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Anabolic-androgen steroids: A possible independent risk factor to Cardiovascular, Kidney and Metabolic Syndrome

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ABSTRACT

Millions of individuals make illicit use of anabolic-androgenic steroids (AAS), remaining a public health issue. It often leads to detrimental effects, including cardiovascular and renal diseases, besides hormonal and metabolic imbalances. The objective of this review is to emphasize the contribution of oxidative stress and inflammation to these effects and connect the findings of experimental animal studies with the alterations found in clinical contexts, in AAS users. The study's results showed that AAS promotes a redox disruption and a pro-inflammatory state on organs that are involved in important physiologic processes. These drugs increase inflammatory high-sensitivity C-reactive protein (hs-CRP) and cytokines that contribute to the progression of atherosclerosis, cardiovascular disease risk or endpoints, including stroke, myocardial infarction and death. In the kidney, the AAS increase proteinuria and structural damage. Studies have linked AAS abuse with high BP, low HDL-C levels, high triglyceride levels and impaired fasting blood glucose that characterize Metabolic syndrome. Overall, the studies indicate that oxidative stress, apoptosis, and AAS-mediated inflammation play a significant role in tissue damage, regardless of the dose and duration of exposure, and we point it as a putative independent risk factor to Cardiovascular, Kidney and Metabolic syndrome.

1. Introduction

Anabolic-Androgen Steroids (AAS) abuse remains a public health problem affecting millions of people around the world. The AAS have well-established clinical applications, being used in the treatment of medical conditions such as male hormone replacement therapy, hypogonadism and delayed puberty (Rogol, 2005), cachexia associated with chronic diseases, such as cancer and acquired immunodeficiency syndrome, some forms of anemia (Basaria et al., 2001), osteoporosis, hereditary angioedema, neuromuscular disorders, and Turner Syndrome (Akyurek and Dunn, 2006; Menke et al., 2010; Moore et al., 1976). In these situations, AAS are administered in carefully controlled doses, with the aim of restoring or improving compromised physiological functions (Baytugan and Kandemir, 2024). However, indiscriminate use and/or in high doses (abuse), much higher than those recommended clinically, is a growing problem, especially in non-medical contexts, such as for aesthetic purposes and enhancement of athletic performance, thereby promoting body image ideals (Bird et al., 2016; Bond et al., 2022). This use/abuse is associated with a wide range of adverse effects, creating a typical phenotype, including disproportionate muscle hypertrophy, gynecomastia, skin changes such as severe acne, stretch marks, and testicular atrophy in men (Rahnema et al., 2014). In women, it leads to signs of virilization, such as deepening of the voice and increased body hair (Bienenfeld et al., 2019). In both sexes, psychiatric and reproductive changes occur, which can seriously compromise the health of users (Butzke et al., 2022).

Although the AAS were greatly used by athletes and bodybuilders, after the 1980s they were widely used by non-competitive athletes and young to middle-aged men for personal appearance (Kanayama and Pope, 2018). In general, the estimated global lifetime prevalence rate of

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AAS use is 3.3 %, with rates of 6.4 % for males and 1.6 % for females. However, consumption varies extensively across different countries, with rates of up to 25 % in some cases (Bond et al., 2022; de Zeeuw et al., 2023; Hammoud et al., 2023; Sagoe et al., 2014). Therefore, establishing the accurate frequency of AAS use in the general population is challenging.

According to Kanayama and Pope (2018), the prevalence of AAS use/abuse varies widely depending on the population context. The abuse is particularly high among young men, who represent the primary demographic group. Furthermore, the study highlights that epidemiological data may be underestimated due to the stigma and illegality associated with AAS use, which makes it difficult to obtain accurate research results.

In this context, the authors reported significant geographical variations in AAS use. Developed countries, such as the United States, present a higher use rate due to the availability and well-established black market. In contrast, in some developing countries, limited access may restrict the use, although exceptions exist due to the lack of strict regulations in certain regions (Kanayama and Pope, 2018).

Furthermore, globalization and e-commerce have increased the availability of AAS, reducing some of the previously observed geographic differences. For example, an increase in the abuse of AAS has been observed in regions of Asia and the Middle East, where cultural and social practices related to body image may influence this trend. However, it is recognized that the abuse of AAS is a global phenomenon with variable prevalence rates influenced by cultural, social, and economic factors. Therefore, understanding these factors could help to effectively address the public health challenges associated with the abuse of AAS (for extensive review, see (Kanayama and Pope, 2018).

The simultaneous use of multiple types of anabolic steroids, commonly referred to as "stacking," is a widespread practice among users aiming to take advantage of the aesthetic and physical performance enhancing effects of these substances (McVeigh et al., 2012). These combined effects often outweigh the risks associated with isolated use, increasing the likelihood of serious and irreversible complications (Sagoe et al., 2015). With polypharmacy, there is also the possibility of chemical interactions between other medications and AAS that can manifest adverse effects that can lead to death of the individual (Pagonis et al., 2006). Thus, the main and combined effects of these substances' use should attract the attention of physicians, policy makers and public health authorities. Faced with specific needs, physicians may inadvertently administer medications to AAS users, triggering unintended adverse interactions that can be harmful. Therefore, it is important to gather a history of use of different substances commonly used by AAS users, as such information can guide the medical clinic in the diagnosis and prescription of "safe" medications during possible treatments (Kimergard et al., 2014; Sagoe et al., 2015).

The most used AAS are Nandrolone Decanoate (ND), Boldenone Undecanoate (BU), oxandrolone (OXA) and stanozolol (ST) and they were banned by sports federations (World Anti-Doping Agency, 2024). These drugs may present molecular deleterious effects, such as causing inflammatory effects and increasing oxidative stress, which can damage various tissues, as presented in this review.

As previously described in the literature, AAS comprises a broad category of synthetic compounds derived from testosterone (TT). Considering the double effect (anabolic and androgenic) of TT, over time, several structural adaptations have been made with the aim of maximizing its anabolic effects while minimizing its androgenic impact, namely C-17 esterification, demethylated group at C-19, C-17 esters and alkylation at C-17 (for review see (El-Desoky et al., 2016; Hartgens and Kuipers, 2004; Tauchen et al., 2021).

Similar to TT, AAS can directly interact in several tissues with specific plasmatic membrane and/or cytoplasm receptors, acting through non-genomic and genomic mechanisms. The non-genomic pathway is characterized by the binding to receptors coupled with G proteins, triggering faster effects like activating adenylate cyclase, catalyzing the formation of cyclic adenosine monophosphate (cAMP), increasing intracellular calcium, activation of protein kinases such as cyclic adenosine monophosphate-dependent protein kinase (PKA) and protein kinase C (PKC). PKA, in turn, phosphorylates the cAMP response element, acting as a transcription factor. The genomic mechanism, on the other hand, relies on the passage of AAS through the plasma membrane. Within the cytoplasm, AAS binds to the specific androgen receptor, forming a drug-androgen receptor complex, which then migrates to the cell nucleus, initiating gene transcription processes and subsequently protein translation. This modulation thus influences androgen-dependent cellular actions (For extensive review see (Cato et al., 2002; Heinlein and Chang, 2002; LÖSEL et al., 2003)). The mechanisms described above could explain the clinical effects of AAS, but also their adverse effects.

The objective of this review was to gather the available information regarding the effects of AAS in the cardiovascular, renal and metabolic systems, emphasizing the contribution of oxidative stress and inflammation to these effects and connecting the findings from experimental animal studies with the alterations observed in clinical contexts related to AAS abuse.

2. Effects of AAS in the cardiovascular system

Exogenous AAS have been associated with a variety of adverse cardiovascular effects, including dyslipidemia, arterial hypertension, increased atherosclerosis, concentric left-ventricular myocardial hypertrophy, abnormal cardiac remodeling and scarring, arrhythmia, and hypercoagulability (Anawalt, 2019; Baggish et al., 2017; Pope et al., 2014). These pathophysiological changes may be related to AAS use/abuse with a variety of cardiovascular disease (CVD) events or endpoints, including myocardial infarction, stroke, and sudden cardiac death (SCD) (Joury et al., 2022; Montisci et al., 2012; Thiblin et al., 2015) (Fig. 1) and are reported in the literature only in the form of case reports or small clinical studies.

The most frequently reported macroscopic alterations is cardiac hypertrophy in AAS users (Torrisi et al., 2020), however, distinguishing between cardiac hypertrophy caused by AAS use/abuse and "athlete's heart" is a clinical challenge, given that both conditions result in increased left ventricular mass. However, their origins and implications are significantly different. While "athlete's heart" represents a beneficial physiological adaptation to intense exercise, characterized by symmetric hypertrophy and preservation of diastolic function, AAS-associated hypertrophy is often associated with pathological changes, including fibrosis, diastolic dysfunction, and increased risk of arrhythmias (Achar et al., 2010). Studies such as those by Albano et al. (2021) and Patanè et al. (2020) highlight the adverse cardiovascular effects of AAS use, including disproportionate myocardial remodeling and predisposition to heart disease (Albano et al., 2021; Patanè et al., 2020). Differentiating these conditions requires careful evaluation using imaging methods, such as echocardiography and cardiac magnetic resonance imaging, combined with a detailed anamnesis to identify the use of illicit substances and the sporting context.

