AAS AND ORGANS DAMAGE: A FOCUS ON NANDROLONE EFFECTS

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ABSTRACT

AAS are being used widespread in order to improve athlete’s performance, due to its capacity to improve muscle mass and strength, furthermore, recent studies showed a diffuse abuse by adolescents for libido reasons and appearance. Nandrolone is one of the most abused AAS in the world, it has a similar structure and chemical characteristic compared to testosterone. It interacts with different receptors in our body, such as estrogen, progesterone and testosterone, that’s why Nandrolone side effects affect the whole human body. Different proteins and genes are involved in testosterone biosynthesis of testosterone, with different regulatory pathways. In this review we focused on nandrolone related male reproductive system damage, in order to show the heavy consequences of AAS abuse. Nandrolone regulates MMP-2 and it could lead to spermatogenesis impairment. Oxidative stress and increase of Ros production could play a leading role in Nandrolone related infertility. Different oncogenic pathways are activated by Nandrolone and more studies need to be done to clear up correlations between this molecule and cancer. Leydig cell testosterone production is influenced by Nandrolone, moreover it was demonstrated to be a dose-dependent mechanism, despite multiple proteins and genes involved in this process were discovered, other regulatory proteins, genes and pathways, need to be studied in deep.

Keywords: anabolic androgenic steroids, nandrolone decanoate, athletes, testis, organ damage.

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Introduction

Anabolic androgenic steroids (AAS), represent a large group of synthetic derivatives, produced to prolong the biological activity of the molecule in vivo, as well as to develop molecules that are more anabolic and less androgenic45. AAS are used for therapeutic reasons in lots of diseases, such as growth delay, osteoporosis, hypogonadism and anemia in elderly people45. However, AAS are commonly used in order to improve athlete’s performance, due to its capacity to improve muscle mass and strength; furthermore, recent studies showed a diffuse abuse by adolescents for appearance and libido matters, and ranked AAS within a selection of most frequently abused 19 drugs between adolescents, including cocaine, heroine, cannabis and ecstasy14. AAS abuse side effects act on almost all body tissues, indeed most of them have androgen receptors55. Side effects include: aggressive behavior, violence, suicide attempts, liver failure, acne, decrease of HDL, hepatic adenoma, hypogonadism65.

The testosterone, nandrolone, methandienone, and methenolol, are the most commonly abused androgens and among them nandrolone differs from
stanozolol because of its capacity to be converted in estrogens by aromatase, meanwhile stanozolol is a non-aromatizable androgen (8) (table 1).

Nandrolone is one of the most frequently abused AAS in the world (9), furthermore it is responsible of apoptosis induction of spermatogenic cells in rats (10), and influences leydig cells production of testosterone (11).

Recent studies involving bodybuilders and AAS abusers proved that Nandrolone decanoate (ND) leads to several changes in sperm such as decrease sperm count and percentage and azoospermia. In this review we focused on Nandrolone molecule, its effects on Leydig cells function and its influence on reproductive system, in order to show the consequences of its abuse.

Nandrolone effects on organs

As we mentioned before, nandrolone is one of the AAS most frequently abused in the world (9). Its structure is pretty similar to testosterone and the basic chemical structure is a perhydrocyclopentanophenantrene ring system which comprises of three fused six-membered rings (A, B and C) and one five-membered ring (D) (fig. 1) (12).

Hormone synthesis begins in citosol and it is completed in mithocondria. Star (steroidogenic acute regulatory protein) protein, transfers cholesterol to the inner membrane, and during its delivery cholesterol is converted in progrenenlone by a cytochrome P450, family II, subfamily A, polypeptide I (CYP II A1) (13). Pregnenolone is then transported to endoplasmic reticulum and converted to progesterone by 3b-hydroxysteroid dehydrogenases (HSD3b). The maturation of progesterone to androstenedione is catalyzed by the 17a-hydroxylase/C17-20lyase (CYP17A1 also known as P45017A1) and finally to testosterone by 17b-hydroxysteroid dehydrogenase (17bHSD), steroid dehydrogenase specific for androgens production (14).

