

Anabolic androgenic steroids effects on the immune system: a review

Review Article

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Abstract: Androgenic anabolic steroids (AAS) are synthetic derivatives of the male hormone testosterone. AAS are used by athletes and recreational users of all ages to enhance their athletic performance and/or physical appearance. While several adverse effects of AAS abuse have been described, their effect on the immune system has not been clearly elucidated. The literature generally indicates that supraphysiologic doses of AAS with an intact steroid nucleus are immunosuppressive, that is they reduce immune cell number and function. While those with alterations to the steroid nucleus are immunostimulatory as they induce the proliferation of T cells and other immune cells. Specifically, several common AAS have been shown to adversely influence lymphocyte differentiation and proliferation, antibody production, Natural Killer Cytotoxic activity and the production of certain cytokines, thereby altering the immune reaction. These effects may be profound and long lasting depending on the dosing regime, types or combinations of AAS used and the extent and duration of AAS abuse. Nevertheless, the effects of long term use of supraphysiologic doses of AAS on the immune system remain uncertain.

Keywords: *Androgenic anabolic steroids • Lymphocytes • Immune system • Natural killer • Performance*

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1. Introduction

Androgenic anabolic steroids (AAS) are either produced endogenously or exogenously where the synthetic derivative of the male hormone testosterone is an example of the latter. They were first developed in the late 1930s in an effort to treat hypogonadism and chronic wasting [1]. Their use rapidly spread and after World War Two athletes were openly using AAS for performance enhancement, however a 1972 study suggested that AAS had nothing more than a placebo effect as participants reported comparable performance enhancement when injected with a placebo [2]. However, there was little scientific evidence to suggest AAS had performance enhancing effect [2]. Consequently for years, the scientific community debated the utility of AAS, despite the fact that studies published had used

inconsistent controls, insignificant doses and in some cases anecdotal reports [3]. However, recent studies that have been tightly controlled scientific investigations have shown that the use of certain AAS, namely nandrolone decanoate and testosterone enanthate does increase athletic performance by building muscle mass and strength [4–8].

While there is anecdotal evidence indicating the widespread usage of AAS among both recreational and professional athletes (20–90%), studies suggest that usage is no higher than 6% [9]. In 2001, it was reported that in the US 1–2% of adolescent girls and 4–6% of adolescent boys had used an anabolic steroid at least once [10], while another study found that non medical use of AAS among college students was at or less than 1% [11]. According to the 2006 Monitoring the Future Study, a US survey of middle and high school students, 1.6% of 8th graders, 1.8% of 10th graders and 2.7%

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of 12th graders reported having used steroids at least once in their lifetime [12] indicated that there was an increase in the perceived risk of steroid abuse (56.8% to 60.2%) amongst 12th graders [12].

The US congress declared anabolic steroids a controlled substance (Schedule III, *Controlled Substances Act*) [13] following growing concerns about AAS abuse and any harmful long-term effects. However, the definition of anabolic steroids did not extend to “prohormones” that act as steroid precursors and once metabolized, may pose similar health risks. In 2003 the *Controlled Substances Act* was amended to include these “prohormone” supplements, such as androstenediones, androstenediols and norandrostenediones, as they potentially may act as a steroid hormone. Consequently, the Anabolic Steroid Control Act 2004 was introduced, taking effect on 20 January 2005. It effectively reclassified steroid prohormones as controlled substances by amending section 201(g) of the *Controlled Substances Act* and section 1903 of the *Anabolic Steroids Control Act* 1990. This meant that the sale of steroid precursors was prohibited without a prescription. Now, only persons registered as dispensers are allowed to distribute the steroids and their precursors.

There are a number of substances that fall under the broad term “anabolic agents”. In the World Anti-Doping Agency (WADA) list of prohibited substances they are categorized into either AAS or Other Anabolic Agents. The category AAS is further divided into exogenous, those not found naturally in the body and endogenous, those naturally found within the body. Examples of prohibited substances that fall within each of these categories are shown in Table 1.

Anabolic agent category	Examples
Anabolic Androgenic Steroids (AAS)	
a. Exogenous	Stanozolol Nandrolone Oxandrolone Trenbolone Metandienone (Dianabol)
b. Endogenous	Testosterone Androstenediol Androstenedione Dehydroepiandrosterone
Other anabolic agents	Including but not limited to: Clenbuterol Zeranol Zilpaterol

Table 1. Examples of the prohibited substances classified under the three sub categories of the anabolic agents classification [144].

Continuing evidence shows that AAS use and abuse remains. WADA reported that accredited testing laboratories found that 46.8% of adverse analytical findings in 2007 were AAS. In addition, it has been reported that up to 78.4% of steroid users are non-competitive bodybuilders and non-athletes [14]. Given the prevalence of AAS use and the lack of information about their effects on the immune system the purpose of this review is to survey the current literature and provide a comprehensive analysis of the reported effects of AAS on the immune system.