2.1. Cardiac effects

Cardiovascular system is one of the systems affected by the harmful effects of AAS that has been widely studied, as summarized in Table 1. AAS promotes changes in the oxidative metabolism and inflammatory biomarkers in the heart and vessels of both animals and humans. The study's results showed that AAS promotes a redox disruption and a pro-inflammatory state on organs that are involved in important physiologic processes. These alterations may be the cause of pathological effects of AAS on different organs (Kutscher et al., 2002; Parssinen and Seppala, 2002; Torrisi et al., 2020).

Previous studies from our laboratory (Bissoli et al., 2009a; Franquni et al., 2013; Nascimento et al., 2016; Uggere de Andrade et al., 2011)

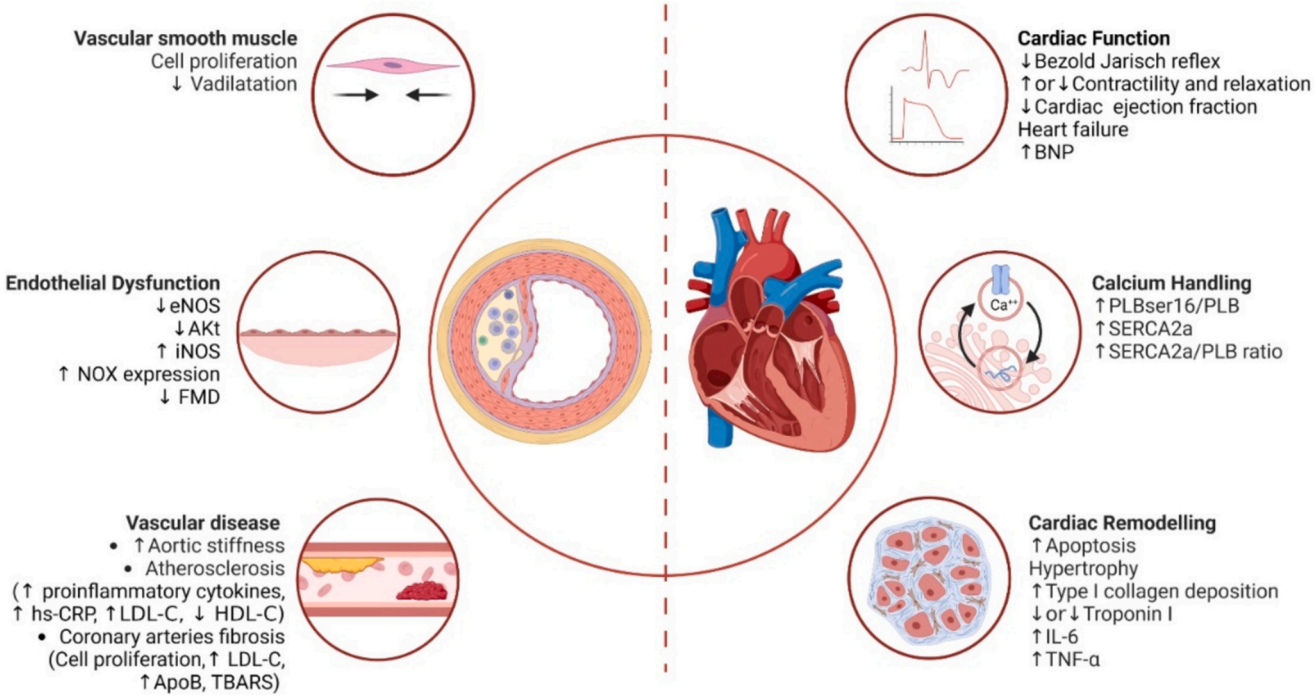

Fig. 1. Structural changes induced by AAS in heart and vessels. Apolipoprotein B (ApoB); B-type natriuretic peptide (BNP); endothelial nitric oxide synthase (eNOS); flow-mediated dilatation (FMD); high density lipoprotein-cholesterol (HDL-C); high-sensitivity C-reactive protein (hs-CRP); inducible nitric oxide synthase (iNOS); interleukin (IL); low density lipoprotein-cholesterol (LDL-C); protein kinase B (AKt); ratio p-Phospholamban (PLB ser16/PLB); ratio sarcoplasmic/endoplasmic reticulum calcium ATPase2 / Phospholamban (SERCA2a/PLB); sarcoplasmic/endoplasmic reticulum calcium ATPase2 (SERCA2a); thiobarbituric acid-reactive species (TBARS); tumor necrosis factor alpha (TNF-α).

and others (do Carmo et al., 2011; Rocha et al., 2007) contributed on demonstrating the induction of cardiac hypertrophy and hemodynamic changes after the use of high doses of AAS, and the participation of the renin angiotensin system (RAS) and inflammation on these parameters. In the literature, the role of angiotensin II receptors (AT1R) in cardiac remodeling is well established, which may be related to collagen synthesis and deposition and cardiac fibrosis, increased ROS and inflammation (Brilla et al., 1994a; Ichihara et al., 2001; Mori et al., 2013).

In a study aimed to address AAS and RAS, carried out by our group, male Wistar rats were treated with ND (20 mg/kg, for 4 weeks). After the experimental period, the ND group presented increased deposition of matrix type I collagen, increased cardiac angiotensin-converting enzyme (ACE) activity, myocyte hypertrophy, reduced Interleukin 10 (IL-10) and troponin I levels, and increased levels of pro-inflammatory cytokines (TNF-α and IL-6). These results indicate that AAS caused injury to the heart, associated with a pro-inflammatory state. Additionally, in the same study, ND caused reduction in the Bezold Jarisch reflex (BJR) control of heart rate (HR) and blood pressure (BP). These changes led to arterial hypertension, which was independent of changes in vascular reactivity, since the treatment did not change the sensitivity of the mesenteric bed to phenylephrine and acetylcholine (Franquni et al., 2013). It is interesting to highlight that these effects are time- and dose-dependent; eight weeks of ND (10 mg/kg) treatment affected the BJR (Bissoli et al., 2009), whereas four weeks of treatment had no effect (Andrade et al., 2008). In line with this, current AAS abuse was associated with increased 24-h BP and increased aortic stiffness (Rasmussen et al., 2018). In addition to BJR impairment, the baroreflex is another important cardiovascular reflex altered by AAS (Beutel et al., 2005; Camara Puppin et al., 2019; Engi et al., 2012; Kouidi et al., 2021; Santos et al., 2018).

In AAS users, tonic cardiac autonomic regulations, heart rate variability (HRV) and electrical activity have been studied.

A previous study from Bissoli's group showed that high doses of AAS increased the susceptibility for vasovagal syncope and autonomic dysfunction in AAS abusers by the head-up tilt test (HUTT) (Camara Puppin et al., 2019). More recently, the effects of AAS use on athletes' cardiac autonomic activity in terms of baroreflex sensitivity (BRS), and HRV were studied by Kouidi et al. The results showed lower BRS and baroreflex effectiveness index and increase in sympathetic activity in AAS abusers. These altered cardiovascular autonomic modulations were associated with indices of early LV diastolic dysfunction (Kouidi et al., 2021).

Studies have evaluated the electrical activity of the heart and autonomic tonus in AAS abusers and non-users, using different indexes that are related to arrhythmia and SCD. The presence of abnormal signal-averaged electrocardiogram (SAECG) measures increases the risk of conduction abnormalities as they provide a re-entry mechanism for ventricular tachycardia (Gatzoulis et al., 2018), and QT prolongation and increased QT dispersion are predictors of risk of arrhythmias and SCD in different patient populations (Tse and Yan, 2017). The reduction in HR from the peak exercise rate in the first, second, third, and fifth minutes following the end of the exercise stress test is known as the heart rate recovery index (HRRI) and it is an important measure of autonomic nervous system dysfunction and is directly related to parasympathetic activity.

These indexes were found altered in AAS users, as described in the following studies. Sculthorpe et al. (2010) evaluated the AAS cardiac toxicity in regular prolonged users of AAS compared to weight-trained age-matched non AAS-using controls. For that, the researchers used SAECG arrhythmias. The results demonstrated that, at rest, there was a nonsignificant trend for a higher incidence of abnormal SAECG in AAS, but after exercise, the AAS users displayed a significantly higher incidence of abnormal SAECG compared with controls. In the other study, electrocardiogram was continuously recorded and they analyzed electrocardiographic ventricular repolarization QT parameters. Among the parameters analyzed, AAS users have a significantly longer rate-corrected QT interval (QTc) interval and higher QT dispersion, at rest and after moderate exercise training, when compared with AAS-free

Table 1
Studies involving Cardiovascular System and AAS. ↑increase, ↓decrease and = not alter. NADPH oxidase (NOX); oxygen peroxide (H_2O_2); Nitric Oxide (NO); Induzível Nitric Oxide (iNOS); Phenylephrine (PE); Acetylcholine (Ach); Flow-mediated dilatation (FMD),Angiotensin-converting enzyme (ACE); Total Antioxidant Capacity (TAC). High-sensitivity C-reactive protein (hs-CRP), Catalase (CAT); Superoxide dismutase (SOD); Superoxide anion (O_2^-); marker of lipid peroxidation (TBARS and MDA); Advanced oxidative protein products (AOPP); Glutathione Reductase (GR); Glutathione Peroxidase (GPx); Interleukin-6 (IL-6), Interleukin-1 beta (IL-1β); Tumor Necrosis Factor alpha (TNF-α); Heat Shock Protein 90 (HSP90); Reduced Glutathione (GSH); Total Thiol (T-SH); Reactive Oxygen Species (ROS), Mice deficient in low-density lipid receptors ($LDLr^{-/-}$); signal-averaged electrocardiogram (SAECG); heart rate recovery index (HRRI), head-up tilt test (HUTT), sudden cardiac death (SCD). *: indexes that are related to arrhythmia and SCD.

