8}.

The androgens act on intracellular androgenic steroid receptors, which are translocated to the nucleus and link androgen response elements on DNA, inducing androgen-stimulated genes in-

involved in cell growth and development. An unregulated growth stimulus to hepatocytes is the likely cause of nodular regeneration and hepatic tumors related to anabolic steroid use⁵.

Other hepatic harms associated with AAS abuse include liver alterations such as subcellular changes of hepatocytes, hepatocellular hyperplasia, and general hepatic damage determined by increased liver enzymes: alkaline phosphatase, lactate dehydrogenase (LDH), aspartate aminotransferase (AST), alanine aminotransferase (ALT), gammaglutamyltransferase (GGT) and conjugated bilirubin. Some growths, initially believed to be hepatocellular carcinomas, were benign hyperplastic lesions, which regressed with the discontinuation of AAS use^{22,40}.

A 2011 research⁴¹ conducted in Brazil on 180 non-competitive bodybuilders, 95 AAS users and 85 non-users, asymptomatic of any liver alteration, suggested that anabolic steroids could induce toxicant-associated fatty liver disease (TAFLD) development in 12.6% of the 95 AAS users. Another 2015 study⁴² on 182 asymptomatic recreational AAS using bodybuilders, showed that they were affected by liver injuries including hepatotoxicity, fatty liver, and liver neoplasm.

Dietary Supplements Induced-Liver Injury

Dietary supplements induced- liver injury (DILI) (i.e. hepatocellular hepatitis, spontaneous hepatic rupture, cholestatic hepatitis, hepatocellular adenoma and carcinoma, peliosis hepatis and focal nodular hyperplasia) has been associated with products containing AAS such as androstenedione, dehydroepiandrosterone, desoxymethyltestosterone, stanozolol, methasterone, methylepithiostanol⁴².

Herbal and dietary supplements related liver toxicity range from 2% to 16% of all cases of hepatotoxicity identified in Western countries Registries. It is worth to note that in the Spanish DILI Registry the cases of hepatotoxicity due to AAS has recently increased: from 5 cases in 1994-2009 to 15 cases in 2010-2013 (from 1% to 8% of the total cases) with a mean age of 32 years^{42,43}. Hepatocellular damage was the most frequent liver injury followed by cholestatic injury⁴³. Dietary supplements containing methylepithiostanol, known to induce hepatotoxicity, were seized in Spain and withdrawn in 2013⁴³. The exact mechanism of toxicity is unclear, but the genotyping of patients with cholestatic hepatitis due to AAS suggests that these substances could

inhibit biliary transporter proteins such as ATP8B1/ABCB11, similarly to cases of benign intrahepatic cholestasis type 1 or 2⁴². In the USA, a total of 10 AAS DILI cases involved young men who took these products unsupervised. The principal drug responsible for liver injury was methasterone⁴³.

The oral designer steroid methasterone, also known as methylrostanolone, is an example of the hepatotoxicity associated with the consumption of 17-alpha alkylated steroids. This steroid was sold in the USA as a dietary supplement until 2012 and is a methylated (17-alpha alkylated) version of the injectable steroid drostanolone⁴⁴.

Many designer AAS remain available in over-the-counter dietary supplements that are legally sold in countries, where specific compounds have been prohibited. Currently, unscheduled designer steroids include: dimethazine (17aa oral androgen with high potential for hepatotoxicity, studied in humans in Italy for its anabolic effects, and detected in dietary supplements); methylepithiostanol (17aa oral androgen with high potential for hepatotoxicity, detected in dietary supplements); methoxygonadiene (progestin related to levonorgestrel, effects of oral consumption unknown); methylclostebol (17aa oral androgen with high potential for hepatotoxicity, detected in dietary supplements); methylstenbolone (17aa oral androgen with high potential for hepatotoxicity, detected in dietary supplements); metobolan/trestione (closely related to MENT/trestolone, strong androgen)⁴⁴.

Cholestasis

Cholestasis due to the C-17 substituted androgens is not well established, but high doses of those compounds cause a similar cholestasis in some animal models, and may be due to reduced bile salt transporter proteins and disruption of intrahepatic microfilaments^{5,24,40}.

