

# Anabolic androgenic steroid-induced hepatotoxicity

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## ABSTRACT

Anabolic androgenic steroids (AAS) have been abused for decades by both professional and amateur athletes in order to improve physical performance or muscle mass. AAS abuse can cause adverse effects, among which are hepatotoxic effects. These effects include cholestatic icterus and possibly peliosis hepatis and hepatocellular carcinoma or adenoma. In particular, 17 $\alpha$ -alkylated AAS appear to be hepatotoxic, whereas nonalkylated AAS appear not to be. The 17 $\alpha$ -alkyl substitution retards hepatic metabolism of the AAS rendering it orally bioavailable. The mechanism responsible for the hepatotoxicity induced by 17 $\alpha$ -alkylated AAS remains poorly understood. However, oxidative stress has been repeatedly shown to be associated with it. In this manuscript we present a hypothesis which describes a potential mechanism responsible for AAS-induced hepatotoxicity, based on several observations from the literature which suggest oxidative stress being a causal factor.

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## Introduction

Anabolic androgenic steroids (AAS) are synthetic derivatives of the male sex hormone testosterone. Numerous synthetic derivatives have been developed and researched since the initial isolation and synthesis of testosterone in 1935 in order to dissociate the unwanted androgenic effects, such as male pattern baldness and prostate hypertrophy, from the anabolic (muscle building) effects. Moreover, efforts have been made to increase the oral bioavailability of AAS, since they are rapidly metabolized by the liver and therefore do not exert a pharmacological effect when taken orally. These efforts successfully lead to the finding that 17 $\alpha$ -alkyl substitution (see Fig. 1) of an AAS markedly decreased its rate of degradation in the liver, rendering it orally bioavailable. Unfortunately, this structural modification appears to be inextricably linked to hepatotoxicity. As a result, oral administration of AAS in clinical practice is not commonly used and intramuscular injection or dermal application of gel formulations are favored. Obviously, the oral route of administration is the preferred route by most patients, especially compared to intramuscular injection.

Nevertheless, contrary to the low frequency of usage in clinical practice, 17 $\alpha$ -alkylated AAS are commonly used to increase physical performance or enhance muscle mass by athletes. A review by Sjögqvist et al. in 2008 estimates between one and three million

people in the USA to have abused AAS [1]. This prevalence of AAS usage outside clinical practice is alarming as AAS abuse has been linked to several adverse effects, including, but not limited to, cardiomyopathy [2], atherogenic alterations in serum lipid levels [3], hypogonadism [4], acne vulgaris [5], gynecomastia [6] and hepatotoxicity [7]. The latter commonly involving cholestatic icterus [8], as well as peliosis hepatis [9,10], hepatocellular carcinoma [11] and adenoma [12] in rare cases.

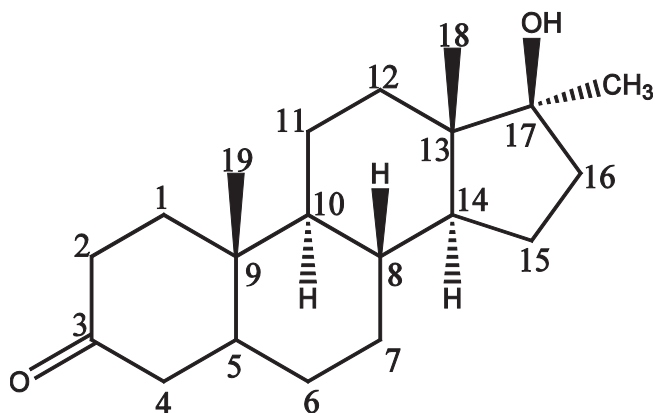
In this manuscript we present a hypothesis which describes the mechanism behind AAS-induced hepatotoxicity based on several observations from the literature. Briefly, these observations are that: (1) AAS-induced hepatotoxicity has been repeatedly shown to be associated with oxidative stress in hepatic cells; (2) androgen receptor (AR) activation can lead to an increase in reactive oxygen species (ROS); (3) AR activation increases mitochondrial  $\beta$ -oxidation; (4) antioxidants have been found to exhibit a hepatoprotective against AAS-induced hepatotoxicity; (5) metabolic resistance and androgenic potency appear positively correlated with the degree of hepatotoxicity. In the following sections we provide background information and elaborate on these observations in order to arrive at our hypothesized mechanism of how AAS possibly induce hepatotoxicity.

## Oxidative stress

In 1985 Helmut Sies defined oxidative stress as a disturbance in the prooxidant-antioxidant balance in favour of the former [14].

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**Fig. 1.**  $\alpha$ -Alkylation at position C-17 allows the compound to have oral bioavailability due to retardation of a major deactivating pathway, namely oxidation of the 17 $\beta$ -hydroxyl group catalyzed by one of the 17-hydroxysteroid dehydrogenase isozymes to form low active 17-keto steroids [13].