Article	Treatment	Rodents	Results
Frankenfeld et al., 2014a	Nandrolone decanoate	Male rats	↑mRNA NOX ↑H_2O_2 ↓GPx and SOD
Chaves et al., 2013	Nandrolone decanoate	Male rats	Cardiac hypertrophy Reversal of the benefits of exercise
Vasilaki et al., 2016	Nandrolone decanoate	Rabbits	↑Focal fibrosis and inflammatory infiltrations ↑lipid peroxidation and troponin I ↓ CAT activity
Franquni et al., 2013	Nandrolone decanoate 20 mg/kg	Male Wistar rats	↑hypertrophy and collagen deposition ↑TNF- α and IL-6 ↓IL-10 and cardiac troponin I
Melo Junior et al., 2018	Nandrolone decanoate 10 mg/kg	Male SHR	↑hypertrophy and collagen deposition ↑TNF- α, ACE, AT1R Enhanced cardiac contractility and relaxation
El-Shamarka et al., 2023	Boldenone 5 mg/kg	Male rats	↑lipid peroxidation, NO, IL-6 and troponin I ↓GSH and SOD
Ronchi et al., 2021	Oxandrolone Low dose: 2.5 mg/kg/day High dose: 37.5 mg/kg/day	Young male Wistar Rats	Low dose: ↑ SOD1 and ↓ catalase expression. High dose. ↑AOPP. Both doses: ↑ ACE expression, collagen deposition
Germanakis et al., 2013	Methanabol and Turinabol Low dose: 0.6 mg/kg/day High dose: 1 mg/kg/day	New Zealand white female rabbits	Low doses of both anabolic steroids: not affect oxidative stress markers. High dose of turinabol: ↑ CAT activity and lipid peroxidation
Barbosa dos Santos et al., 2013	Stanozolol 5 mg.kg^{-}1/day	Male Wistar rats	↑SOD and CAT activity
Kara et al., 2018	Stanozolol 5 mg/kg/day	Male Sprague-Dawley rats	Cardiac apoptosis ↑ protein carbonyl and CAT. Exercise has a protective role in stanozolol induced oxidative stress and apoptosis
Caliman et al., 2017	Nandrolone decanoate 10 mg/kg	Female Wistar rats	Impaired Ach-induced relaxation in mesenteric bed ↓NO ↑iNOS and NOX
Tofighi, 2017		Male Wistar rats	↑Coronary smooth muscle cell, lipid peroxidation and vascular fibrosis
Sun et al., 2013	Nandrolone decanoate 0.2 mL/kg twice a week	Old Male Sprague-Dawley rats	↓eNOS, CAT, MnSOD ↑carbonyl and lipid peroxidation in aorta ↓ ACh sensitivity with mitochondrial remodeling in aorta
de Andrade et al., 2019	Stanozolol	Mice LDLr-/-	↑ lipid deposition, triglycerides and non-HDL cholesterol in the aortas
Rasmussen et al., 2018	**Human studies** (weightlifters or bodybuilders other athletes: AAS abuse)		↑TNF-α. ↑ lipid peroxidation and AOPP
Article			**Results**
Rasmussen et al., 2018			AAS abuse was associated with increased 24-h BP and increased aortic stiffness
Camara Puppin et al., 2019			AAS increased the susceptibility for vasovagal syncope and autonomic dysfunction in AAS users by the HUTT
Kouidi et al., 2021			Lower BRS and baroreflex effectiveness index and increase in sympathetic activity in AAS users
Sculthorpe et al., 2010			After exercise, the AAS users displayed a significantly higher incidence of abnormal SAECG* compared with controls
Maior et al., 2010			AAS users have longer the rate-corrected QT interval (QTc)* interval and higher QT dispersion*, at rest and after moderate exercise training, when compared with AAS-free subjects
Hernández-Guerra et al., 2019			SCD case reports: multi-organ congestion, acute pulmonary edema, cardiomegaly, and coronary atherosclerosis
Montisci et al., 2012			cardiac hypertrophy, interstitial and replacement fibrosis, and vascular dysfunction
Ahlgrim and Guglin, 2009			AAS users developed heart failure with ↓ an ejection fraction
Severo et al., 2013			↑platelet counts, hs-CRP levels, ↓HDL cholesterol and FMD.
de Souza et al., 2019			↓FMD associated with ↑ sympathetic nerve activity
Souza et al., 2023			↑ Pericoronary fat attenuation (mPFA) and systemic inflammatory cytokine profile (IL- 1, IL-6 and IL-10)

subjects (Maior et al., 2010). An increased QT interval has been associated with abnormal depressed parasympathetic activity after exercise, indicating that the parasympathetic tone may provide a physiological antiarrhythmic effect during this period, which may be lost upon AAS use (Sundaram et al., 2008). More recently, HRRI was reported to be significantly lower in AAS users, compared with the control group (Baytugan and Kandemir, 2024). So, these noninvasive tools can early assessment the risk for SCD of AAS users.

Moreover, several cases of SCD in humans are reported in the literature, shown by morphological analysis of cardiac tissue and toxicological analysis of biofluids (Darke et al., 2014; Fineschi et al., 2006; Fineschi et al., 2001; Hernández-Guerra et al., 2019). Montisci et al. (2012) reported four cases, where SCD was the cause of death in three of them. Cardiac hypertrophy, interstitial and replacement fibrosis, and vascular dysfunction were common factors (Montisci et al., 2012). Additionally, Hernandez-Guerra and coworkers (2019) presented a case-report regarding a SCD case of a 24-year-old male AAS abuser (STZ, ND and TT detected in circulation) that presented multi-organ congestion,

acute pulmonary edema, cardiomegaly, and coronary atherosclerosis (Hernández-Guerra et al., 2019). As abovementioned, AAS use/abuse promote dyslipidemia, HTN, atherosclerosis, concentric left ventricular myocardial hypertrophy, abnormal cardiac remodeling and scarring, arrythmia, and hypercoagulability, all of which contributing as risk factors for SCD (Montisci et al., 2012). Recently, micro RNAs (miRNAs) have risen as potential early biomarkers, as they are more stable molecules that have a great discriminatory potential, and to which exist analytical techniques that allow their identification and profiling (Esposito et al., 2021). Using miRNAs profiling may enable the identification of an AAS-induced damage fingerprint, improving the accuracy of forensic determination of AAS as the cause of SCD (for extensive review, see (Esposito et al., 2021).

To further understand the effect of AAS on the cardiovascular system in a state of hypertension, Bissoli's group evaluated the cardiac effects of ND in hypertensive animals (male SHR rats). ND treatment (10 mg/kg, twice a week for four weeks) amplified hypertension, increased cardiac contractility and relaxation and hypertrophy. These functional and structural changes were accompanied by an increase in deposition of cardiac collagen, increased levels of inflammatory biomarkers (TNF-α) and of components of the renin angiotensin system (ACE, AT1R). Also, the proteins involved in the regulatory mechanism of cellular calcium were also increased as shown by an increase in the ratio p-Phospholamban (PLB)ser16/PLB, sarcoplasmic/endoplasmic reticulum calcium ATPase2 (SERCA2a) and SERCA2a/PLB protein levels. Reinforcing the role of the RAS in the changes observed, a group of animals was treated with ND and enalapril (ACE inhibitor). This ACE inhibitor prevented the changes caused by ND, except for TNF-α (Melo Junior et al., 2018). In another study, we demonstrated that enalapril was able to prevent cardiac hypertrophy, elevation in mean arterial pressure, and an impairment of BJR in male Wistar rats treated with ND (Uggere de Andrade et al., 2011). As observed in male SHR rats, regulatory components of cytosolic calcium, such as SERCA2a and p-PLB, play important roles in modulating the contractility and relaxation effects of ND in female Wistar rat (Nascimento et al., 2016).

Despite ND promoting pathological cardiac hypertrophy, treatment with ND enhanced cardiac contractility and relaxation in our studies (Melo Junior et al., 2018; Nascimento et al., 2016). However, Trifunovic et al. (1995) observed that treatment with ND in male rats caused cardiac hypertrophy and reduction of myocardial contractility of the left ventricle (LV) (Trifunovic et al., 1995) and, in humans, there are reports in the literature that the AAS users developed heart failure with an ejection fraction ranging from 10 to 20 % (Ahlgrim and Guglin, 2009; Jamal et al., 2022). AAS users demonstrated relatively reduced LV systolic and diastolic function when compared to non-users, suggestive of an association between long-term AAS use and myocardial dysfunction (Rasmussen et al., 2018). The adverse effects of AAS seem to depend on the temporal relationship of use. It is known that AAS users make use of these drugs in cycles, for a long time, most of the time associated with physical exercise (Hartgens and Kuipers, 2004).