2. AAS Structures

AAS can be divided into two main groups those with alkylation of the 17- α position with the ethyl or methyl group or those with esterification of the 17- β -hydroxyl group [15]. These modifications enable these substances to have a prolonged physiological effect for up to several months. Consequently, any observed variations in the effect of AAS may be due to structural variations in the AAS molecules. The 17- β groups are highly soluble with a slower rate of absorption in the blood circulation. Therapeutically, they have been shown to increase hormone levels in patients with hypogonadism [16]. However sustained duration of these androgens without absorption can increase susceptibility to coronary thrombosis [17]. The structure of the 17- α group makes it highly intolerable to degradation by the liver thus increasing its half life. This is mainly due to the presence of the methyl group which inhibit binding to the active sites of enzymes, hence they are more toxic [18]. The mode of administration of the 17- β is usually through injection while 17- α is administered orally [19]. Examples of structures of common AAS are shown in Figure 1.

3. Mechanism of Action

Testosterone is a C-19 steroid hormone that exists both free (unbound) and bound to plasma proteins. Although approximately 38% of testosterone is bound to the protein albumin, the major binding protein is sex hormone binding globulin (SHBG), which binds 60% of testosterone [20,21]. The remaining (~2%) testosterone is unbound within the plasma. According to the free hormone hypothesis, it is the unbound testosterone that elicits the physiological response by binding to the androgen receptor where it is converted by 5 α -reductase to the more active dehydrotestosterone [22]. The physiological mechanism of action of AAS

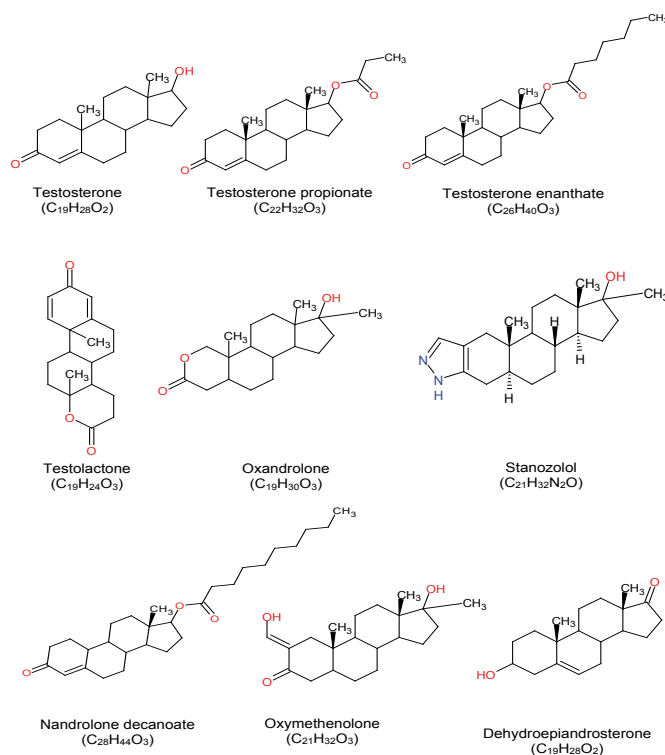


Figure 1. The chemical structure of testosterone and its common derivatives adapted from [19].

depends on the specific AAS molecule, as modifications of the AAS to form for example esters contributes to variations in binding specificity to receptors or steroid metabolizing enzymes [22-24].

The mechanism of action of all AAS is similar to all other steroid hormones in that it binds to an intracellular protein, known as an androgen receptor, in target tissues (Figure 2) to form an androgen receptor complex in the cell nucleus [25]. The androgen receptors are a type of nuclear receptor [26] that is structurally organized into different domains. These domains are a variable N-terminal region, a central and highly conserved cysteine rich DNA-binding domain (DBD) and a C-terminal ligand binding domain (LBD). Within the LBD and N-terminal domains, there are additional domains known as hormone dependent transcriptional activation domains [27,28]. After translocation into the nucleus, the steroid-receptor complex binds to palindromic DNA sequences. Specifically, they bind to hormone response elements (HRE), which are hexanucleotide halves arranged as inverted repeats and separated by three nonconserved base pairs [26]. While the sixth base pair of each half palindrome is not well conserved, it is not essential for specific binding [29]. Nevertheless, steroid receptors can bind to chromatin due to the orientation of HRE in nucleosomes. Interaction between the steroid-receptor complex and the HRE is coordinated

by two steroid-receptor specific zinc fingers formed by cysteines in the DBD and amino acids at the adhering region [26].

Direct contact of the nuclear receptors with transcription factors, or indirectly by coactivators, facilitate transcription and translation [30]. Gene transcription is achieved by this receptor complex translocating to binding sites on the chromatin, promoting gene transcription and the consequent synthesis of messenger RNA from DNA in the cell nucleus [25] to initiate protein synthesis [31]. Chaperone proteins associated with the receptors keep them in an inactive state until a steroid binds [32]. This results in the production of proteins that cause long lasting physiological effects and are considered 'genomic actions' [33,34]. While other investigators have demonstrated that steroid receptors also give rise to 'non genomic' effects [26,35,36] where they may act directly on the cell membrane, altering its physiochemical properties and allowing steroids to intercalate into the phospholipid bilayers at high concentrations [26].

