Effects of anabolic androgenic steroids on the reproductive system of athletes and recreational users: a systematic review and meta-analysis.

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Abstract

Background Anabolic androgenic steroids (AAS) are testosterone derivatives used by athletes and recreational users, in order to improve athletic performance and/or enhance appearance. AAS use may have serious and potentially irreversible adverse effects on different organs and systems, including the reproductive system.

Objective This systematic review and meta-analysis aimed to critically assess the impact of AAS use on the reproductive system of athletes.

Methods An electronic literature search was conducted using the databases MEDLINE, CENTRAL and Google Scholar. Studies were included when the following criteria were fulfilled: participants were athletes or recreational users of any age, gender, level or type of sport; subjects used any type, dose, form or duration of AAS; AAS effects on the reproductive system of athletes, were assessed as stated by medical history, clinical examination, hormone and/or semen analysis. Random-effects meta-analysis was performed to assess the weighted mean difference of serum gonadotropin (LH, FSH) and testosterone levels compared to baseline, during the period of AAS use, as well as following AAS discontinuation.

Results Thirty-three studies (3 randomized clinical trials, 11 cohort, 18 cross-sectional and 1 non-randomized parallel clinical trial) were included in the systematic review (3,879 participants; 1,766 AAS users and 2,113 non-AAS users).The majority of the participants were men; only 6 studies provided data for female athletes. A meta-analysis (11 studies) was conducted, of studies evaluating serum gonadotropin and testosterone levels in male subjects: (1) prior to, and during AAS use (6 studies, n=65 AAS users; 7 studies, n=59, evaluating gonadotropin and testosterone levels respectively), (2) during AAS use and following AAS discontinuation (4 studies, n=35; 6 studies, n=39, respectively), as well as (3) prior to AAS use and following AAS discontinuation (3 studies, n=17; 5 studies, n=27, respectively). During AAS intake, significant reductions in LH (WMD: -3.37 IU/l, 95%CI: -5.05 to -1.70, p <0.001), FSH (WMD: -1.73 IU/l, 95%CI: -2.67 to -0.79, p <0.001) and endogenous testosterone levels (WMD: -10.75 nmol/L, 95% CI: -15.01 to -6.49, p<0.001) were reported. Following AAS discontinuation, serum gonadotropin levels gradually returned to baseline values within 13-24 weeks, whereas serum testosterone levels remained lower as compared to baseline (WMD: -9.40 nmol/L, 95% CI: -14.38 to -4.42, p<0.001). Serum testosterone remains reduced up to 16 weeks following discontinuation of AAS. In addition, AAS abuse resulted in structural and functional sperm changes, reduction of testicular volume, gynecomastia, as well as clitoromegaly, menstrual irregularities and subfertility.

Conclusion The majority of AAS users demonstrated hypogonadism with persistently low gonadotropin and testosterone levels, lasting for several weeks to months after AAS withdrawal. AAS use results in profound and prolonged effects on the reproductive system of athletes and potentially on fertility.

Key points

This is the first systematic review and meta-analysis, of the effects of AAS use on the reproductive system of athletes.

AAS use results in a state of prolonged hypogonadotropic hypogonadism in males; gonadotropin levels recover after 13-24 weeks whereas serum testosterone remains reduced up to 16 weeks following discontinuation of AAS.

AAS use is associated with changes in sperm characteristics, reduction of testicular volume and gynecomastia in men, as well as clitoromegaly and menstrual irregularities in women and subfertility in both sexes.

1 Introduction

First identified in 1935, testosterone is the principal hormone controlling the development of androgenic masculinizing effects in the male body, along with its anabolic properties that increase lean muscle mass [1]. The anabolic androgenic steroids (AAS) are testosterone derivatives that were developed in 1950 in an attempt to maximize anabolic and minimize androgenic effects of testosterone, reduce the rate of its hepatic inactivation and decrease its aromatization to estradiol [2]. AAS formulations may be self-administered orally, parenterally by intramuscular injection, and transdermally in the form of a patch or topical gel.

Empirical evidence in the past, suggested that AAS were mostly used by top-level competitive athletes and especially weightlifters, bodybuilders and track athletes [3]. However, nowadays, AAS are widely used, not only by athletes involved in recreational and minor-league sports but also by non-athletes. Interestingly, at least four out of five AAS users are not competitive athletes but rather men who desire what they perceive to be an “enhanced” appearance [4].

It is estimated that 2.9% to 4.0% of Americans have used AAS at some time in their lives, while high rates of AAS use have also been reported in many other regions of the world, such as Scandinavia, Brazil, British Commonwealth countries and in continental Europe[5]. A meta-analysis indicated that the global lifetime prevalence rate of AAS use is 3.3%, with a higher rate of 6.4% in males compared to 1.6% in females [4]. In addition, AAS use appears more prevalent among teenagers compared to those older than 19 years; notably, 4% to 6% of high school boys have used AAS [4].