Intimately involved in oxidative stress are molecules named reactive oxygen species (ROS). ROS include both free radical (e.g. superoxide [ $O_2^-$ ]) and non-radical molecules (e.g. hydrogen peroxide [ $H_2O_2$ ]) which participate in radical reactions. These molecules are highly reactive and can therefore damage other molecules such as lipids, proteins, carbohydrates and nucleic acids. Subsequently, ROS are involved in various disease pathologies. Nevertheless, they also play an important role in many vital physiological processes [15]. As such, both too high and too low levels of ROS can be detrimental to health.

Intracellular ROS can originate from various enzyme-catalyzed reactions such as cellular respiration and other metabolic processes, but also from radiation. The primary source of intracellular ROS is derived from cellular respiration and localized to the mitochondrion [16]. Specifically, superoxide is produced as a result of a slight electron leakage from the electron transport chain directly onto  $O_2$ . Although superoxide itself is not a strong oxidant, it is a precursor to most other ROS and is involved in the propagation of oxidative chain reactions [17]. Since ROS are inherently linked to aerobic metabolism, cells have evolved defense mechanisms to regulate their levels. Superoxide is rapidly converted into oxygen and hydrogen peroxide by the superoxide dismutases. Following this conversion, catalase converts hydrogen peroxide to water in the cytosol, whereas the glutathione peroxidases are responsible for this conversion in the mitochondria as they lack catalase [18]. The subsequent conversion of hydrogen peroxide to water is important as hydrogen peroxide is the precursor of the highly reactive hydroxyl radical ( $\cdot OH$ ).

Given that ROS mainly arise as a by-product of metabolic processes, the liver's central role in food, hormone and drug metabolism makes it no surprise that various liver pathologies are (causally) involved with oxidative stress. Some examples include metal-induced hepatotoxicity [19], NSAID-induced hepatotoxicity [20], alcohol-induced hepatotoxicity [21], and possibly AAS-induced hepatotoxicity [22].

### A role for ROS and CPT1 in AAS-induced hepatotoxicity?

AAS-induced hepatotoxicity has been repeatedly shown to be associated with oxidative stress in hepatic cells [23–26] as well as in other cell types [22]. Of particular interest are ROS. Researchers have linked AR activation in a human prostate carcinoma epithelial cell line (22Rv1) to an increase in basal ROS [27]. This increase might be the result of an AR-mediated effect on the outer membrane carnitine palmitoyltransferase I (CPT1). CPT1 is the

rate-limiting enzyme in the process of mitochondrial fatty acid (FA)  $\beta$ -oxidation [28]. The researchers observed this increase in basal ROS after addition of the synthetic 17 $\alpha$ -alkylated AAS methyltrienolone (R1881) [27]. Further addition of the AR antagonist bicalutamide significantly reduced this effect, thus suggesting an AR-mediated effect. Later research by the same group applying a similar study design confirmed these results and also demonstrated that the increase in ROS is due to an AAS-induced increase in mitochondrial FA  $\beta$ -oxidation of fatty acids (FA) [29]. Notably, they found an increase in CPT1 mRNA levels. Moreover, the 17 $\alpha$ -alkylated AAS fluoxymesterone and methylandrostanolone have been shown to increase CPT1 activity in rat liver [30]. An AAS-induced increase in CPT1 with a subsequent increase in mitochondrial  $\beta$ -oxidation might also underlie its beneficial effects in a non-alcoholic fatty liver disease rat model [31]. Additionally, ultrastructural alterations of rat hepatocytes being treated with the 17 $\alpha$ -alkylated AAS fluoxymesterone and methylandrostanolone showed clear signs of mitochondrial degeneration as evidenced by swelling of the mitochondria and only slightly defined cristae [32]. Oxidative stress has been reported to induce inner membrane remodeling of the mitochondria [33], thus supporting the idea that the observed mitochondrial degeneration might be the result of oxidative stress. In sum, androgens might induce hepatotoxicity due to an increase in ROS which can result from an increase in CPT1 activity and subsequent augmented mitochondrial FA  $\beta$ -oxidation. As such, the commonly observed oxidative stress in hepatic cells might be casually involved.

It is also interesting to note that some antioxidants have been found to exhibit a hepatoprotective effect against AAS-induced hepatotoxicity. In an observational cohort study of 320 athletes, a positive effect on hepatic markers of liver damage was found in AAS users who used a multivitamin and phospholipid complex (compound N) when compared to AAS users who did not use compound N [34]. Compound N contains polyenylphosphatidylcholine (a mixture of polyunsaturated phosphatidylcholine [PC]), a substance which has been found to attenuate lipid peroxidation and liver fibrosis [35]. Additionally, compound N contains a vitamin B complex which might potentiate the antioxidative effect of PC. Moreover, silymarin (a mixture of various flavonoids extracted from *Silybum marianum*) showed hepatoprotective effects in rats treated with the 17 $\alpha$ -alkylated steroid methandienone [36]. Silymarin also is known for its antioxidant activity [37]. These observations further strengthen the view that oxidative stress might play a causative role in AAS-induced hepatotoxicity.