The development of cholestatic jaundice is predictable and related to dose and treatment duration: it may occur in a low percentage of patients assuming 17aa steroids like methyltestosterone, danazol, methandrostenolone, stanozolol or oxymetholone^{2,5,8}. Cholestatic jaundice is usually transient and can be completely healed upon discontinuation of AAS⁸. Bland cholestasis due to anabolic steroids use may occur in patients, who do not report anabolic steroids consumption or who are taking herbal or a dietary supplement, containing anabolic steroids not declared on label, as a mean to increase muscle strength⁵.

Liver Peliosis

Prolonged use of oral anabolic steroids has been linked to vascular changes in the liver referred to as peliosis hepatis. Peliosis hepatis is a rare syndrome, firstly noted in 1952, in which blood-filled enlarged sinusoids and cysts form in the liver^{5,24,40}. These lesions can be asymptomatic⁴⁰. The liver may be enlarged, deep red in color and fragile. Peliosis hepatis most typically occurs in patients with advanced wasting diseases, but it has also been associated with prolonged use of anabolic steroids for aplastic anemia and hypogonadism treatment as well as for enhancing performance and muscles in body building⁵.

Case reports⁴⁰ usually show mortality from internal hemorrhage or hepatic failure secondary to blood-filled cysts. Peliosis associated with anabolic steroids may or may not fully revert by stopping these drugs⁵. The mechanism by which peliosis affects steroids users is not known yet since it has been observed either on 17 α steroids or testosterone (which is not 17-alpha-alkylated) users⁸.

Hepatic Tumors

Human liver expresses estrogen and androgen receptors and experimentally both androgens and estrogens have been implicated in stimulating hepatocyte proliferation, probably causing liver tumor⁴⁵. Patients treated with anabolic androgenic steroids are all at risk of developing liver tumors⁴⁶.

The most serious complication of anabolic steroid use is the development of hepatic adenoma (benign liver neoplasm) or hepatocellular carcinoma (HCC) (first reported in the 1950s)⁸. Adenomas rarely turn into malignant tumors, but present risks of sudden rupture and bleeding, leading to hemoperitoneum, a life-threatening complication⁴⁰. Usually, a spontaneous regression of this tumor may occur, when the anabolic steroids are stopped or after early detection^{5,8,40}.

The hepatic tumors arise in patients on long-term androgenic steroids therapy, usually for 2 to 4 years or over⁸, and occasionally in athletes or body builders illicitly abusing anabolic steroids⁵. For patients with pre-existing pathologies (e. g. hepatitis B or C, Fanconi's anemia) the liver tumor appearance can be shorter^{8,46}. Both parenteral and oral androgens may induce hepatic neoplasms⁴⁶. The type of neoplasm can be associated with the type of androgen. Among the oral 17 alpha-alkylated steroids associated to liver tumors in patients

(n=36) treated for Fanconi's anemia (FA), there are oxymetholone, methyltestosterone, norethandrolone, methandienone, oxandrolone; among the oral 1-methyl type there is methenolone; and among the parenteral 17-beta-ester type there are nandrolone decanoate, testosterone cypionate, enanthate, and propionate⁴⁶. HCC in FA patients were 58%, and adenomas were 36%. Among the patients (n = 97) with liver tumors, who received androgens for other pathologies (non-FA aplastic anemia and non-aplastic anemia disorders), there are the following 17 alpha-alkylated steroids: oxymetholone, methyltestosterone, danazol, methandrostenolone, norethandrolone, fluoxymesterone, stanozolol, ethylestrenol, methandienone, oxandrolone; among the oral 1-methyl type there are methenolone and mesterolone; among the parenteral 17-beta-ester type there are nandrolone decanoate, nandrolone phenpropionate, testosterone enanthate, propionate, and not-specified⁴⁶. In non-FA aplastic anemia group HCC were 60% and adenomas 30%; in non-aplastic anemia group, HCC were 44% and adenomas were 42%. Oxymetholone was the most used in FA and non-FA groups; methyltestosterone and danazol were common in non-FA individuals⁴⁶.

Case Reports of AAS Hepatotoxicity

As regards to the use of anabolic steroids in sports, especially in male bodybuilders, and subsequent development of hepatocellular carcinoma or adenomas, several case reports have been recently reported (Table I).