Androgen pathway involves steroid binding the androgen receptors (ARs), an intracellular ligand-activated transcription factor, and member of the nuclear receptor family, acting on the genome. After complex AR-steroids formation, it migrates into the nucleus and it binds to palindromic DNA sequences, and specifically, to hormone response elements (HRE) initiating gene transcription. Genomic action of ARs is modulated by a large variety of coregulators, which are proteins that target gene expression by enhancing (coactivator) or restraining (corepressor) transcription.

However, AASs may also have a direct rewarding or hedonic properties, mediated not so much by their genomic effects, but by non genomic effects (39), characterized by:

1) rapid onset (seconds to minutes) that is faster than genomic mechanism,
2) insensitivity to inhibition of RNA and protein syntesis,
3) effects produced by steroids unable to access the nucleus either covalently linked to membrane impermeable macromolecules or in cell lacking a nucleus),
4) not usually blocked by classical antagonists due to different stereoidal specificity from classical cognate nuclear receptors. These mechanisms are responsible of smooth muscle relaxation, neuromuscular and junctional signal transmission and neuronal plasticity (37,38).
Endogenous nandrolone is produced in several biochemical reactions, that's why, its main metabolite, norandrosterone, can be detected in human urine\textsuperscript{14}.

ND interacts with various receptors, such as estrogen, progesterone and testosterone\textsuperscript{16} and despite ND has a low affinity to Androgens receptors (AR), it was proved that by injecting a higher dose of ND in rats this lower affinity can be overcome\textsuperscript{17}. Other effects of ND injection in rats, such as testosterone level in serum, sperm DNA fragmentation level, sperm concentration, were proved to be receptor and dose-independent. Then, this observation could give some evidences about two different models of action, receptor-dependent and receptor independent. Nevertheless, the two mechanisms in testis could be strictly linked. Because of ND influence on AR activity, several effects in different tissues and organs were established. Two weeks of daily ND administration causes alterations in mRNA expression of dopamine receptors, furthermore ND could influence expression of serotonin receptors but these data are not significant yet. These data could suggest a influence between ND and some functions such as aggressive behavior, memory and hippocampal plasticity. Due to its anabolic activity, ND was proved to increase the volume but not the length of of the proximal and distal convoluted tubules of the mouse kidney, furthermore, some studies said that ND doses administered over a long period is responsible for selective nephrotoxicity\textsuperscript{19}. Prolonged administration of nandrolone in mice causes a dose-dependent oxidative stress and damage: in mice kidneys treated with ND was shown an increase of marker of lipid peroxidation (MDA) and a decrease of antioxidant enzymes activity, such as GR and GPx\textsuperscript{18}.

Other recent studies findings supported the idea that oxidative stress plays a leading role in Nandrolone mediated neurotoxicity\textsuperscript{20}. Indeed ROS represent a serious hazard for cells because of their oxidizing ability to damage proteins, lipids and DNA\textsuperscript{21-23}. Recent studies proved that androgens are neuroprotective in case of minimal oxidative stress levels, otherwise, by the time stress levels are elevated, androgens increase oxidative stress damage. Moreover, with a similar mechanism, ND injection in mice impairs Cardiac function and his ability to react to oxidative stress.

Moreover, other studies showed that ND could have serious side effects on other organ systems, such as in liver, which could lead to liver failure, a decrease in high-density lipoprotein (HDL) levels and hepatic adenomas\textsuperscript{24}.

**Testosterone biosynthesis in Leydig cells**

The endocrine system consists of dynamic biological processes involved in the regulation of a complex array of physiologic activities. The synthesis of sex steroids in male is elaboratively regulated by various local factors including testicular paracines/autocrines in addition to the negative feedback through the hypothalamic-pituitary-gonadal (HPG) axis\textsuperscript{25}. It is commonly known that gonadotropic hormones, such as LH and FSH, influence testis activity and growth. Testosterone production and secretion is carried out by groups of cells called Leydig cells, interposed between the seminiferous tubules, and whose activity is regulated by the pituitary hormone LH\textsuperscript{29}. LH is essential for Leydig cell function because it is the main stimulating hormone of androgen production by adult Leydig cells. LH release from the pituitary gland, may in turn result in a decreased testosterone level, and as a result, testicular atrophy occurs\textsuperscript{30}. Leydig cells synthetize testosterone, using steroidogenesis machinery composed by cholesterol transporters\textsuperscript{33-35}, steroidogenic enzymes, and many regulatory molecules. In the testis, it was established that the binding of LH to its G protein-coupled receptor (LHR) on Leydig cells acutely increases the levels of cAMP and cytoplasmic Calcium, which are both required for steroidogenesis\textsuperscript{35}.