Ronchi and collaborators (2021) recently investigated the potential influence of RAS, oxidative stress and AAS, however, focusing on OXA. The results demonstrated subclinical alterations after OXA chronic treatment in both low- and high-doses (2.5 and 37.5 mg/kg/day, respectively), *via* gavage for 4 weeks in Wistar rats. The results showed the presence of pathological cardiac remodeling without modifications in physiological parameters (cardiac autonomic tonus and cardiac contractility), but with changes in the expression of calcium-handling proteins. OXA increased superoxide dismutase 1 (SOD 1) and decreased catalase expression only in the low-dose group, and advanced protein oxidation products (AOPP) were increased in high-dose. These modifications could be related to increased ACE expression, a possible cause to collagen deposition in the cardiac tissue (Ronchi et al., 2021).

In continuing the review of studies concerning the impact of AAS on the cardiovascular system, we observed evidence that reinforces the earlier discussion. High doses of AAS frequently lead to a disruption in redox homeostasis, which can consequently contribute to inflammation (Magalhães et al., 2020).

The redox homeostasis in the heart was evaluated in ND treated male Wistar rats (10 mg/Kg, once a week) for 8 weeks (Frankenfeld et al., 2014a). The ND treatment promoted an increase in cardiac oxygen peroxide (H_2O_2) production. Trying to understand the mechanism involved, the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (NOX) mRNA levels and antioxidant enzymes activity were evaluated. The levels of NOX were increased in heart, however without changes in oxidative stress biomarkers including glutathione (GSH) peroxidase (GPx), SOD and catalase activities.

The effects of AAS on the cardiovascular system are influenced not only by dosage and duration, as previously noted, but also by the route of administration, thus adding complexity to the outcomes. For instance, Vasilaki and collaborators (2016) investigated the cardiac effects of ND on young rabbits (Vasilaki et al., 2016). The study administered ND in two doses, low (4 mg/kg) and high (10 mg/kg), that were applied two days per week for 6 months. Interestingly, it also evaluated the influence of the route of administration (intramuscular and subcutaneous), as well as a washout period (4 months). In the high-dose groups, focal fibrosis and inflammatory infiltrations of cardiac tissue were observed. In addition, thiobarbituric acid-reactive species (TBARS) levels were significantly increased in the high-dose groups, while catalase activity decreased. Intramuscular administration appeared to further increase inflammation, as represented by telomerase activity in peripheral blood monocytes (PBMNs). These molecular changes were accompanied by functional alterations. Animals treated with higher anabolic doses exhibited a more pronounced deterioration of global myocardial performance indexes (the main echocardiographic index) compared with low-dose treated animals. The high-dose subcutaneous group showed significantly higher plasma troponin I levels, which continued to rise during the washout period and correlated with a sharp increase in B-type natriuretic peptide (BNP) levels. Therefore, subcutaneous administration seems to be more deleterious to the cardiovascular system, as oxidative stress, telomerase activity and biochemical markers do not return to normal values during the washout period.

In turn, BU was studied in adult male rats (5 mg/kg, intramuscular, weekly) for 2 months. The BU treated animals showed elevated serum biochemical parameters, such as aspartate aminotransferase (AST), creatine phosphokinase (CPK) and deviations in lipid profiles. Moreover, elevated levels of tissue malondialdehyde (MDA), nitric oxide (NO), and interleukin-6 (IL-6), along with lower levels of GSH and SOD, were observed among the oxidative and inflammatory parameters. These changes also led to structural alterations, as evidenced by distorted cardiac histopathological images and upregulated cardiac troponin I (El-Shamarka et al., 2023).

Methanabol (methandienone), used as an aid to muscle growth (Mosler et al., 2012) and Turinabol, which is a chloride-substituted compound of methandienone (Ho et al., 2007) represent other examples of synthetic oral AAS formerly used by bodybuilders and athletes, until regulatory measures were implemented due to their reported abuse and adverse effects. An experimental study conducted on rabbits evaluated cardiac function with these drugs, using methanabol (0.6 or 1 mg/kg/day) or turinabol (0.6 or 1 mg/kg/day) (Germanakis et al., 2013). The protocol was 1 month of treatment followed by a washout period of 1 month and then another month of treatment. The study shows that acute treatment with AAS promotes ventricle function impairment, due to an increase in LV mass and cardiac ejection fraction. Although the washout period promoted a reversion in those effects, curiously the second administration of AAS increased the cardiac output and decreased myocardial mass. AAS led to negative effects, however methanabol trends worse myocardial indexes. Regarding the systemic oxidative stress, low doses of both studied AAS did not significantly affect any of the oxidative stress markers monitored. Nonetheless, the high-dose of turinabol increased catalase activity and TBARS, while no effect was observed on reduced GSH and total antioxidant capacity

(TAC) levels. On the other hand, the high-dose of methanabol exhibited a significant trend for reduction of TAC levels, while GSH increased.

Within the scope of the review, which aims to investigate the relationship between AAS, oxidative stress and inflammation, we found studies that evaluated the effect of exercise on the role of AAS in these systems. Rats that received ND (10 mg/kg, twice a week) were exercised with resistance training twice a week for 4 weeks. The exercise control group promoted physiological myocyte hypertrophy but did not change the other parameters. However, the association of ND with exercise impaired the effect of resistance exercise by inducing increased myocyte hypertrophy, deposition of matrix type I collagen, TNF-α, ACE activity, and consequently led to hypertension. This was associated with decreased IL-10 and impairment in the BJR when compared with the exercise and control groups (Lima et al., 2015). Similar response occurred when ND was combined with aerobic exercise. The combination of swimming training and ND caused an increase in the heart collagen concentration and cardiac hypertrophy, associated with activation of the cardiac RAS (AT1R expression, aldosterone synthase), and inflammatory markers (TGFA and osteopontin) (Rocha et al., 2007). Chaves and collaborators (2013) evaluated the impact of the association between treadmill (5 days a week for 10 weeks) with ND (10 mg/kg, weekly for 7 weeks). After that period, the heart was excised and the ischemia-reperfusion was evaluated in the isolated heart. The results of the study showed that association between exercise and ND promotes a reversion of the benefits of exercise. That can be observed by the decrease in oxidative markers (GPx and SOD activity). Also, a cardiac hypertrophy was noted in ND-treated animals (Chaves et al., 2013).

Meanwhile, since ST is a widely used 17α-alkylated AAS derivative, Kara et al. (2018) conducted a study with male Sprague-Dawley rats, treated with ST (5 mg/kg, subcutaneously, for 28 days) (Kara et al., 2018). This administration triggers apoptosis, as evidenced by apoptosis parameters (TUNEL assay and cytochrome-c immunohistochemical staining intensity), along with increasing protein carbonyl content and catalase levels. In this study, swimming training was able to protect the effects of ST induced oxidative stress and apoptosis. Even though, in another study, the SOD and catalase activity increase promoted by ST (5 mg.kg, subcutaneous, for 42 days) was not prevented by swimming training (Barbosa dos Santos et al., 2013). Besides the dose and physical training between the two studies were similar, there is a difference in the experimental period of treatment, making it difficult to compare the parameters between them. This suggests that ST may demonstrate time-dependent effects, although it remains unclear whether dose-dependency also plays a role, as both studies evaluated the same dosage of 5 mg/kg.

2.2. Vascular effects

The development and progression of CVD is strongly associated with systemic and local inflammation. So, AAS abuse can lead to endothelial damage and dysfunction, as well as traditional risk factors for cardiovascular diseases. This can occur when AAS lead to an imbalance in the production or bioavailability of endothelium-derived NO, resulting in a decreased vasodilator response and a prothrombotic and proinflammatory endothelium (Caliman et al., 2017; Severo et al., 2013; Sun et al., 2013), causing endothelial dysfunction.

During the inflammatory process induced by AAS, there is an increase in the production of pro- and anti-inflammatory mediators, such as high-sensitivity C-reactive protein (hs-CRP) and cytokines (Severo et al., 2013; Souza et al., 2023). In the literature, it is well known that proinflammatory cytokines (*e.g.,* IL-1α, IL-1β, IL-6, IL-12, IL-15, IL-18, and TNF-α) (for more details see (Tedgui and Mallat, 2006) contribute to the progression of atherosclerosis, and that C-reactive protein (CRP) is a biomarker of cardiovascular risk (Tajfard et al., 2019). Finally, the evidence shows that AAS are capable of increasing the concentrations of low-density lipoprotein-cholesterol (LDL-C), stimulating the inflammatory response and promoting oxidative stress, effects demonstrated in the studies that are detailed below. All these processes are crucial for the development of atherosclerosis (Schramm et al., 2012).