A 35-year-old bodybuilder, completely asymptomatic, after taking high doses of AAS (oral stanozolol and oxymetholone; parenteral nandrolone decanoate, testosterone enanthate and methenolone enanthate) for more than 15 years, developed hepatic adenomas secondary to AAS abuse. Withdrawal of anabolic steroids, after 4 years, promoted the slight reduction of the size of the tumors and liver function improvement. Nevertheless, the individual has been included in a liver transplantation program because of the severe hepatomegaly and of the risk of lesions in the liver to turn into malignant³².

Another case involved a bodybuilder, 23 years old, who self-administered high doses of oral stanozolol and oxymetholone, parenteral nandrolone decanoate, testosterone phenylpropionate and boldenone. After 6 months upon AAS and 1 month of diuretics and restrictive diet, the patient started to show symptoms such as asthenia and

Table I. Hepatotoxicity due to AAS: case reports

Sport	Gender and age (years)	AAS	Duration of intake	Pathol. findings	Reference
Bodybuilder	Male, 35	Stanozolol Oxymetholone Nandrolone decanoate Testosterone enanthate Methenolone enanthate	15 years	Hepatomegaly Hepatic adenomas	Socas et al ³²
Bodybuilder	Male, 23	Stanozolol Oxymetholone Nandrolone decanoate Testosterone phenylpropionate Boldenone	6 months	Hepatomegaly Hepatic adenomas	Socas et al ³²
Bodybuilder	Male, 28	Androstenedione Nandrolone	6 years	Hepatic adenoma	Pais-Costa et al ⁴⁷
Bodybuilder	Male, 37	Testosterone propionate Testosterone phenylpropionate Testosterone isocaproate Testosterone decanoate Trenbolone acetate 5-alpha-androstanediol Boldenone Methandriol dipropionate 17alpha-methyl-5alpha-androstano [3,2-c]pyrazole-17beta-ol 17beta-hydroxy-17alpha-methyl- 2-oxa-5alpha-androstane-3-one Oxymetholone Methandienone Spironolactone Mesterolone Fluoxymesterone	5 years	Hepatocellular carcinoma	Hardt et al ⁴⁸
Bodybuilder	Male, 24	Testosterone	16 years	Hepatocellular carcinoma	Kesler et al ³⁵
Bodybuilder	Male, 29	Nandrolone decanoate Testosterone propionate Testosterone phenylpropionate Testosterone isocaproate Testosterone decanoate	6 years	Hepatomegaly Hepatocellular carcinoma	Solbach et al ³⁶
	Male, 25	2alpha-17alpha-dimethyl- etiocholan-3-one,17beta-ol	3 weeks	Cholestatic hepatitis	El Sherrif et al ⁴⁹
	Male, 45	2alpha-17alpha-dimethyl- etiocholan-3-one,17beta-ol	9 days	Cholestatic hepatitis	El Sherrif et al ⁴⁹
	Male, 18	2 alpha,17alpha-dimethyl-17beta- hydroxy-5 alpha-androstan-3-one	2 weeks	Cholestatic hepatitis	Awai et al ⁵⁰

anorexia; analysis reported hypernatraemia, renal failure and muscular damage. A mild hepatomegaly was detected as well as adenomas without any malignancy that after 1 year showed a decrease in the size and analytical values close to normal upon AAS cessation³².

A giant hepatic adenoma was detected in a bodybuilder, 28 years old, who reported the consumption of oral androstenedione and intramuscular nandrolone over 6 years. The risk for large adenoma is malignant transformation, hemorrhage

and sometimes death. After surgical treatment and stopping the intake of AAS, 6 months later there were no signs of further focal lesions⁴⁷.

Chronic intake of AAS and development of HCC was observed on a 37 years old bodybuilder who reported a huge consumption of AAS for 5 years: testosterone propionate, phenylpropionate, isocaproate and decanoate, trenbolone acetate, 5-alpha-androstanediol, boldenone and methandriol dipropionate, 17alpha-methyl-5alpha-androstano[3,2-c]pyrazole-

17beta-ol, 17beta-hydroxy-17alpha-methyl-2-oxa-5alpha-androstane-3-one and oxymetholone or methandienone. Furthermore, he used spironolactone, mesterolone, fluoxymesterone, and torasemide (diuretics) eighteen hours before competition. A daily intake of other substances such as amino acid, vitamins, and mineral tablets, thyroxine, and growth hormones was also reported. It has to be noticed that this case regards a polydrug use pattern and the precise mechanisms of HCC development remain unclear⁴⁸.