cGMP signaling also stimulates testosterone production, meanwhile, phosphodiesterases have a regulatory function in Leydig cells. The transient synthesis of steroids in response to LH occurs through modulation of a key factor, steroidogenic acute regulatory protein (StAR), whose expression highly correlates with testosterone level. StAR is the rate limiting factor in hormone-dependent steroidogenesis because it is essential for the transport of cholesterol, the precursor of all steroids hormones, from the outer to the inner mitochondrial membrane. The cAMP pathway induces StAR expression and steroidogenesis through activation of several transcription factors. Besides Star activation also requires de novo synthesis of the nuclear receptor 4A1 (NR4A1) also known as NUR77\textsuperscript{39}.

Moreover, induction of NUR77 and STAR involves the Calcium pathway and an increase in intracellular Calcium is nonetheless required. Indeed, cytosolic calcium concentration increases in parallel with testosterone production probably
coming from both extracellular environment and from intracellular calcium store (i.e. endoplasmic reticulum). IGF 1 plays a relevant role in control of Leydig cells number and development. Its production take place also in the testis, in Sertoli, Leydig and peritubular cells derived from immature testis and cultured in vitro\textsuperscript{(40)}. In several studies conducted in IG1F1 knockout mice, failure in maturation of Leydig cells and a reduction of production of testosterone was testified, due to a deregulated expression of testosterone and metabolizing enzymes\textsuperscript{(40)}.

Furthermore, androgens have an autocrine regulation of steroidogenesis in Leydig cells: a recent study showed AR physically interacts with Nur77, and inhibits the transactivation of Nur77 on steroidogenic enzyme gene promoters, eventually resulting in decreased steroidogenesis in Leydig cells. These findings strongly support the local action of androgen/ AR on testicular steroidogenesis, and may provide an insight into its regulatory mechanism with regard to the steroidogenesis in Leydig cells.

**Nandrolone effects on testis and Leydig cells function**

Androgen action in the testis, as in other tissues, is mediated through androgen receptor (AR) transcriptional activation. Inside Sertoli cells, testosterone is selectively bound to the androgen receptor and activation of the receptor will result in initiation and maintenance of the spermatogenic process and inhibition of germ cell apoptosis\textsuperscript{(39)}. In testes, ARs are expressed in the somatic Leydig, peritubular myoid and Sertoli cells as well as in rete testis, the epithelial cells of the epididymis, and prostate\textsuperscript{(40)}. Nandrolone interferes with testis growth, development and function. Its administration in rats causes morphological changes, such as reduction of number and size of Leydig cells, cytoplasmic vacuolization, as well as lipid droplet deposition. Moreover, it induces testicular damage by triggering oxidative stress, inflammatory cytokines, matrix metalloproteinases, cell adhesion molecules, apoptotic markers, and DNA damage.

Several studies described quantitative changes in nandrolone decanoate treated rats such as reduction of testis volume and seminiferous tubule length. Moreover, ND treatment in rats decreased number of spermatocytes and spermatids in sperm, and induced sperm DNA fragmentation\textsuperscript{(47)}.

Other studies demonstrated sperm alterations in other parameters, such as sperm count, mobility and morphology. Typical Histological findings were found in Nandrolone treated rats, such as diameter length decrease in seminiferous tubules, as well as basement membrane flattening, degeneration of interstitial cells.

Recent studies showed that despite high doses of Nandrolone may negatively influence Leydig cells, sperm cell and testosterone concentration, in both mature and immature rats, otherwise number of Sertoli cells, testis size and seminiferous diameter changes were observed just in long immature rats. It’s commonly known that Nandrolone decrease testosterone levels. This could be an expected finding since AAS activate negative feedback loop of HPA/HPG axis and decrease LH and FSH levels\textsuperscript{(41, 42)}. Otherwise a recent study using in vitro cell cultures, showed no testosterone levels decrease with low doses of Nandrolone, disappearing with higher doses: it’s a dose-increase dependent trend. These changes run together with modifications in Star and CYP17A1 genes and proteins expression.