Experimental studies have supported vascular damage from the use of AAS in different preparations, including resistance and conductance vessels. Our previous work showed that chronic exposure of female Wistar rats to ND (20 mg/kg/week for 4 weeks) impaired the reactivity of the mesenteric vascular bed to the vasodilator agonist (acetylcholine). The endothelium-dependent NO component was reduced in rats treated with ND. Endothelial dysfunction observed in rats treated with ND was associated with decreased phosphorylation sites of endothelial nitric oxide synthase (eNOS) (Ser1177) and protein kinase B (Akt) (Ser473) and upregulation of inducible nitric oxide synthase (iNOS) and NOX expression. Most of the aforementioned effects appear to involve the NO pathway, which leads to the modulation of other molecular pathways, for example, increased iNOS inflammatory pathway and generation of oxidative stress through increased expression of NOX (Caliman et al., 2017).

Similarly, treatment with 20 mg/kg of ST for 8 weeks demonstrated an increased area of lipid deposition in the aortas, triglycerides and non-high density lipoprotein-cholesterol (HDL—C) levels in mice deficient in LDL receptors ($LDLr^{-/-}$), that could be attributed to a modification of circulating levels of cytokines and systemic oxidative stress, indicated by an increase in the level of pro-inflammatory markers (TNF-α) in plasma, as well as oxidized LDL (OxLDL), TBARS and AOPP in the liver (de Andrade et al., 2019).

In other study, Tofighi and colleagues (2017) observed that male Wistar rats treated with chronic consumption of ND (10 mg/kg, 3 times per week) for 6 weeks, led to coronary smooth muscle cell proliferation and lipid peroxidation; significant rise in levels of apolipoprotein B (ApoB), LDL, and cholesterol, as well as severe fibrosis in around coronary arteries. These findings strongly support the idea that ingestion of ND by sedentary rats and exercise induced an abnormality mediated by oxidative stress, which manifested itself in increased lipid peroxidation (Tofighi, 2017).

Additionally, ND treatment associated to exercise increased biomarkers of oxidative stress (Carbonyl, TBARs) and inhibited exercise-induced increases in the expression of eNOS, heme oxygenase-1 (HO-1), catalase, and mitochondrial manganese superoxide dismutase (MnSOD). ND alone did not alter these biomarkers. Furthermore, it also attenuated the elevated expression of peroxisome proliferator-activated gamma coactivator 1-alpha (PGC-1α) and mitofusin-2, and further increased LC3II conversion, beclin1, ATG7 and dynamin-related protein-1 expression from Sprague-Dawley rats. These results demonstrate that ND attenuates aortic adaptations to exercise by regulating dynamic mitochondrial remodeling, including downregulation of mitochondrial biogenesis and intensive autophagy (Sun et al., 2013).

Consistent with experimental studies, evidence from clinical studies has shown that weightlifters or bodybuilders and users of AAS (age range 18 to 45 years) have greater mean pericoronary fat attenuation (mPFA) and a greater systemic inflammatory cytokine profile (IL-1, IL-6 and IL-10), suggesting that AAS can induce coronary atherosclerosis through coronary and systemic inflammation (Souza et al., 2023).

Another study observed that AAS abuse in male users (age 27 ± 4 years) is associated with retrograde and oscillatory shear rate (SR) of the brachial artery, which was associated with increased sympathetic flow. Users had higher HR, systolic BP, diastolic BP, mean arterial pressure, LDL-C, muscular sympathetic nerve activity (MSNA) (bursts/min), MSNA (bursts/100 heartbeats), resting diameter and retrograde velocity diameter peak. In contrast, flow-mediated dilation (FMD) and allometric-scale FMD were reduced. Furthermore, AAS appears to lead to inflammation characterized by an increase in hs-CRP. These changes may have the potential to increase the early risk of atherosclerotic disease in young AAS users (de Souza et al., 2019). Study with young male athletes (mean age 22 ± 3 years; range 18 to 35 years) who use AAS show important changes in blood lipids and in inflammatory markers, compatible with increased cardiovascular risk. Furthermore, this profile

is accompanied by reduced endothelial function. The aforementioned effects may be related to increased platelet counts and hs-CRP levels, as well as reduced HDL-C in users of AAS. In addition, flow-mediated dilation was significantly reduced and positively associated with HDL-C levels (Severo et al., 2013).

Finally, based on knowledge about the effects of AAS on vessels, and that the relationship between inflammation and atherosclerosis in the coronary and cerebral arteries could occur through the same mechanisms (Libby, 2021; Parikh et al., 2020), we can suggest that this process is related to cardiovascular events observed due to the abusive use of AAS.

3. Effects of AAS in the kidney

Considering that one of the main androgenic hormones is TT itself, the literature has described different influences between androgens and estrogens on the regulation of BP and renal function (Elliot et al., 2007; Melo Junior et al., 2020; Stringer et al., 2005). Besides that, studies have observed that androgen or specifically TT in renal tissue increases pro-apoptotic and pro-fibrotic signaling (Metcalfe et al., 2008; Verzola et al., 2009), attenuates the pressure-natriuresis relationship (Reckelhoff et al., 1998), increases the RAS (Nwia et al., 2023), enhances proximal tubule transport (Quan et al., 2004), contributes to increased ET-1 expression (Kalk et al., 2009), which subsequently, the development of renal dysfunction leads to increased oxidative stress and inflammatory cytokines (*e.g.*, TNF-α, IL-1β, and IL-6) (El-Reshaid et al., 2018; Herlitz et al., 2010). Moreover, studies in experimental rat models have observed that castration reduced constrictor responses in the kidney, with chronic TT treatment reversing this response reduction (Song et al., 2006).

On the other hand, in humans, TT administration induces hypertension, although the mechanisms are not fully understood, suggesting that androgens may directly contribute to this increase in BP in humans (Dubey, 2002; Song et al., 2006).

However, regarding AAS specifically (Table 2), few studies have been conducted to confirm whether there is a relationship with the same responses observed with TT, in addition to nephrotoxicity and various effects resulting from the administration of supraphysiological doses. Thus, the discussion of studies on AAS in renal tissue follows below to further understand the results found in the literature, considering the different methodologies employed.

The majority of renal complications with AAS occur after chronic use and range from a mild reversible increase in serum creatinine and urea to focal segmental glomerulosclerosis (FSGS) and irreversible chronic kidney disease (Al-Hwiesh et al., 2022). Due to glomerular injury caused by increased hemodynamic stress occurring in FSGS, modification in the structural and functional characteristics of podocytes (primary form) is observed, which in turn, function to maintain the selective permeability of the glomerular filtration barrier. Additionally, an adaptive response caused by increased glomerular capillary pressure, increased demand for glomerular filtration rate (GFR), and reduction in the number of nephrons (secondary form) is observed. In clinical practice, patients with post-adaptive FSGS typically develop subnephrotic proteinuria. Immunosuppressants are used for the primary form, RAAS blockers for the secondary and post-adaptive form, in addition to treatment of the underlying cause (Al-Hwiesh et al., 2022; Herlitz et al., 2010). Moreover, Herlitz et al. (2010) suggest that abuse of vitamin supplements, creatine monohydrate, and protein, combined with the use of AAS, may contribute to renal injury due to high protein intake leading to glomerular hyperfiltration, and improper use of creatine leading to interstitial nephritis, both of which leading to FSGS (Al-Hwiesh et al., 2022; Ardalan et al., 2012; Martin et al., 2005).

In a comparative study of dose and treatment period with ND using adult male Wistar rats, the animals were divided into 5 groups: (1) control; (2) 3 mg/kg/week for 6 weeks; (3) 3 mg/kg/week for 12 weeks; (4) 15 mg/kg/week for 6 weeks; (5) 15 mg/kg/week for 12 weeks.

Table 2
Studies involving kidney and AAS. 8-Hydroxy-2'-deoxyguanosine (8-OHdG); Advanced oxidative protein products (AOPP); Blood Urea Nitrogen (BUN); Catalase (CAT); Creatinine (Cr); Glutathione Peroxidase (GPx); Glutathione Reductase (GR); Heat Shock Protein 90 (HSP90); Interleukin-1 beta (IL-1β); marker of lipid peroxidation: thiobarbituric acid-reactive species and malondialdehyde (TBARS and MDA); *N*-acetyl β-glucosaminidase (NAG); Reactive Oxygen Species (ROS); Reduced Glutathione (GSH); Superoxide anion (O2-); Superoxide dismutase (SOD); The volume of the distal and proximal convoluted tubules (DCT and PCT); Total Thiol (T-SH); Tumor Necrosis Factor alpha (TNF-α); Uric Acid (UA).