In the short-term, AAS use results in few serious medical consequences, but their long-term use has been associated with several debilitating physical and psychological adverse effects and even increased mortality. Specifically, adverse effects may range from development of acne or gynecomastia, to serious and life threatening, such as increased risk of cardiovascular disease and hepatic carcinoma [6, 7].

Normal gonadal function depends on the presence of intact hypothalamic pituitary gonadal (HPG) axis activity through secretion of gonadotropin releasing hormone (GnRH) by the arcuate nucleus of the hypothalamus, as well as gonadotropins by the pituitary (follicle stimulating hormone, FSH and luteinizing hormone, LH). AAS use produces dose-dependent depression of gonadotropin release either by direct action on the pituitary or by suppression of the hypothalamic GnRH release. In males, reduced gonadotropin secretion results in decreased intratesticular and peripheral testosterone concentrations, leading to AAS-induced hypogonadotropic hypogonadism manifesting with testicular atrophy, oligospermia, azoospermia, and other sperm abnormalities [8]. Some male AAS abusers experience lack of libido, erectile dysfunction or even gynecomastia. Effects on the prostate gland include hyperplasia, hypertrophy and possibly cancer [9]. In females, the changes most often attributed to AAS abuse are menstrual irregularities (delayed

menarche, oligomenorrhea, secondary amenorrhea), dysmenorrhea, anovulation, clitoral hypertrophy, libido changes and uterine atrophy, with many of them being permanent [10].

Although some narrative reviews have been published on the field [9, 11-13], to our knowledge, this is the first systematic review and meta-analysis using explicit methodology to critically examine AAS effects on the reproductive system of both male and female athletes.

2 Methods

2.1 Protocol and registration

The protocol of the study has been submitted to the PROSPERO international prospective register of systematic reviews (registration number: CRD42015017099) and the guidelines of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses statement (PRISMA) were followed [14].

2.2 Inclusion and exclusion criteria

Studies were included in the systematic review when the following criteria were fulfilled: participants were athletes or recreational users of any age, gender, level or type of sport; AAS use of any type, dose, form or duration; AAS effects on the reproductive system of athletes, were assessed as stated by medical history, clinical examination, hormone analysis and/or semen analysis. Medical history and/or clinical examination referred to assessment of specific signs and symptoms, such as testicular or clitoris size, regularity of menstruation and changes in libido. At least one of the following three hormone values had to be reported: LH, FSH and/or testosterone. Semen analysis included measurement of different sperm characteristics, such as sperm concentration, motility and morphology. No language, publication date or publication status restrictions were imposed. All study designs were eligible except for case reports, case series, reviews and meta-analyses. For studies on overlapping populations, the one providing the most complete data was included in the analysis. In the meta-analysis, studies were included when the mean hormone values and the standard deviation, or the necessary data to compute them, were provided for at least two time points, i.e. at baseline, at the end of AAS use and/or at the end of the period of AAS discontinuation.

2.3 Study selection and data extraction

Eligible studies were identified by searching electronic databases, scanning reference lists of included articles and also after screening references of pertinent reviews. The search was applied to Medline (PubMed), Cochrane Central Registry of Controlled Clinical Trials (CENTRAL), and Google Scholar (from inception to August 2016). The algorithm

"(anabolic OR androgenic OR AAS) AND (reproduction OR fertility OR hormone OR semen OR sperm OR hypogonad*)" was used to search for all relevant studies in the aforementioned databases. Screening of the retrieved records (titles, abstracts, full-texts) and data extraction of the included studies was performed independently in an unblended standardized manner by two reviewers (M.A.C., P.A.C.). Disagreements between reviewers were resolved by consensus. If no agreement could be reached, then a third author (S.T.) decided.

2.4 Risk of bias assessment

Risk of bias of the included studies was assessed by the Cochrane Collaboration Tool for Randomized Controlled Trials (RCT) [15]. The domains used pertain to randomization and allocation concealment (selection bias), blinding of participants and personnel (performance bias), blinding of outcome assessment (detection bias), incomplete outcome data (attrition bias), selective reporting (reporting bias) and other bias. This tool assigns a judgment of high, unclear, or low risk of bias for each item. To draw conclusions about the overall risk of bias for each study, it is necessary to summarize assessments across the different domains. The RTI Item Bank tool which consists of 13 questions was used for the assessment of risk of bias in observational studies [16, 17]. Each study was given a score and graded as high, unclear, or low risk of bias based on the number of critical appraisal items met. The cut off score was determined, based on previous systematic reviews and meta-analyses [18, 19] as follows; 0.00 to 0.30, high risk; 0.31 to 0.70, unclear risk and 0.71 to 1.00, low risk of bias.