A recent study showed a significant decrease in Star and CYP17A1 genes expression in mice treated with Nandrolone compared with control trained mice, a significative increase of Star and CYP17A1 genes expression in trained mice with no treatment compared with sedentary control, a significative increase of HSD3B1 in nandrolone treated mice, sedentary and trained, compared with not treated control\textsuperscript{(41, 42)}; these data are in agreement with previous studies that showed a increased CYP17A1 gene expression in trained mice treated with linoleic conjugated acid. Moreover, in nandrolone treated mice, both trained and sedentary, a traslocation of MUC1 protein from cytoplasm to nucleus was observed\textsuperscript{(47)}. Traslocation of MUC 1 to nucleus suggests a possible oncogenic role of this protein. Nandrolone decanoate seems to regulate MMP secretion, disrupting Sertoli-germ cell adhesion with consequences on spermatocytes maturation and impairment of blood-testis barrier\textsuperscript{(44, 46)}.

It was proved that aromatizable and non aromatizable androgens like Nandrolone and Stanolozol, could promote leydig cell tumor development trough the induction of aromatase, estrogen and IGF-1 expression\textsuperscript{(40)}. As we mentioned before, administration of Nandrolone leads to increase of lipid peroxidation, heat shock proteins, induces DNA fragmentation and apoptosis.
A recent study shows a significant activity of caspase 3 activity and decrease of germ cell layers in trained rats treated with nandrolone. Although recent studies have shown that exercise training enhanced antioxidant enzyme activities and reduced lipid peroxidation in vital tissues of animals, the testes contain a low amount of antioxidant enzymes compared to other tissues such as the liver and kidney. The testicular mitochondrial membrane is rich in polyunsaturated lipids and the testes may actually be more vulnerable to peroxidative injury. Oxidative stress has been shown to be a major cause of male infertility; a large proportion of infertile men have elevated levels of seminal ROS. Several forms of sperm DNA damage are caused by ROS, e.g. chromatin cross-linking, chromosome deletion and DNA strand breaks (apoptosis)\(^{52-61}\).

**Conclusion**

Recent data could suggest an influence between ND and some functions such as aggressive behavior, memory and hippocampal plasticity. Moreover, prolonged administration of nandrolone in mice causes a dose-dependent oxidative stress and damage, that could lead to nephrotoxicity and impair cardiac response to oxidative stress. Besides, Nandrolone leads to relevant damages in male reproductive system. Both macroscopic and microscopic findings were found, such as reduction of volume of the testis, reduction of weight, as well as flattening of basement membrane of tubule and degeneration of interstitial cells. It decreases Leydig cell function and testosterone production, activating negative feedback loop, decreasing LH and FSH levels, and causes changes in genes and proteins, such as Star and CYP17A1, production in Leydig cell with a dose-dependent mechanism.

Trough MMP it could damage Blood-Testis barrier and impair spermatogenesis. It was proved that Nandrolone can activate some biochemical signalling pathways, such as, IGF1, aromatase, MUC1, and be involved in cancerogenesis. Nandrolone causes an increase of ROS and oxidative stress, in most of tissues and organs with increase of lipid peroxidation and apoptosis and this mechanism, could be a major cause of male infertility caused by Nandrolone. Nandrolone influence on testosterone biosynthesis should be investigated in order to clarify the mechanisms and find the regulatory genes and proteins involved in. Is not clear how exercise could affect nandrolone mediated oxidative stress, that’s why more studies need to be done in this field.

Scientific studies should study in deep the correlations between Nandrolone, tumor development and implicated biochemical mechanisms. People, adolescents, all AAS abusers should be strictly informed about AAS side effects and be aware of all the consequences due to this substance affecting the whole body, that could lead to infertility and severe alterations in testis, Leydig cell function, as well as almost all body tissues and organs\(^{52-74}\).

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