T lymphocytes have been proposed to be regulated in the immature stage since they possess functional androgen receptors [37]. Therefore in the immature stage of development androgen receptors are present on the T cell, however, testosterone binding is no longer observable in mature peripheral T cells [37], consequently mature T lymphocytes have been suggested to contain

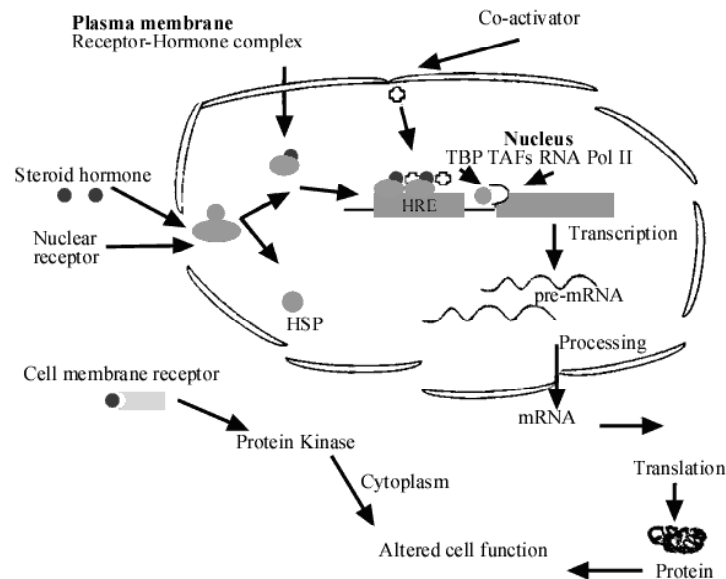


Figure 2. Molecular mechanism of androgenic anabolic steroid [145].

no androgen receptors. However rationale for this outcome may be explained by the presence of inactive androgen receptors [38] on these lymphocytes and the effect of AAS on intracellular signaling pathways on immune cells. Benten *et al.* [38] found that testosterone also induced a rapid rise in Ca^{2+} concentration in murine T lymphocytes, presumably due to a Ca^{2+} influx triggered by testosterone binding to receptors on the outer surface of T lymphocytes. Similarly, Machelon *et al.* [39] demonstrated that androstenedione treatment caused an increase in Ca^{2+} concentration following both an influx of Ca^{2+} and mobilization of Ca^{2+} from the endoplasmic reticulum. Since treatment with pertussis toxin and specific phospholipase C (PLC) inhibitor U-73,122 abolished this effect, the intracellular mobilization of Ca^{2+} probably involves PLC activation and pertussis toxin sensitive G proteins. However, the study failed to find an increase in Ca^{2+} concentration following the addition of testosterone [39]. In a later study, Benten *et al.* [38] again observed that the binding of testosterone to these cell surface receptors rapidly increased the concentration of intracellular free Ca^{2+} . Binding of testosterone-bovine serum albumin Fluorescein isothiocyanate (BSA-FITC) occurred on almost all intact T lymphocytes and was specific for testosterone since BSA-FITC without conjugated testosterone did not adhere to T lymphocytes [38]. In addition, Benten *et al.* [38] found that these testosterone binding sites are functionally coupled with Ca^{2+} channels in the plasma membrane [38]. The Ca^{2+} influx proceeds through Ca^{2+} channels that are non-voltage-gated,

but Ni^{2+} blockable [38]. The presence of testosterone receptors in the immature T cell membranes support growing evidence that several cell membranes are associated with receptors for steroid hormones [40,41] including androgens [42,43]. Hence, this data supports earlier findings that lymphocytes contain testosterone receptors in their plasma membranes [44], suggesting the existence of a novel nongenomic testosterone signaling pathway involving Ca^{2+} as an intracellular mediator. Thus it appears AAS may have both short duration and long duration effects.

In contrast, supraphysiologic doses of AAS may saturate the androgen receptor system, causing the down-regulation of androgen receptors. Consequently, aromatase, an intracellular enzyme, may convert high levels of AAS into estrogen hormones. These newly formed female sex hormones may then competitively bind to estrogen receptors and migrate to the cell nucleus to form estrogen receptor complexes, much like androgen receptor complexes.

4. Therapeutic use of AAS

A *physiological* dose represents the amount required to restore the androgen concentration to that normally found in someone of a particular age. The physiological range of testosterone for an adult male is 300-1000 ng/dl [45]. Accordingly, a *supraphysiologic* dose means a higher concentration in the body than would normally be found. AAS abuse refers to the regular use of supraphysiologic doses of AAS.