Article	Treatment	Rodents	Results
Hoseini et al., 2009	DECA (3 mg/kg)	Female bulb-c mice	↑ Kidney Weight and Volume ↑ The volume of the distal and proximal convoluted tubules (DCT e PCT)
Frankenfeld et al., 2014b	DECA (1 mg.100 g^{1-}/Kg)	Adult male Wistar	↓ CAT ↑↑ Carbonyls ↓ Thiol residues
Riezzo et al., 2014	DECA (3,75 mg/Kg/week) (10 mg/Kg/week)	Male CD1 mice	↑ TBARS ↓GR e GPx ↑ IL-1β, TNF-α, HSP90 Renal structural damage
Tsitsimpikou et al., 2016	DECA (4 mg/Kg/2× per week) (10 mg/Kg/2× per week)	Male Rabbits	↑ Urea e Creatinine ↑ TBARS ↓ GSH Renal structural damage
Dornelles et al., 2017	BU e ST (1,25 mg/Kg) (2,5 mg/Kg) (5 mg/Kg)	Male Wistar	↑ EROs e TBARS ↓ GSH e T-SH
Kahal and Allem, 2018	DECA (30 mg/Kg/week)	Male mice	Renal structural damage
Tofighi et al., 2018	DECA (10 mg/Kg)	Male Wistar	↑ Cistatin C e Urea ↓ Creatinine Clearance ↑ Gene expression of nephrin and podocin ↑ 8-OHdG Renal structural damage
Lima et al., 2020	DECA (20 mg/Kg/week)	Female Wistar	↑ Creatinine Clearance Proteinuria ↑ TBARS e AOPP ↓ SOD activity Renal structural damage
Salem and Alnahdi, 2020	DECA (3 mg/Kg/week) (15 mg/Kg/week)	Male Wistar	↑ Serum Creatinine, Urea, Uric Acid and *N*-acetyl β-glucosaminidase ↑ Superoxide anion ↑ lipid peroxidation ↓ SOD and CAT activity
Memudu and Dongo, 2023	TU (120 mg/Kg)	Male Wistar	↑ MDA activity ↓ SOD activity Renal structural damage
Article	**Treatment**	**Human**	**Results**
Kantarci et al., 2018	Testosterone enanthate DECA Methandrostenolone BU	Young Athletes	↑ BUN e Creatinine ↑ Renal Tissue Volume Renal structural damage
El-Reshaid et al., 2018	Testosterone injections up to 250 mg/day Multiple AAS	Adult males	Focal segmental glomerulosclerosis Nephroangiosclerosis Chronic interstitial nephritis Acute interstitial nephritis Nephrocalcinosis with chronic interstitial nephritis
Ali et al., 2020	Testosterone proprionate Nandrolone twice a week 250	Adult males	Focal segmental glomerulosclerosis Membranous glomerulonephritis

(*continued on next page*)

Table 2 (*continued*)

Article	Treatment	Rodents	Results
	Stanozolol 250 mg of once a week		Tubulointerstitial nephritis Nephrocalcinosis
Ozkurt et al., 2023	Testosterone propionate Drostanolone propionate Stanozolol Oxandrolone Boldenone	Adult males	Proteinuria and albuminuria ↓eGFR Renal injury

Initially, it showed that regardless of time, both high-dose groups had increased serum levels of creatinine and urea. On the other hand, it significantly attenuated SOD and catalase activity in renal tissues. Meanwhile, the low-dose groups only showed a significant difference in the 12-week treatment, which there was an increase in serum uric acid and *N*-acetyl β-glucosaminidase (NAG), in addition to increased superoxide anion (O_2^-) and lipid peroxidation. Furthermore, the low-dose also showed attenuated SOD and CAT activity. It is thus understood that even with a non-abusive dose, if used chronically, it can lead to renal damage and oxidative stress. Whereas with abusive dose, regardless of time, previously described damages will occur (Salem and Alnahdi, 2020).

This fact can be attributed to the kidneys being linked to oxidative stress, which is characterized by disruption of redox signaling. This imbalance leads to an increase in ROS, such as O_2^- and H_2O_2, which are produced by enzymes such as NOX and xanthine oxidase (XO), as well as can be produced by the natural process of energy generation in mitochondria. It is important to note that NOX is one of the major sources of O_2^-, which can be activated through AngII (Bhatia et al., 2012; Jones, 2008; Reckelhoff et al., 2019; Salem and Alnahdi, 2020).

Similarly, a comparative dose-time study investigated two drugs: BU or ST. In this study, 60-day-old Wistar rats were divided into: (P1) 5 mg/kg (4 weeks); (P2) 2.5 mg/kg (8 weeks); (P3) 1.25 mg/kg (12 weeks); and the control group. The animals were treated once a week. Firstly, it was observed that treatment with BU led to an increase in ROS and TBARS in groups P1 and P2; in antioxidant parameters such as GSH and total thiol (T-SH), a reduction in GSH was observed in all groups (regardless of dose and treatment time), and T-SH showed reduction only in group P3 (low-dose and prolonged treatment). On the other hand, treatment with ST was associated with an increase in ROS in groups P2 and P3, an increase in TBARS only in P3 (low-dose and prolonged treatment), reduction in GSH in groups P2 and P3, and a reduction in T-SH in all groups (regardless of dose and treatment time). Overall, the results indicated that both treatments led to depletion of antioxidant defenses (GSH and T-SH) in the kidneys of the animals, thus compromising the ability to combat ROS, which may have led to lipid peroxidation. It is suggested that even at low doses, AASs should only be used for therapeutic purposes (Dornelles et al., 2017).

These data are consistent with another study that evaluated ND in male CD1 mice (8-10 weeks old), which compared a sedentary group with a treadmill exercise group treated with 3.5 mg/kg/week, and a group that performed treadmill exercise but was treated with 10 mg/kg/week. The animals were treated for 42 days and the results showed that the groups receiving ND exhibited oxidative damage in renal tissue, as evidenced by an increase in the level of MDA (lipid peroxidation) and a reduction in the activity of antioxidant enzymes (glutathione reductase (GR) and GPx) in the examined groups, causing a decreased ability of the kidney to eliminate toxic H_2O_2 and lipid peroxides. Moreover, an increase in pro-inflammatory cytokines (TNF-α, IL-1β, and HSP90) was observed in mesangial cells (Riezzo et al., 2014).

There is increasing evidence that AASs affect the production of pro-inflammatory cytokines and activate inflammatory signaling cascades, resulting in significant differences in the ability of certain organs to tolerate injuries. Experimental studies show that both AAS administration and intense exercise increase renal damage in response to toxic renal injury. In addition, androgens induce oxidative stress, possibly through depletion of reduced GSH, suggested in some studies as a contributing factor to TNF-α-induced cell death (Cerretani et al., 2013; Metcalfe et al., 2008; Nagai et al., 2002; Turillazzi et al., 2011). These data suggest that TNF-α-mediated injury in renal cells appears to play a role in activating both intrinsic and extrinsic pathways of apoptosis during prolonged periods of injury.

Regarding the structural evaluation of the kidneys in the study by Riezzo et al. (2014), regardless of dose and exercise, the groups presented certain impairments, ranging from hypertrophy, fragmentation of glomeruli, vacuolation in the proximal convoluted tubule, to focal collapse of the tuft with hypertrophy and hyperplasia of overlying visceral epithelial cells, perihilar segmental sclerosis, mild interstitial fibrosis, eventually resulting in FSGS (Riezzo et al., 2014).

Another study associated ND (10 mg/kg for 6 weeks) with and without resistance exercise in male Wistar rats. This study showed that regardless of exercise, the ND-treated groups exhibited an increase in plasma levels of Cystatin C and Urea. Creatinine clearance in the exercise-associated group showed an even greater reduction than established with treatment alone when compared to the control group. Meanwhile, both groups showed increased gene expression of podocytes (nephrin and podocin), and 8-hydroxy-2'-deoxyguanosine (8-OHdG), a biomarker frequently used to assess oxidative DNA damage. Furthermore, there was increased cell proliferation and fibrosis in different parts of the nephron in the AAS-treated groups. The reduction in creatinine clearance and increase in Cystatin are indicative that ND use, regardless of whether the individual engages in resistance exercise or not, leads to impairment in renal function (Tofighi et al., 2018).

Additionally, the results found by Tofighi and collaborators (2018) corroborate with other studies (Asadi et al., 1994), which describes that androgens induce the transcription of various genes in mouse kidneys, as well as a parallel relationship between the overexpression of nephrin mRNA and renal failure. Meanwhile, to detect possible genotoxic damage caused by AAS treatment, Pozzi et al. (2013) treated Wistar rats (8 weeks old) with ND at doses of 5 and 15 mg/kg subcutaneously, and they were euthanized 24 h after exposure to the drug. Renal tissue was subjected to the single-cell assay (comet assay), which showed an increase in DNA strand breaks in renal cells after exposure to the drug at the higher dose (Pozzi et al., 2013). These genotoxic effects are suggested due to the action of metabolic activators and an indirect process occurring in the redox cycle, as well as in the generation of ROS.

Similarly, Frankenfeld et al. (2014b) also demonstrated in the kidney that ND promoted reduction in catalase activity, a significant increase in carbonyls, which are typically formed by the process of protein oxidation, and a reduction in thiol residues, mainly found in proteins and low molecular weight metabolites such as GSH, which can be reversibly oxidized by ROS (Frankenfeld et al., 2014b). Evidently, these results indicate that chronically administered supraphysiological doses of ND are capable of causing an imbalance in the redox system in renal tissue, characterizing a state of oxidative stress.