Another case of HCC secondary to AAS was observed in 2004 on a young bodybuilder 24 years old that started testosterone abuse (200 mg/week) since age 17. At that time a hepatic adenomatosis was revealed. The individual persisted in AAS consumption in 2013 developed chronic kidney disease, coronary artery disease and was diagnosed with HCC³⁵.

A 29 years old male professional body builder presented severe hepatomegaly with signs of HCC after 6 years of AAS abuse including nandrolone decanoate, 4 ester based testosterones (testosterone propionate, testosterone phenylpropionate, testosterone isocaproate, testosterone decanoate), methandienone, stanozolol. Other substances assumed by the sportsman were human growth hormone, insulin, tamoxifen and diuretics (aldosterone and thiazide). The case required liver transplantation³⁶.

Hepatotoxicity due to dietary supplements ingestion containing AAS was detected in 2 males, 25 and 45 years old respectively. The dietary supplement contained the AAS 2alpha-17alpha-dimethyl-etiocolan-3-one, 17beta-ol. It is possible that the AAS could have inhibited expression of the biliary transporter proteins ATPB81/ABCB11 and therefore induced cholestatic hepatitis⁴⁹.

Adolescents with pre-existing liver disease, such as non-alcoholic fatty liver disease (NAFLD), are more easily at risk of hepatotoxicity due to AAS intake. This was showed by a case report concerning a young man 18 years old who developed cholestatic hepatitis, with hepatocellular and intracanalicular cholestasis, following to AAS 2 alpha, 17alpha-dimethyl-17beta-hydroxy-5 alpha-androstan-3-one consumption for 2 weeks preceding the onset of symptoms⁵⁰.

AAS Misuses Potentially Responsible for Hepatotoxicity

The following modalities of consumption are probably or consistently related to hepatotoxicity

since the way in which AAS are used, misused or abused can compromise liver function.

Polydrug Use

Athletes (usually body builders and weightlifters) are inclined to polydrug use for reducing side effects and boosting AAS effects^{2,51}. The polydrug or polypharmacy behavior, such as the concomitant consumption of different licit and illicit substances^{2,6,9,29,31,51} may contribute to the toxicity of AAS⁵¹, making hard to ascertain a causal relationship between a specific substance and its consequent adverse effects¹⁹.

AAS Dependence

AAS use is often related to misuse of prescription drugs (such as sedatives or analgesics) and current or past dependence on illicit substances like cocaine, heroin, and opioids^{3,4,6,18,25,27,37,52-54}. Individuals using high doses of anabolic steroids for a long time may develop depressive symptoms, anhedonia, fatigue, impaired concentration, and even suicidality due to steroid withdrawal. It has been noted that these withdrawal effects may contribute to a syndrome of dependence²². A role of reinforcement of compulsive exercise in AAS dependence has also been suggested¹⁸.

AAS Pattern of Use

Doses of AAS that exceed 10 to 100 times the physiological limits or doses usually prescribed for pathologies and used with high frequency may cause a variety of clinical effects, which often are not taken into the right consideration, even if they can seriously endanger life. To hypothetically minimize side effects or maximize the drug effects, AAS abusers follow different patterns of use: pyramiding (gradually increasing the doses and then a tapering phase), cycling (cycles of several weeks and periods of abstinence), stacking (users combine different injectable and oral AAS)^{2,14,37}, blast and cruise or bridging (never stop AAS but alternate periods of high doses and lower doses) and blitz-cycles (quick change of AAS to prevent tolerance and androgen receptor down-regulation)⁵¹. It is not scientifically proven that these patterns of use are helpful to avoid severe adverse effects of these substances^{2, 14,37}.

AAS as Image Performance Enhancing Drugs

AAS are reported in the literature also for being included as a subset of the numerous group of substances, licit or illicit, such as GH

(Growth Hormone), insulin, erythropoiesis-stimulating agents, stimulants, cutting drugs (synthetic thyroid hormones), ancillary agents (sildenafil, tamoxifen, human chorionic gonadotropin etc.) supplements (e.g. creatine, protein powder, L-carnitine), diuretics, recreational drugs and analgesics^{4,18,51}. All these substances are variously reported under similar names considering either the scopes of boosting muscles and performance or the cosmetic finalities⁵⁵: PED (Performance Enhancing Drugs)^{4,56}, PEA (Performance-Enhancing Agents)³¹ or PES (Performance Enhancing Substances)⁵⁷, IPED (Image and Performance-Enhancing Drugs)⁵¹, APED (Appearance and Performance Enhancing Drug)²¹ or PAED¹⁹, Body Image Drugs⁵⁸. Most of AAS and IPED are easily available on thousands of web sites where are marketed without any warning about the eventual harmful effects but, on the contrary, favorably supporting them⁵⁶. Legal IPED such as dietary supplements are frequently contaminated by AAS^{51,59}. In particular, it has been reported that dietary supplements are daily used by 33% of the adults and are contaminated with anabolic steroids in the 12.5% of the samples²¹. This poses severe health risks to the unaware consumer.