While administering AAS orally is the most convenient, a steroid must be chemically modified to prevent degradation by the liver before the steroid reaches the bloodstream [46,47] and for this reason modifications to testosterone are either the alkylation of the 17- α position with the ethyl or methyl group or esterification of the 17- β -hydroxyl group thereby making these AAS readily injectable [15], further prolonging their physiological effect.

AAS exert a significant effect on the body and, as such, they are useful in treating a wide range of diseases. AAS cause both the increased synthesis and decreased catabolism of muscle proteins [34]. They trigger muscle hypertrophy and protein synthesis, resulting in greater muscle mass and strength, and reduce recovery time by blocking the catabolic effects of cortisol [4,5,47-49]. As a result, AAS are useful in treating chronic wasting conditions such as cancer and AIDS [50,51].

In the clinical setting AAS use has been for the purpose of treating renal failure, anemia, hypogonadal states, bone marrow failure, children with growth failure, delayed puberty, acquired immunodeficiency syndrome and late stages of breast cancer [52]. Anabolic steroids have also been used for hormone replacement therapy in men, gender dysmorphia (by producing secondary male characteristics) and as a male contraceptive [53,54]. However as AAS has been shown to affect bone remodeling and growth, erythropoiesis [55-58], hair growth (pubic, beard, chest, limb), vocal cord size, libido, penis or clitoral size and sperm production [19] these doses need to be carefully monitored in the clinical setting.

4.1 Non medical use and abuse of AAS

The use of AAS for non medical purposes, such as improving body composition and image, increasing physical strength or assisting in recovery from injury, is controversial due to the potential adverse health effects and unfair performance advantage it offers [7,8]. Anabolic steroids have the potential to be of benefit to athletes in a wide variety of sports by increasing strength, power and body mass [6,22,59].

Nearly 30% of people who use AAS at supraphysiologic doses experience subjective adverse effects [60]. The specific health risks are a function of the steroids used, dose, duration of use and route of administration. Taking high doses of AAS orally for long periods can cause liver damage as the steroids are metabolized (17- α -alkylated) in the digestive system to increase their bioavailability and stability [61]. Over time, abuse has been associated with lower levels of liver enzymes, peliosis hepatitis, hepatocellular carcinoma, hepatic angiosarcoma [52,58,62]. Wilm's tumor, a

cancerous tumor of the kidney, has also been linked with the extended use of AAS [52].

The effects of AAS on the cardiovascular system are dose-dependent. Common side effects include increased risk of cardiovascular disease, coronary artery disease, harmful changes in cholesterol levels and alterations in the heart structure [52,63,64]. These changes may lead to hypertension, cardiac arrhythmias, congestive heart failure, heart attacks or sudden cardiac death [65]. Although these structural changes are also apparent in non-drug using athletes, use of AAS accelerate the process [66].

In men, use of AAS causes testicular atrophy due to a suppression of natural testosterone levels, inhibiting sperm production [58,67]. Depending on the AAS dose used and the length of time of use, testicular size may return to normal after discontinuation of the steroid [68]. Men also experience reduced sexual function, temporary infertility, gynecomastia (caused by high levels of circulating estrogen due to increased conversion of testosterone to estrogen by the enzyme aromatase) and folliculitis [52,69]. Other effects include, accelerated bone maturation, increased frequency and duration of erections and premature sexual development. Acne is also a common problem due to the abnormal stimulation of the sebaceous glands by high levels of testosterone [58,70]. Female-specific side effects include increases in body hair, deepening of the voice, enlarged clitoris and temporary decreases in menstrual cycles. When taken during pregnancy, AAS can affect fetal development by causing the development of male features in the female fetus and female features in the male fetus [71].

Psychogenic side effects have been reported following AAS use, particularly when used in supraphysiologic doses. Specifically, investigators have found increased aggression and irritability have been associated with AAS abuse [72,73]. Depression, psychosis and muscle dysmorphia ("reverse anorexia") have also been reported [52]. However, some of these effects may be mitigated through the use of exercise and/or supplementary drugs, for example AAS users may increase their cardiovascular exercise to counteract changes in the left ventricle [70,74]; aromatase inhibitors or selective estrogen receptor modulators may be used to prevent the aromatisation of androgens and any associated side effects [75] and "post cycle therapy" (PCT) consists of a combination of a selective estrogen receptor modulator (SERM), an aromatase inhibitor and human chorionic gonadotropin where it aims to counter the suppression of natural testosterone by returning the body's endogenous hormonal balance to normal and restoring proper function of these glands. PCT is used after each cycle of AAS use [74].

5. The Immune System

The immune system is an intricate network of cells and molecules that act in unison to protect the body against infections and toxins. It is comprised of the innate and adaptive immune system. At the innate level rapid, efficient and non specific immune response against pathogens is achieved through the action of Natural Killer (NK) lymphocytes, dendritic cells, macrophages and neutrophils. While specific long lasting immune response to pathogen requires the recruitment of adaptive immune cells (T and B lymphocytes) *via* cytokine and chemokine production.