In a study conducted with another drug, adult male Wistar rats were administered testosterone undecanoate (TU) at 120 mg/kg to evaluate potential differences in treatment effects over 3 weeks, with and without a 1-week washout period. This study was able to show that the group treated with TU had increased MDA activity (lipid peroxidation) and reduced endogenous SOD activity. This imbalance in redox activity and increased oxidative stress led to disruption of the integrity of glomerular epithelial cells and renal tubules, characterized by loss of the basement membrane for the epithelial cells. However, although the drug withdrawal period was not able to normalize oxidative stress parameters, it was able to show improvement by reducing MDA activity and increasing SOD activity. Thus, they were able to present a progressive repair of the AAS-mediated distortion of glomerular cells and renal tubular epithelial cells (Memudu and Dongo, 2023). On the other hand, Kahal and Allem (2018) showed in adult male mice that after 3 months of treatment with

ND (30 mg/kg, once a week), the renal structure exhibited: glomerular atrophy and fragmentation, tubular wall rupture, vacuolar degeneration of the epithelium lining the proximal convoluted tubules, hemorrhage between the tubules, some areas of necrosis, and vascular congestion. While the other group treated for 3 months but with a 6-week washout period, it was not able to reverse the structural damage in the kidney (Kahal and Allem, 2018).

Furthermore, Tsitsimpikou et al. (2016) treated male rabbits (10-15 weeks old) with low- and high-dose (4 mg/kg and 10 mg/kg, respectively) twice a week *via* intramuscular and/or subcutaneous injection for six months. This study observed that ND increased serum Urea and Creatinine levels in the groups, and in the high-dose groups regardless of the treatment form, increased TBARS and attenuated GSH. In addition, histopathological evaluation revealed hyperemia, fibrosis, and moderate interstitial inflammation. It is worth noting that the subcutaneously treated group also showed vascular congestion and increased vascular density (Tsitsimpikou et al., 2016). Moreover, these findings are consistent with those of Tousson et al. (2016), which treated male rabbits (9-10 months old) with BU (5 mg/kg), exhibiting increased biochemical parameters of renal function, such as serum urea and creatinine (Tousson et al., 2016). This suggests that AAS alters both renal function and structure, regardless of the drug and dose used, possibly as a result of increased oxidative stress.

It is also noteworthy that there has been a significant increase in AAS use among women, with ND being one of the most commonly used. However, few studies have evaluated the potential renal effects in women. Therefore, Lima et al. (2020) carried out a study using female Wistar rats (2 months old) that were treated for 8 weeks with ND (20 mg/kg/week) (Lima et al., 2020). This study demonstrated that chronic treatment in female rats resulted in: reduced renal function, due to increased creatinine clearance and marked proteinuria; increased collagen deposition, indicating renal fibrosis; renal hypertrophy; increased protein oxidation and lipid peroxidation; and a reduction in SOD activity. Additionally, the treatment with ND (3 mg/kg) in female bulb-c mice prompted changes in kidney, namely increased weight and volume, as well as the volume of the distal and proximal convoluted tubules (DCT and PCT, respectively) (Hoseini et al., 2009).

Overall, the studies indicate that oxidative stress, apoptosis, and AAS-mediated inflammation play a significant role in renal tissue damage, regardless of the dose and duration of exposure. However, the mechanisms involved in nephrotoxicity are still not fully understood. Therefore, further studies are needed to elucidate the possible pathways directly linked to renal impairment established by AAS.

4. Effects of AAS in metabolic (dys)regulation

Given the association between cardiovascular disease and metabolic syndrome (Guembe et al., 2020), an association between AAS and the development of metabolic syndrome is plausible. Metabolic syndrome is a condition that includes high BP, low HDL-C levels, high triglyceride levels and impaired fasting blood glucose. As the BP component was focused on the previous sections, we will now discuss the currently existing evidence focusing on the metabolic alterations associated with AAS usage (summarized in Fig. 2).

In an analytical observational study of 1.5 years, Iñigo and collaborators (2000) studied the effects of a mix of AAS on the metabolic parameters of 39 male bodybuilders (Iñigo et al., 2000). They reported that 6 weeks of AAS usage (total mean dose of 2.928 g) resulted in decreased serum levels of HDL-C, along with increased levels LDL-C, AST and alanine transaminase (ALT), the latest two associated with liver damage. Later, and in line with this, Hartgens and colleagues (2004) studied the effect of AAS mix in lipoproteins (Hartgens and Kuipers, 2004). In this prospective non-blinded study, the authors reported that both 8 and 14 weeks of AAS usage led to decreased circulating levels of HDL-C, HDL2-C and HDL3-C, besides decreased levels of apolipoprotein A-1 (ApoA-1) and lipoprotein (a) (Lp(a), an independent risk factor for vascular disease development (Vinci et al., 2023), which was coincidental with increased serum levels of ApoB. After 6 weeks of AAS withdrawal, only HDL3-C and ApoB were normalized to baseline levels in both AAS durations.

The consistent decrease in HDL-C observed in both studies is in line with increased oxidative burden, as HDL-C is a known important antioxidant, as oxidative modifications in HDL modify its capacity to activate the eNOS (Mineo and Shaul, 2012). HDL-C also works in reverse cholesterol transport, removing cholesterol from cells and tissues and delivering them to the liver, by binding to ApoA-1 (Mendivil et al., 2016), which was also found decreased in these studies, which goes in line with the cardiovascular implications of AAS usage. Furthermore, the levels of Lp(a) are also associated with dysmetabolism, as decreased levels of Lp(a) have been associated with decreased risk of cardiovascular events (*e.g.*, myocardial infarction), but were shown to be related to a higher prevalence of diabetes (Langsted et al., 2021).

Although the AAS mix is the closest to what is found in AAS abuse scenarios, the effect of individual AAS must be evaluated to understand the contribution of each of them to the effects observed. Kuipers and his team (1991) evaluated the effects of ND on the lipid profile and liver function of bodybuilders. In this double-blind crossover study, the authors observed that 8 weeks of ND administration (200 mg + 100 mg/week for 7 weeks) decreased the serum levels of HDL-C, compared to the

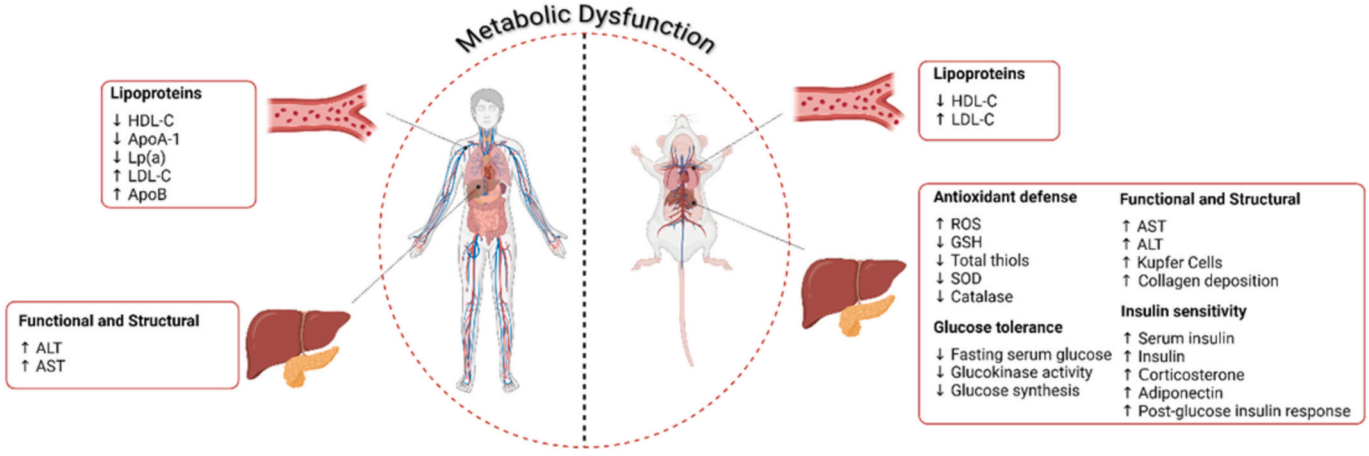

Fig. 2. Metabolic dysfunction in human and animal models. Alanine transaminase (ALT); apolipoprotein A-1 (ApoA-1); apolipoprotein B (ApoB); aspartate aminotransferase (AST); high density lipoprotein-cholesterol (HDL-C); lipoprotein (a) (Lp(a)); low density lipoprotein-cholesterol (LDL-C); reactive oxygen species (ROS); reduced glutathione (GSH); superoxide dismutase (SOD).

placebo-treated group (Kuipers et al., 1991). Moreover, Hartgens and colleagues (2004) conducted a randomized double-blind placebo-controlled study, where bodybuilders were treated with a weekly dose of 200 mg of ND for 8 weeks and observed that AAS-treated subjects presented decreased Lp(a) circulating levels, compared with the group's baseline levels. Moreover, the same effect was observed in the placebo-treated group (Hartgens and Kuipers, 2004). The abuse of ST, another commonly found AAS, was also studied in both human (Thompson et al., 1989) and animal (Dornelles et al., 2017) models. Thompson et al. (1989) demonstrated that daily oral administration of 6 mg of ST for 6 weeks led to a decrease in HDL-C, HDL2-C, HDL3-C, and ApoA-1 serum levels, concomitantly with an increase in LDL-C and ApoB levels (Thompson et al., 1989).