Further Complications of AAS

Prolonged use of AAS may cause several complications. Genitourinary and reproductive in women: menstrual irregularities, uterine atrophy, clitoral hypertrophy, teratogenicity, hirsutism breast atrophy, infertility, deepening of voice. In men: testicular atrophy, hair loss, prostatic hypertrophy, prostatic carcinoma, decreased reproductive hormones, impotence, gynecomastia, anabolic steroid induced hypogonadism, renal carcinoma. Metabolic: glucose intolerance, increased LDL cholesterol, decreased HDL cholesterol. Cardiovascular: stroke, myocarditis, thrombosis, atherogenesis, hypertension, left ventricular hypertrophy, arrhythmic episodes, polycythemia, heart failure. Skeletal-muscular: premature growth cessation, tendon damage, ligament injuries, rhabdomyolysis, disc herniation. Neurological: cephalgia, drowsiness, loss of concentration. Psychiatric and behavioral: irritability, aggressiveness, depression, maniac behavior, paranoid psychoses, suicide thoughts, AAS and multiple drug dependence. On the skin: acne, allergic cutaneous eruption, sebaceous cysts, alopecia, profuse sweating^{1,2,9,19}.

Conclusions

AAS and polydrug abuse can be harmful to health, causing a variety of side effects including mainly liver injuries, which in some cases can be life threatening. Nowadays also dietary supplements containing designer steroids are a serious health risk for the consumers, who easily buy these products on Internet, without any control. AAS has been detected in vitamin products suggesting that many products may be contaminated with a variety of unlabeled ingredients⁴⁴. World Anti-Doping Agency (WADA) established in 1999 as an international independent agency composed and funded equally by the sport movement and governments of the world, prohibits any AAS use or related compound. DEA constantly include substances into controlled substances list; nevertheless, new compounds frequently appear in the marketplace. The availability of designer steroids over-the-counter for more than 10 years may have promoted significant exposure in the population. Individuals should be warned about the use of either AAS or dietary supplements potentially containing designer steroids, because of the adverse effects and theoretical effects of exogenous synthetic androgens⁴⁴.

Authors Contributions

R.S., M.C.R., L.M., C.M., A.M. and I.P. made substantial contributions to conception and design of the manuscript; R.S. and I.P. performed the literature search. All the authors have been involved in drafting the manuscript and revising it critically for important intellectual content and all of them have given final approval to the version to be published.

Acknowledgements

The authors thank Simonetta di Carlo, Antonella Bacosi, Dr. Adele Minutillo for technical assistance in the manuscript preparation.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

References

- 1) MARAVELIAS C, DONA A, STEFANIDOU M, SPILIOPOULOU C. Adverse effects of anabolic steroids in athletes. A constant threat. *Toxicol Lett* 2005; 158: 167-175.
- 2) BARCELOUX DG, PALMER RB. Anabolic-androgenic steroids. *Dis Mon* 2013; 59: 226-248.