5.1 T Lymphocytes

The T lymphocytes are derived from common lymphoid progenitor cells in the bone marrow [76]. They mature in the thymus to give rise to two subpopulations that express cluster of differentiation four (CD4) or CD8 molecules [77]. These cells are either helper of immune cells (CD4 T cells) or exhibit lysis against pathogens (CD8 T cells). Antigen recognition by these cells is dependant on the type of Major Histocompatibility class (MHC) of molecules on the antigen surfaces. Antigens that express MHC class I are detected by CD8 T cells while CD4 T cells distinguish antigens expressing MHC class II [78]. Further differentiations of CD4 T cells occur under stimulation by cytokines such as interleukin (IL) 12, IL-18 and IL-4. IL-12 and IL-18 induce the production of TH₁ CD4 T cells while IL-4 results in the production of TH₂ CD4 cells [79]. These two distinct subtypes of CD4 T helper cells are responsible for the production of pro-inflammatory like cytokines (TH₁) and anti-inflammatory like cytokines (TH₂) [80]. Cytotoxicity against pathogens is performed by CD8 T lymphocyte, this can be naturally occurring or through antibody dependent cellular cytotoxicity mediated by the Fc gamma receptor III (FcγRIII) [81].

5.2 B Lymphocytes

Importantly B lymphocytes derived from similar pluripotent stem cell as the T lymphocyte are involved in mediating immune response against pathogens and allergic reactions [82]. These lymphocytes express immunoglobulin (Ig) receptor molecules on their cell surfaces that allow non-specific and specific antigen recognition [82]. The different Ig receptor molecules expressed by the B cells are IgM, IgG, IgA, IgE and IgD [83]. These receptor molecules are also antibodies and have neutralization, opsonization and high affinity receptor mediated killing effects on pathogens [84]. Immunological memory is another key component of

these lymphocytes; this responsibility is preferential to IgG molecules [85].

5.3 Natural Killer Lymphocytes (NK)

Another significant lymphocyte which mainly functions both the innate and adaptive immune response is the Natural Killer (NK) lymphocyte. Elimination of virus, tumour cells and the production of cytokines are among the functions of NK lymphocytes [86,87]. Cytokines produced by these cells include interferon-gamma (IFN-γ), tumour necrosis factor alpha (TNF-α) and granulocyte macrophage colony-stimulating factor (GM-CSF) [88]. Similar to the CD8 lymphocyte, NK cells also initiate natural cytotoxicity and ADCC through the FcγRIII receptor or CD16 [89]. NK cells can further be classified in to CD56^{dim}CD16⁺ and CD56^{bright}CD16⁺NK cells [87]. Cytotoxic activity is mainly induced by CD56^{dim}CD16⁺NK cells whereas CD56^{bright}CD16⁺NK cells produce an array of cytokines [90].

Cytotoxic activity entails the ligation of death receptors and the activation of the granule exocytosis pathway [91]. Pro-apoptotic granzymes and perforin are released *via* the granule exocytosis pathway into the target cell where they bind onto organelles in the cytoplasm and induce apoptosis [92].

5.4 Cytokines

Cytokines are important proteins that are responsible for cell to cell communication. Essentially they promote cellular interaction between cells of the immune system and other bodily systems. There are two distinct types of cytokine pro and anti-inflammatory cytokines. Pro-inflammatory cytokines such as IL-2, TNF-α, IFN-γ, IL-12 induce inflammatory responses that are sometimes suppressed by IL-6, IL-4, IL-10, IL-13 anti-inflammatory cytokines [92,93].

6. AAS and the immune system

There is limited investigations that have examined the effects of therapeutic levels of AAS on immune responses [94-97]. Most literature has focused on the short term effects of AAS use without exploring what long term effects, if any, follow AAS abuse. This distinction is significant given that the effects of AAS are often dose dependent and given the side effects of AAS abuse, it seems likely that the immune and/or neuroendocrine systems could be involved as testosterone receptors are present in lymphoid and accessory cells [98], androgen receptors have been shown to be preferentially expressed after cell activation [99,100], suggesting

androgens may preferentially target certain immune cells to regulate their function.

6.1 Animal Studies

As there are ethical issues associated with AAS studies in humans, animal models have been considered an appropriate substitute [52]. Animal studies have found the immune response is affected by supraphysiologic doses of AAS. It has been documented in animal studies that different AAS can act in either an immunostimulatory or immunosuppressive manner [101-103].

In general, AAS have been considered immunosuppressive [99,102]. For example, reduction in immune cell numbers and inhibition of lymphoid regeneration for self healing in mice following multiple treatments with testosterone was reported by Kotani and associates [104]. Weinstein and Berkovich [105] suggested that AAS may exert their effect by enhancing suppressor cell activity as was observed when NZB mice were treated with testosterone.