### Is AR activation involved?

Already in 1964, Marquardt et al. demonstrated failure of non-17 $\alpha$ -alkylated AAS to produce abnormal liver function tests [38]. While 17 $\alpha$ -alkylated AAS-induced hepatotoxicity has been observed in multiple controlled clinical trials [39–41], it has not been observed for non-17 $\alpha$ -alkylated AAS [42], not even with supraphysiological dosages (up to 600 mg weekly) of testosterone [43]. Consequently, it is appealing to speculate a structure-toxicity relationship based on the observation that hepatotoxicity is restricted to 17 $\alpha$ -alkylated AAS. Besides 17 $\alpha$ -alkylation, two other structural alterations have been associated with varying degrees of hepatotoxicity: in combination with 17 $\alpha$ -alkylation, a 3-keto group is thought to be more hepatotoxic than a hydroxyl group at the same position. Additionally, saturation of the A-ring is thought to reduce its ability to cause hepatic dysfunction [44].

Notably, the 3-keto group plays a pivotal role in AR binding [45]. The oxygen atom has two free pairs of electrons and can therefore act as a hydrogen bond acceptor for two polar residues in the ligand-binding domain (LBD) of the AR [46]. Not

surprisingly,  $3\alpha/\beta$  reduction of the group to a hydroxyl group (an important pathway in AAS deactivation [13]) dramatically decreases AR binding for multiple AAS [47]. Furthermore, an important (and rate-limiting) step prior to reduction of the 3-keto group of 3-keto-4-ene AAS, such as testosterone, is reduction of the 4,5 double bond [13]. AAS with a saturated A-ring do not require this step, thus facilitating their metabolism. Therefore, to prevent saturation of the A-ring, AAS have been synthesized with an alkyl group substituent at C-1 (e.g. mesterolone) or an introduction of a 1,2 double bond (e.g. methandienone) to retard their metabolism.

If AR activation is involved in AAS-induced hepatotoxicity, it is to be expected that both resistance to metabolism, as well as potency to induce AR transactivation in hepatic cells, primarily determine the gradation of hepatotoxicity. Various AAS seem to support this line of thinking. R1881, having both a very high affinity for the AR and exhibiting strong resistance to metabolism, has made it an ideal compound for an assay of androgen binding sites [48]. A mammalian reporter gene bioassay also demonstrated its high potency in terms of AR transactivation [49]. Indeed, a clinical trial demonstrated that very low dosages of R1881 ( $\leq 1$  mg daily) were enough to markedly increase hepatic markers of liver damage within two weeks [50]. This led the authors to state that the compound was the most hepatotoxic AAS at the time. Methandienone, a potent  $17\alpha$ -alkylated AAS which also contains a 1,2 double bond to further resist metabolism, also confers notable hepatotoxicity at low dosages [51]. However, the  $17\alpha$ -alkylated AAS oxandrolone exhibits very weak hepatotoxicity, even at high dosages (80 mg daily) [52]. Oxandrolone is fairly resistant to hepatic metabolism since approximately 28% is excreted unchanged and unconjugated in the urine after oral administration in man [53], but it has low affinity for the AR [54] and shows a relative potency in terms of AR transactivation nearly 100 times lower than that of R1881 in the same mammalian reporter gene bioassay mentioned earlier [49]. Its resistance to metabolism therefore lends itself to be hepatotoxic as it can lead to high exposure of hepatic cells to the compound, but its relatively weak potency of AR transactivation makes it so that high dosages are required. Additionally, oxymetholone also has a very low affinity for the AR [55], exhibiting a relative potency similar to that of oxandrolone in the same bioassay [49] and displays hepatotoxicity in a minority of patients, despite high dosages (100–150 mg every day) [56,57].

## Conclusion

In sum, we propose that AR-transactivation in hepatic cells leads to upregulation of CPT1, the rate-limiting enzyme in the process of mitochondrial FA  $\beta$ -oxidation. This, in turn, leads to an increase in ROS which leads to mitochondrial degeneration of hepatic cells, ultimately advancing to the clinical signs of hepatotoxicity observed with the usage of  $17\alpha$ -alkylated AAS. This hypothesis helps to explain why resistance to hepatic metabolic deactivation, as well as androgenic potency, appear so strongly linked to hepatotoxicity. Moreover, it clarifies why some antioxidants have been found to exhibit a hepatoprotective effect against AAS-induced hepatotoxicity. It is appealing to speculate that antagonistic targeting of the AR specifically in hepatic cells might relieve  $17\alpha$ -alkylated AAS from their hepatotoxic effects. Based on the limited clinical data and animal models, the effects of antioxidants in AAS-induced hepatotoxicity warrant further exploration, as they might be a cheap and safe strategy to ameliorate the hepatotoxic effects of AAS.

## Conflict of interest statement

The authors declare that they have no conflict of interest.

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