- 3) LIDDLE DG, CONNOR DJ. Nutritional supplements and ergogenic aids. *Prim Care* 2013; 40: 487-505.
- 4) POPE HG JR, WOOD RI, ROGOL A, NYBERG F, BOWERS L, BHASIN S. Adverse health consequences of performance-enhancing drugs: an endocrine society scientific statement. *Endocr Rev* 2014; 35: 341-375.
- 5) Liver disease research branch of the division of digestive diseases and nutrition of the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK). Anabolic steroids. *LiverTox*, United States National Library of Medicine, 2016.
- 6) KANAYAMA G, HUDSON JI, POPE HG JR. Illicit anabolic-androgenic steroid use. *Horm Behav* 2010; 58: 111-121.
- 7) NIESCHLAG E, NIESCHLAG S. Testosterone deficiency: a historical perspective. *Asian J Androl* 2014; 16: 161-168.
- 8) SHAHIDI NT. A review of the chemistry, biological action, and clinical applications of anabolic-androgenic steroids. *Clin Ther* 2001; 23: 1355-1390.
- 9) QUAGLIO G, FORNASIERO A, MEZZELANI P, MORESCHINI S, LUGOBONI F, LECHI A. Anabolic steroids: dependence and complications of chronic use. *Intern Emerg Med* 2009; 4: 289-296.
- 10) HARTGENS F, KUIPERS H. Effects of androgenic-anabolic steroids in athletes. *Sports Med* 2004; 34: 513-554.
- 11) FRATI P, BUSARDÒ FP, CIPOLLONI L, DOMINICIS ED, FINESCHI V. Anabolic Androgenic Steroid (AAS) related deaths: autptic, histopathological and toxicological findings. *Curr Neuropharmacol* 2015; 13: 146-159.
- 12) BUSARDÒ FP, FRATI P, SANZO MD, NAPOLETANO S, PINCHI E, ZAAMI S, FINESCHI V. The impact of nandrolone decanoate on the central nervous system. *Curr Neuropharmacol* 2015; 13: 122-131.
- 13) DRUG ENFORCEMENT ADMINISTRATION (DEA). Steroids. In: DEA. *Drugs of abuse*. Drug enforcement administration, United States department of justice, 2015.
- 14) NATIONAL INSTITUTE ON DRUG ABUSE (NIDA). *Drug facts: anabolic steroids*. NIDA, 2016.
- 15) EUROPEAN COMMISSION. *Study on Doping Prevention*. Publications office of the European Union, 2014.
- 16) BARROSO O, MAZZONI I, RABIN O. Hormone abuse in sports: the antidoping perspective. *Asian J Androl* 2008; 10: 391-402.
- 17) BÜTTNER A, THIEME D. Side effects of anabolic androgenic steroids: pathological findings and structure-activity relationships. *Handb Exp Pharmacol* 2010; 195: 459-484.
- 18) HILDEBRANDT T, SHOPE S, VARANGIS E, KLEIN D, PFAFF DW, YEHUDA R. Exercise reinforcement, stress, and β -endorphins: an initial examination of exercise in anabolic-androgenic steroid dependence. *Drug Alcohol Depend* 2014; 139: 86-92.
- 19) NIESCHLAG E, VORONA E. Doping with anabolic androgenic steroids (AAS): adverse effects on non-reproductive organs and functions. *Rev Endocr Metab Disord* 2015; 16: 199-211.
- 20) FIELD AE, SONNEVILLE KR, CROSBY RD, SWANSON SA, EDDY KT, CAMARGO CA JR, HORTON NJ, MICALI N. Prospective associations of concerns about physique and the development of obesity, binge drinking, and drug use among adolescent boys and young adult men. *JAMA Pediatr* 2014; 168: 34-39.
- 21) HILDEBRANDT T, LAI JK, LANGENBUCHER JW, SCHNEIDER M, YEHUDA R, PFAFF DW. The diagnostic dilemma of pathological appearance and performance enhancing drug use. *Drug Alcohol Depend* 2011; 114: 1-11.
- 22) HALL RC, HALL RC. Abuse of supraphysiologic doses of anabolic steroids. *South Med J* 2005; 98: 550-555.
- 23) SCHEUER PJ. Long-term effects on the liver. *J Clin Pathol Suppl (R Coll Pathol)* 1975; 9: 71-74.
- 24) NERI M, BELLO S, BONSIGNORE A, CANTATORE S, RIEZZO I, TURILLAZZI E, FINESCHI V. Anabolic androgenic steroids abuse and liver toxicity. *Mini Rev Med Chem* 2011; 11: 430-437.
- 25) LINDQVIST AS, MOBERG T, ERIKSSON BO, EHRNBORG C, ROSÉN T, FAHLKE C. A retrospective 30-year follow-up study of former Swedish-elite male athletes in power sports with a past anabolic androgenic steroids use: a focus on mental health. *Br J Sports Med* 2013; 47: 965-969.
- 26) HAKANSSON A, MICKELSSON K, WALLIN C, BERGLUND M. Anabolic androgenic steroids in the general population: user characteristics and associations with substance use. *Eur Addict Res* 2012; 18: 83-90.
- 27) CORNFORD CS, KEAN J, NASH A. Anabolic-androgenic steroids and heroin use: a qualitative study exploring the connection. *Int J Drug Policy* 2014; 25: 928-930.
- 28) KOKKEVI A, FOTIOU A, CHILEVA A, NOCIAR A, MILLER P. Daily exercise and anabolic steroids in adolescents: a cross-sectional European study. *Subst Use Misuse* 2008; 43: 2053-2065.
- 29) IP EJ, LU DH, BARNETT MJ, TENEROWICZ MJ, VO JC, PERRY PJ. Psychological and physical impact of anabolic-androgenic steroid dependence. *Pharmacotherapy* 2012; 32: 910-919.
- 30) NAKHAEI MR, PAKRAVAN F, NAKHAEI N. Prevalence of use of anabolic steroids by bodybuilders using three methods in a city of Iran. *Addict Health* 2013; 5: 77-82.
- 31) IP EJ, TRINH K, TENEROWICZ MJ, PAL J, LINDFELT TA, PERRY PJ. Characteristics and behaviors of older male anabolic steroid users. *J Pharm Pract* 2015; 28: 450-456.
- 32) SOCAS L, ZUMBADO M, PÉREZ-LUZARDO O, RAMOS A, PÉREZ C, HERNÁNDEZ JR, BOADA LD. Hepatocellular adenomas associated with anabolic androgenic steroid abuse in bodybuilders: a report of two cases and a review of the literature. *Br J Sports Med* 2005; 39: e27.