Fuji and associates [102] conducted an experiment on four groups of mice: 4 week old irradiated and marrow reconstituted mice treated and not treated with testosterone and 9 week old normal mice treated and not treated with testosterone. In the irradiated and marrow reconstituted mice, treatment with 5-20 mg of testosterone severely depleted the number of lymphocytes in the thymus-independent areas in the spleen, lymph nodes and Peyer's patches. Lymph follicles were also comparatively small in number and in size. Myelopoiesis was more active in testosterone treated mice. Also, the number of plaque forming cells in the spleens of mice treated with 1-20 mg of testosterone was significantly lower ($P < 0.005$) than that in the untreated group. This difference was also noted in the normal mice treated with testosterone. In normal testosterone-treated mice, most of the germinal centers in the lymph nodes were surrounded by a relatively thin and poor outer collar of small lymphocytes. Fuji and associates [102] suggested that testosterone had a greater influence on stem cells than mature lymphocytes. They demonstrated that a single dose of testosterone could inhibit the differentiation of certain stem cells in the B cell population. This inhibition remained thirty days after the original treatment. Immune function was completely restored after ninety days.

Further evidence for AAS mediated immunosuppression has been demonstrated by Deschaux and co-workers [101]. They conducted a study testing the effect of sex steroids on natural killer (NK) cells in mice. In the absence of thymosin, they found that testosterone significantly increased NK cell cytotoxicity ($P < 0.01$). However, when administered

with thymosin, testosterone depressed the natural killer cytotoxic activity when compared to the increase achieved by treatment with thymosin alone. In an earlier study, Deschaux *et al.* [106] had already demonstrated that testosterone inhibits the immunostimulation of antibody production.

Mendenhall and co-workers [103] conducted a study to evaluate the AAS modulation of immune function. Thirty rats were intramuscularly administered 1.1 mg/kg/day of steroids dissolved in corn oil for 10 days. Immune function was assessed at peak areas of induration (24 hours) using delayed cutaneous hypersensitivity (DCH) responses to intradermal phytohemagglutinin. The rats were tested for 2 weeks prior to treatment (to create a baseline) and then 5 and 10 days after treatment. Testosterone, testosterone propionate, testolactone, oxandrolone and stanozolol were all shown to inhibit the immune response. These AAS were selected due to a variety of structural differences. Testosterone propionate belongs to the 17- β hydroxyl group with propionate ester group on carbon 17; it is highly soluble and remains in circulation for a lengthy period [107]. Testolactone and antineoplastic agent inhibits the conversion of androgens into estrogen; it has a six lactone ring instead of 5 noticed in most testosterone [108]. Oxandrolone is a derivative of the 17- α alkyl group with a ketone on the 3rd carbon, it is administered orally [19]. Lastly stanozolol has the greatest anabolic to androgen ratio it is distinctive from the other alkyl substitutes by the presence of N_2O group [19]. After 5 days, testolactone showed the greatest depression in immune response and stanozolol the smallest (from 67% to 17%). However, after day 10, immune function in rats treated with testolactone, oxandrolone and stanozolol improved while those treated with testosterone and testosterone propionate remained depressed.

Mendenhall *et al.* [103] also used intact and castrated rats to test the effects of endogenous gonadal hormones. The rats were treated for 8 days with oxandrolone (1.1 mg/kg/day), testosterone (1.1 mg/kg/day), or oxandrolone combined with physiologic amounts of testosterone (15 μ g/day). After 8 days of treatment with oxandrolone, intact animals experienced a 41% increase in T cell proliferation function. Conversely, rats treated with testosterone experienced a 36% reduction in T cell proliferation function. Rats treated with oxandrolone and physiologic testosterone gave results that were not significantly different to baseline. Therefore, any net immunostimulation was abolished by testosterone. In general, castration resulted in an increase in immune responses. Supraphysiologic doses of AAS, as found during abuse, may mimic medical castration by suppressing serum levels of gonadotropins (follicle-

stimulating hormone and luteinizing hormone) and testosterone [109]. However, treatment with oxandrolone returned the DCH response in castrated rats to baseline while treatment with oxandrolone and physiologic testosterone produced an even greater suppression (45%). Therefore only a direct immunosuppressive effect was observed. These results appear to be linked to the integrity of the steroid nucleus as those with an intact steroid nucleus consistently produced an immunosuppressive effect, while those with structural alterations produced immunostimulation.

In a study conducted by Hughes *et al.* [52], mice were given nandrolone decanoate or oxymethenolone in doses approximating those chronically abused in humans. The mice were injected with AAS every other day for 10 days before and 6 days after administration of sheep red blood cells [52]. Nandrolone decanoate and oxymethenolone significantly inhibited the plaque forming cell response, while testosterone had a minor effect. Nandrolone decanoate together with oxymethenolone resulted in a 45% additive inhibition. Therefore, supraphysiologic doses of both 17- β and 17- α esterified AAS, nandrolone decanoate and oxymethenolone, significantly inhibit antibody production.