Rodents treated with AAS also demonstrate alterations in lipoproteins. Intermediate and supraphysiological doses of ND (5.3 mg/kg/week and 10.3 mg/kg/week) increased the number of Kupffer cells (KC), plasma levels of AST and ALT, decreased total and fractions of cholesterol and triglycerides, and reduced the number of uninucleated hepatocytes (Vieira et al., 2008). Interestingly, these alterations, especially in KC, are closely associated with non-alcoholic fatty liver disease (NAFLD). This is because they facilitate the recruitment of monocytes responsible for increasing pro-inflammatory cytokines and promoting the progression of hepatic steatosis to fibrosis (Kazankov et al., 2019; Remmerie et al., 2020). In fact, the abuse of AAS has been related as a possible direct risk factor to the development of NAFLD in humans (Dickerman et al., 1999; Pertusi et al., 2001; Schwingel et al., 2011). The pathophysiology of liver diseases is intricately associated with inflammatory processes, increased oxidative stress, fatty acid oxidation, cytokine-induced injury, hyperinsulinemia, lipid peroxidation, extracellular matrix remodeling, and alterations in immune system function (Pouwels et al., 2022). The use of AAS contributes to many of the aforementioned factors, *e.g.*, dysregulates the activity of key oxidant and antioxidant enzymes, namely increasing the activity of NOX while decreasing the activity of SOD and Catalase, decreased levels of GSH, an essential thiol compound in the non-enzymatic antioxidant system, in Wistar male rats treated with 10 mg/kg for 8 weeks (Frankenfeld et al., 2014b).

Regarding the effects of AAS on glucose metabolism, ND (10 mg/kg) reduced fasting serum glucose and glucokinase activity, increased serum insulin levels, decreased glucose synthesis from pyruvate and increased serum glycerol concentration in male Wistar rats treated for 8 weeks (Frankenfeld et al., 2014a). Additionally, low- and high-dose of ND (3 mg/kg and 15 mg/kg, respectively) were associated with reduced plasma concentrations of insulin, corticosterone, and adiponectin in male Sprague–Dawley rats (Alsiö et al., 2009). Moreover, the same study reported alterations in genes associated with cardiovascular, kidney and metabolic diseases such as 5α-reductase I (5α-RedI), 11β-hydroxylase (11β-OHase), 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGCR), adrenocorticotropic hormone receptor (ACTH receptor) and β3-adrenoceptor in different tissues.

Moreover, to examine the effect of AAS in glucose homeostasis, Cohen and Hickman (1987) evaluated the effect of AAS mix in bodybuilders and observed that 200 mg/day of AAS for up to 7 years led to reduced glucose tolerance and increased post-glucose insulin response (Cohen and Hickman, 1987). A more recent cross-sectional, case-control study by Rasmussen and collaborators (2017) was in line with this, showing that both current and former AAS users present a lower insulin sensitivity (measured by the Matsuda index) than non-AAS users (Rasmussen et al., 2017). Also, Urtado and colleagues (2011) also observed in rats that treatment with ND (0.1 mg/kg) result in decreased insulin sensitivity and glucose tolerance (showed by a decrease in the "area above the curve" of the glucose and insulin tolerance tests) (Urtado, 2011).

As far as we know, few studies have been performed to evaluate the effect of anabolic steroid abuse on metabolic syndrome-related features, most of them from the 20th century. Moreover, some studies investigate specific anabolic steroids, while others show the effects of a combination of AAS and other drugs. In any of these cases, the studies consistently describe a decrease in HDL-cholesterol, hepatic overload and ROS production, both in humans and animals.

5. Discussion and conclusions

After decades of studying the deleterious effects of the use/abuse of AAS, these drugs remain a public health problem. This review highlighted the role of AAS-induced oxidative stress and inflammation in several cardiovascular, renal, hepatic, and metabolic disturbances, both in humans and animal models. Besides testosterone, Nandrolone decanoate, Stanozolol, Boldenone Undecanoate and Oxandrolone are the most prevalent drugs related in studies regarding the deleterious effects of AAS and death.

Recently the scientific community established the term CKM (Cardiovascular, Kidney, and Metabolic Syndrome) to denote the complex intersection of cardiovascular, renal, and metabolic diseases. Molecular alterations described in the development of this syndrome include hyperglycemia, insulin resistance, heightened activity of the renin-angiotensin-aldosterone system, the generation of advanced glycation end-products, oxidative stress, lipotoxicity, endoplasmic reticulum stress, abnormalities in calcium handling, malfunctioning of mitochondria, impaired energy production, as well as persistent chronic inflammation (Sebastian et al., 2024). Thus, we propose and draw attention to the misuse of AAS as an independent risk factor for the development of CKM.

Our proposal is strongly supported by the findings presented throughout this review and summarized below:

The use/abuse of AAS induces severe deleterious effects on the cardiovascular system. These drugs cause structural and functional impairments, such as cardiac remodeling, alterations in proteins involved in calcium mobility, hypertension, and arrhythmias (Baytugan and Kandemir, 2024; Bissoli et al., 2009; Brilla et al., 1994; do Carmo et al., 2011; Franquni et al., 2013; Gatzoulis et al., 2018; Ichihara et al., 2001; Maior et al., 2010; Mori et al., 2013; Nascimento et al., 2016; Rocha et al., 2007; Sundaram et al., 2008; Uggere de Andrade et al., 2011); increased levels of proinflammatory cytokines, endothelial dysfunction and atherosclerosis (Caliman et al., 2017; Lima et al., 2015; Schramm et al., 2012; Severo et al., 2013; Sun et al., 2013; Tajfard et al., 2019). Moreover, the use/abuse can lead to vascular accidents, myocardial infarction and sudden death (Esposito et al., 2021).

Regarding the kidneys, rodents treated with AAS at doses mimicking abusive human use exhibited renal injury, including the loss of podocytes replaced by collagen (Kantarci et al., 2018; Lima et al., 2015; Memudu and Dongo, 2023; Riezzo et al., 2011; Tofighi, 2017; Tsitsimpikou et al., 2016). Additionally, the use/abuse can result in glomerular fragmentation, vacuolation in the proximal convoluted tubule, perihilar segmental sclerosis, and an increase in markers of acute renal injury, which may progress to chronic renal failure. In fact, the kidney is a highly affected organ by AAS use/abuse in both animals and humans. El-Reshaid et al. (2018) followed AAS-using bodybuilders for six years and observed that 25 patients developed kidney disease during the study. The main alterations found included nephroangiosclerosis, chronic interstitial nephritis, acute interstitial nephritis, nephrocalcinosis with chronic interstitial nephritis, membranous glomerulopathy, crescentic glomerulopathy, and sclerosing glomerulonephritis. These findings in human studies are similar to those reported in animal models. Tissue alterations are closely related to systemic changes, or *vice versa*.

Importantly, the hepatic overload caused by excessive AAS administration whether through very high doses or recurrent use over long periods alters markers of liver damage and disrupts endocrine function, including insulin resistance and glucose tolerance.

An alarming point to be considered, given the severity of adverse reactions of these drugs use/abused, is the difficulty in creating and implementing public policies for the adoption of educational actions

that can increase the population's awareness of the serious health consequences. Interventions, through public health policies, could prevent or even mitigate the abuse of AAS. In fact, a vast amount of AAS users don't notify this substance's use to their physicians (Pope et al., 2014; Pope et al., 2004). This number of underrated AAS use cases can explain the low amount of AAS-induced SCD cases reported, compared with what would be expected. Associated with this, it is necessary to standardize early markers of physiological and molecular changes caused by AAS can be identified. Among these markers, we could mention tonic cardiac autonomic regulations, heart rate variability and electrical activity, biochemical markers and more recently, miRNAs. In this sense, miRNAs could be considered as an important molecular biomarker of diseases (Hata, 2013; Joladarashi et al., 2014; Song et al., 2015; Wong et al., 2016; Zheng et al., 2016). For example, miR-122 is involved in liver injury caused by drug assumption. Several studies reported elevated plasma levels of miRNAs distinct after acute myocardial infarction, ischemia, cardiac fibrosis and arrythmia suggesting a role as valuable prognostic biomarkers (Hata, 2013; Joladarashi et al., 2014; Song et al., 2015), Wong et al., 2016). Therefore, as these pathologies are common adverse effects in AAS users, could be considered for detecting side effects of AAS. This hypothesis was tested by Sessa et al. (2020) in an interesting post-mortem study. The authors demonstrated a overexpressed miRNA (miRNA hsa-miR-21-5p, miR-205) at the kidney level in AAS use (with a post-mortem toxicological positive test for anabolic agents) and CKD (men who had died of cardiac arrest after a long period of CKD) groups compared to the control (health men who had died in car accidents) (Sessa et al., 2020).

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Declaration of competing interest

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Data availability

No data was used for the research described in the article.

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