- 33) KANAYAMA G, BROWER KJ, WOOD RI, HUDSON JI, POPE HG JR. Anabolic-androgenic steroid dependence: an emerging disorder. *Addiction* 2009; 104: 1966-1978.
- 34) TRIPATHI A, TEKKALAKI B, SAXENA S, DANDU H. Iatrogenic dependence of anabolic-androgenic steroid in an Indian non-athletic woman. *BMJ Case Rep* 2014; 2014: bcr2013202472.
- 35) KESLER T, SANDHU RS, KRISHNAMOORTHY S. Hepatology: hepatocellular carcinoma in a young man secondary to androgenic anabolic steroid abuse. *J Gastroenterol Hepatol* 2014; 29: 1852.
- 36) SOLBACH P, POTTHOFF A, RAATSCHEN HJ, SOUDAH B, LEHMANN U, SCHNEIDER A, GEBEL MJ, MANN S, VOGEL A. Testosterone-receptor positive hepatocellular carcinoma in a 29-year old bodybuilder with a history of anabolic androgenic steroid abuse: a case report. *BMC Gastroenterol* 2015; 15: 60.
- 37) WOOD RI. Anabolic-androgenic steroid dependence? Insights from animals and humans. *Front Neuroendocrinol* 2008; 29: 490-506.
- 38) TOUSSON E, EL-MOGHAZY M, MASSOUD A, EL-ATRASH A, SWEEF O, AKEL A. Physiological and biochemical changes after boldenone injection in adult rabbits. *Toxicol Ind Health* 2016; 32: 177-182.
- 39) BOND P, LLEWELLYN W, VAN MOL P. Anabolic androgenic steroid-induced hepatotoxicity. *Med Hypotheses* 2016; 93: 150-153.
- 40) MODLINSKI R, FIELDS KB. The effect of anabolic steroids on the gastrointestinal system, kidneys, and adrenal glands. *Curr Sports Med Rep* 2006; 5: 104-109.
- 41) SCHWINGEL PA, COTRIM HP, SALLES BR, ALMEIDA CE, DOS SANTOS CR JR, NACHEF B, ANDRADE AR, ZOPPI CC. Anabolic-androgenic steroids: a possible new risk factor of toxicant-associated fatty liver disease. *Liver Int* 2011; 31: 348-353.
- 42) GARCÍA-CORTÉS M, ROBLES-DÍAZ M, ORTEGA-ALONSO A, MEDINA-CALIZ I, ANDRADE RJ. Hepatotoxicity by dietary supplements: a tabular listing and clinical characteristics. *Int J Mol Sci* 2016; 17: 537.
- 43) ROBLES-DÍAZ M, GONZALEZ-JIMENEZ A, MEDINA-CALIZ I, STEPHENS C, GARCÍA-CORTES M, GARCÍA-MUÑOZ B, ORTEGA-ALONSO A, BLANCO-REINA E, GONZALEZ-GRANDE R, JIMENEZ-PEREZ M, RENDÓN P, NAVARRO JM, GINES P, PRIETO M, GARCIA-ELIZ M, BESSONE F, BRAHM JR, PARANÁ R, LUCENA MI, ANDRADE RJ; SPANISH DILI REGISTRY; SLATINDILI NETWORK. Distinct phenotype of hepatotoxicity associated with illicit use of anabolic androgenic steroids. *Aliment Pharmacol Ther* 2015; 41: 116-125.
- 44) RAHNEMA CD, CROSNOE LE, KIM ED. Designer steroids over the counter supplements and their androgenic component: review of an increasing problem. *Andrology* 2015; 3: 150-155.