In summary, the literature indicates that supraphysiologic doses of AAS with an intact steroid nucleus are immunosuppressive, while those with alterations to the steroid nucleus are immunostimulatory. Testosterone has been shown to decrease NK activity and the number of lymphocytes by inhibiting the differentiation of stem cells into B lymphocytes, thereby depressing antibody production and resulting in a reduction in immune function. Testosterone propionate similarly depressed the immune system. In contrast, treatment with testolactone, oxandrolone and stanozolol improved immune function. While these effects were long lasting, continuing to influence the immune system several weeks after the administration of a single dose, the effects of various dosing regimes and of treatment with combinations of AAS have not been clearly elucidated.

6.2 Human Studies

Males and females differ in their immune responses, as females have been shown to develop stronger immune responses, have higher concentrations of immunoglobulins and have a higher incidence of autoimmune disorders [99,110]. This suggests that sex hormones play a role in regulating immune function.

Clinical and experimental evidence suggests that gonadal steroids regulate immunological function [95,111]. Some studies suggest that AAS are immunosuppressive [102,112-116], while others suggest

that AAS enhance immune function [117]. However, the nature of their effects on the immune system depends on the type of AAS used and the dose and timing of administration. It has been shown that different AAS can act in either immunosuppressive or immunostimulatory manner [52,102,103,107].

In vitro, sex hormones directly affect immune responsiveness as evidenced by a decrease in antibody formation [118-120]. Similarly, Sthoeger *et al.* [97] have shown that testosterone use inhibits the proliferation and differentiation of B lymphocytes. In addition, Wyle and Kent [121] demonstrated a reduction in lymphocyte stimulation by testosterone. In this particular study, progesterone, testosterone, cortisol, estradiol, 11-desoxycortisol and phytohemagglutinin were used in stimulating lymphocytes, after 72 hours incubation there was a reduction in the concentration of lymphocytes [121]. Proliferative characteristic of lymphocytes was prevented by progesterone, testosterone, cortisol, estradiol, 11-desoxycortisol [121]. Significantly the percentage inhibition of 80 μ g/ml was almost 100% (91.0 $\pm$ 5.0%). This highlights the suppressive effects of testosterone on lymphocyte proliferation. However, the effects of AAS *in vivo* are equivocal. Calabrese and coworkers [117] conducted a study on a group of 24 male bodybuilders who were self administering differing types of AAS, such as nandrolone decanoate, oxandrolone and stanozolol. They were monitored during the peak dosing period of one drug use cycle and compared against non drug using body builders. The study found no significant differences between lymphocyte subpopulations in the different study groups as it was unable to demonstrate any alteration in the relative distribution or number of T lymphocytes, T suppressor/inducer lymphocytes, T cytotoxic/suppressor lymphocytes or activated T lymphocytes. However, these results must be carefully considered as the types and dosages of AAS used varied among each participant in this study. Similarly, the study did not control for training loads and intensities for each subject when determining the effect of strength training on immune responsiveness on the AAS group compared to a non AAS group.

Users of AAS have been found to have abnormal immunoglobulin (Ig) concentrations. Steroid users had the lowest levels of immunoglobulin G (IgG), immunoglobulin M (IgM) and immunoglobulin A (IgA), "significantly lower" than controls for IgA and IgM [117]. Of the Ig isotypes examined, the most substantial depression occurred in the IgA class [117]. Similarly, in a study conducted by Kanda *et al.* [122] spontaneous IgM and IgG production in humans was inhibited by exposure to 1 nM testosterone, which is nearly a physiological dose, suggesting that high doses would potentially adversely affect the immune system.

Grossman and Roselle [96] found that one of the biological actions of gonadal steroid hormones is the modulation of the immune system through the down modulation of T lymphocyte activities. Subsequently, Olsen and Kovacs [123] undertook a study to investigate the effects of androgens on lymphocyte development. It found that androgen receptors were expressed in lymphoid and nonlymphoid cells of thymus and bone marrow, but not in mature peripheral lymphocytes [123]. This suggests that androgens may play a major role in the developmental maturation of T and B lymphocytes, rather than on the mature effector cells. Recent experiments [123] have investigated whether developing lymphoid precursors are the targets of androgen action or whether supporting cells are required. Results from these experiments indicated that cells of the epithelial layer and stromal cells from the bone marrow are intermediary that assist androgens in communicating with lymphocytes [123].

AAS have been found to increase serum hemoglobin concentrations [124]. Consequently endurance athletes began using these agents to improve their aerobic capacity. While the majority of the literature has shown AAS use does not improve endurance performance [124-129], two studies recorded an AAS-induced alteration of hematology in athletes [130,131]. Alen [130] demonstrated an increase in serum hemoglobin concentration and hematocrit, platelets and white blood cell numbers after six months of high dose of AAS. Similarly, Hartgens and co workers [131] found an increase in platelet count after short term dosing (8 weeks) of AAS. The significance of these studies is that they indicate that AAS abuse can potentially affect erythropoiesis and other hematological parameters.

A study by Sulke *et al.* [120] aimed to discover the effect of testosterone on NK cytotoxic activity. It found that testosterone had a limited effect on the level of cytotoxicity observed at physiological concentrations. However, substantial inhibition of NK activity was observed at supraphysiologic concentrations. Similarly, Callewaert *et al.* [132] studied cloned human NK cells treated with testosterone and noted a depression in the mean NK lysis (58.9% to 40.9%) and proliferation ($84\ 533.47 \pm 8222.97$ to $70\ 524.60 \pm 11\ 474.51$) following treatment.

In support, Marshall-Gradisnik *et al.* [133] conducted a double-blind placebo-controlled study of healthy young males who were administered known supraphysiologic doses of testosterone enanthate for six weeks. This study found no significant difference between the placebo and testosterone group for lymphocyte numbers. In contrast the placebo group had a significant increase in NK activity while the testosterone group

had a reduced NK activity after a 10 second Wingate cycle test, as it has been reported previously to be a highly reliable measure of performance and is less likely to be influenced by pacing for example the 30 second Wingate test [134]. These results indicated that although an increase in performance is associated with AAS use, acute NK cytotoxic response following an acute bout of exercise was reduced. The results suggest that the AAS, testosterone enanthate, may reduce the cytotoxic activity of natural killer cells which is responsible for the removal of viral or tumor infected cells and highlights the potential of the immune function to be compromised.

In contrast, Calabrese and coworkers [117] noted a significant augmentation of NK activity in AAS users in their study. However, they observed that while testosterone has been demonstrated to augment NK function *in vitro*, the results of this study should be carefully considered given that the subjects in this study were not using the same AAS or dosing regimes. Therefore, although this study suggests that NK activity may increase in these users, the vast majority of literature indicates that AAS usage may have deleterious effects on immune function and potentiate autoimmune tissue damage. This is supported by the observation that in experimental Coxsackie B viral infection in mice, castration of males leads to reduced mortality, myofiber necrosis, and T cytotoxic lymphocyte activity, while treatment with testosterone results in increased mortality, myofiber necrosis and T cytotoxic lymphocyte activity [135].

When examining the effect of AAS on cytokine production, Hughes and associates [52] determined that supraphysiologic doses of nandrolone decanoate and oxymethenolone enhanced the production of the inflammatory cytokines interleukin-1 beta (IL-1 β) and tumor necrosis factor- α (TNF- α) in human peripheral blood lymphocytes cultures *in vitro*. Furthermore, AAS were found to inhibit interferon gamma (IFN- γ) and corticotropin production by peripheral blood lymphocytes. Araneo *et al.* [136] similarly demonstrated that *in vitro* exposure to dihydrotestosterone was associated with reductions in interleukin-4 (IL-4), interleukin-5 (IL-5) and IFN- γ production by anti-CD3 activated mouse lymphocytes but had no effect on interleukin-2 (IL-2) production. Based on this evidence, a decrease in these cytokines potentially may alter immune function and/or immune cell numbers.

In support of this, Benten *et al.* [38] proposed a mechanism for the observed immunosuppression. They suggested that testosterone receptors present on the surface of T lymphocytes are involved in the production of IL-2, which influences NK activity. NK cells rely on IL-2 production from T lymphocytes to increase numbers

of high affinity IL-2 receptors which subsequently influences NK activity [137,138]. Testosterone enanthate or its metabolites may bind to testosterone receptors on T lymphocytes and may downregulate T lymphocyte activities [96] or prevent the release of IL-2. The receptor may also allosterically prevent adrenaline from binding to T cells reducing IL-2 release, so only low affinity IL-2 receptors are expressed on NK cells causing a reduction in NK activity as previous research has shown low affinity and high affinity IL-2 receptors on NK cells are a regulating factor in NK activity, where upregulation of high affinity IL-2 β receptors enhances NK activity [139-141].

Furthermore, in humans, interleukin-6 (IL-6) production by monocytes was significantly reduced relative to cultures not incubated with testosterone [122]. AAS use upregulates androgen receptor immunoreactivity in the brain [142] and inhibits adrenocorticotrophic hormone (ACTH) production by lymphocytes [143], thus affecting neuroimmune endocrine function.

In summary, the literature concludes that AAS use influences immunological function. However, their effects vary according to the dose and type of AAS administered. The vast majority of studies suggest that AAS use decreases antibody formation, NK activity, T and B lymphocyte maturation and stimulation resulting in immunosuppression. Further, supraphysiologic doses of common AAS have been shown to directly influence

the production of certain cytokines, altering immune function.

7. Conclusion and future research

The results from both animal and human studies suggest that supratherapeutic doses of AAS not only elicit measurable increases in performance, but also are able to elicit adverse side effects on immune function and several other bodily systems. These effects may be profound and long lasting, depending on the extent and duration of AAS abuse. Ideally the effects of AAS abuse would be determined using a double-blind placebo-controlled experimental model. However, this would expose participants to supratherapeutic doses of various AAS that may have adverse effects on their health. Hence, given the ethical complications associated with human studies, future research should focus on using strictly controlled animal studies that examine not only immune cell numbers but also immune function status as this provides an accurate representation of these cells.

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