- 45) GIANNITRAPANI L, SORESI M, LA SPADA E, CERVELLO M, D'ALESSANDRO N, MONTALTO G. Sex hormones and risk of liver tumor. *Ann N Y Acad Sci* 2006; 1089: 228-236.
- 46) VELAZQUEZ I, ALTER BP. Androgens and liver tumors: Fanconi's anemia and non-Fanconi's conditions. *Am J Hematol* 2004; 77: 257-267.
- 47) PAIS-COSTA SR, LIMA OA, SOARES AF. Giant hepatic adenoma associated with anabolic-androgenic steroid abuse: case report. *Arq Bras Cir Dig* 2012; 25: 180-182.
- 48) HARDT A, STIPPEL D, ODENTHAL M, HÖLSCHER AH, DIENES HP, DREBBER U. Development of hepatocellular carcinoma associated with anabolic androgenic steroid abuse in a young bodybuilder: a case report. *Case Rep Pathol* 2012; 2012: 195607.
- 49) EL SHERRIF Y, POTTS JR, HOWARD MR, BARNARDO A, CAIRNS S, KNISELY AS, VERMA S. Hepatotoxicity from anabolic androgenic steroids marketed as dietary supplements: contribution from ATP8B1/ABC11 mutations? *Liver Int* 2013; 33: 1266-1270.
- 50) AWAI HI, YU EL, ELLIS LS, SCHWIMMER JB. Liver toxicity of anabolic androgenic steroid use in an adolescent with nonalcoholic fatty liver disease. *J Pediatr Gastroenterol Nutr* 2014; 59: e32-e33.
- 51) SAGOE D, MCVEIGH J, BJØRNEBEKK A, ESSILFIE MS, ANDREASSEN CS, PALLESEN S. Polypharmacy among anabolic-androgenic steroid users: a descriptive metasynthesis. *Subst Abuse Treat Prev Policy* 2015; 10: 12.
- 52) ARVARY D, POPE HG. Anabolic-androgenic steroids as a gateway to opioid dependence. *N Engl J Med* 2000; 342: 1532.
- 53) DODGE T, HOAGLAND MF. The use of anabolic androgenic steroids and polypharmacy: a review of the literature. *Drug Alcohol Depend* 2011; 114: 100-109.
- 54) GRAHAM MR, DAVIES B, GRACE FM, KICMAN A, BAKER JS. Anabolic steroid use: patterns of use and detection of doping. *Sports Med* 2008; 38: 505-525.
- 55) FRATI P, KYRIAKOU C, DEL RIO A, MARINELLI E, VERGALLO GM, ZAAMI S, BUSARDÒ FP. Smart drugs and synthetic androgens for cognitive and physical enhancement: revolving doors of cosmetic neurology. *Curr Neuropharmacol* 2015; 13: 5-11.
- 56) BRENNAN BP, KANAYAMA G, POPE HG JR. Performance-enhancing drugs on the web: a growing public-health issue. *Am J Addict* 2013; 22: 158-161.
- 57) BUCKMAN JF, YUSKO DA, WHITE HR, PANDINA RJ. Risk profile of male college athletes who use performance-enhancing substances. *J Stud Alcohol Drugs* 2009; 70: 919-923.
- 58) KANAYAMA G, HUDSON JI, POPE HG JR. Culture, psychosomatics and substance abuse: the example of body image drugs. *Psychother Psychosom* 2012; 81: 73-78.
- 59) VAN DER BIJL P. Dietary supplements containing prohibited substances: a review (Part 2). *South African journal of sports medicine* 2014; 26: 87-90.