2.5 Statistical analysis

Quantitative analysis was performed to assess the change from baseline in mean hormone values during the period of AAS use (i.e. prior to, and at the end of a period of active AAS use), as well as following AAS discontinuation (i.e. hormone levels at the end of a period of AAS discontinuation compared to those prior to AAS use and to those at the end of a period of AAS use). P-values lower than 0.05 were considered statistically significant. They were provided with precision at the third decimal point. All p-values lower than 0.001 were reported as <0.001. Random-effects meta-analysis was conducted due to the presence of statistically significant heterogeneity in the included studies. The meta-analysis was based on the inverse variance method for weighting. The Dersimonian and Laird estimator was used to pool mean differences of each study and estimate the overall weighted mean difference (WMD), as well as 95% CI [20]. Heterogeneity was assessed with the Cochran's Q test statistic [21], with a p-value<0.1 denoting statistical significance. The degree of heterogeneity was assessed using the formula: $I^2 = 100\% * \frac{Q-(k-1)}{Q}$, where k represents the number of included studies [22]. The I^2 statistic ranges from 0% to 100% and cut-off values of 25%, 50%, and 75% indicate low,

moderate, high and very high degree of heterogeneity, respectively. Data analyses were conducted using the statistical program Stata (version 13.1, Statacorp, College Station, Texas).

3 Results

3.1 Characteristics of included studies

Based on electronic databases search, 19,112 potentially eligible citations were identified. Thirty-four additional eligible studies were found through other sources. Of these 19,146 citations, 18,978 did not meet the inclusion criteria after reviewing the abstracts. Full text of the remaining 168 citations was examined in more detail. Thirty-five studies met the eligibility criteria. However, in 4 studies overlapping populations were studied. Specifically, the study by Stromme *et al*. [3] referred to the same population as that of Aakvaag and Stromme [23], and therefore only the latter was included in the analysis as it provided more complete data. Studies by Holma and Adlercreutz (1976) and Holma (1977) included the same distinct population, but assessed different outcomes; they were therefore analyzed as a single study [24, 25]. Finally, 33 studies were included in the systematic review. The process of study selection is detailed in the flow diagram provided in Fig.**1**.

One study [26], involved 2 partially overlapping populations for 2 separate experiments (methandienone experiment, n=12 and dehydroepiandrosterone sulphate, DHEAS, experiment n=16). Due to the fact that methandienone is considered a potent AAS, whereas DHEAS is actually a natural weak androgen and hormone precursor, results of the methandienone experiment were included in the main analysis and the DHEAS experiment data were used for sensitivity analysis.

Regarding study design, 11 cohort studies (33%), 18 cross-sectional studies (55%), 3 RCTs (9%) and 1 non-randomized parallel clinical trial (3%) were considered eligible. In the RCTs, either a parallel [23]or a cross-over design was used [26, 27].

The publication year of studies included in the analysis extended from 1972 to 2016, with most studies being published between 1980 and 1990 (n=10, 30%). The majority of studies were conducted in Europe (18 studies, 55%; 8 of which studies from Finland) and USA (11 studies, 33%). The median (25^{th}-75^{th} percentile) duration of the follow-up period was 24.5 (15-42) weeks. The language of all eligible publications was English.

Participants: The total number of participants in the studies included in the systematic review was 3,879 (1,766 AAS users and 2,113 controls), with a mean (standard deviation) age of 28.7 (4.9) years. Most studies involved only men (27

out of 33 studies), 4 studies [28-31] referred only to women, and 2 more studies included both men and women athletes [32, 33]. The most common type of exercise was strength training and particularly bodybuilding, weightlifting and powerlifting. When the comparator group was available, it usually referred to athletes not using AAS.

Type of exposure/intervention: Exposure or intervention in all eligible studies was AAS use. The median (25^{th}-75^{th} percentile) number of different AAS agents per study was 5.5 (3-11) and the median (25^{th}-75^{th} percentile) duration of AAS use was 12 (8-25) weeks. The AAS substances used more often were testosterone esters (94% of studies), methandrostenolone or methandienone (79% of studies), and stanozolol (67% of studies). The large diversity across different studies regarding the dose and route of AAS self-administration, made the conduction of descriptive analysis for these parameters impractical. Characteristics of the included studies are shown in summary in Table **1** and in more detail in the Electronic Supplementary Material Table S1.

Outcome definition and method of assessment: The main outcome was AAS effects on the reproductive system of athletes, as assessed by medical history and/or clinical examination (in 23/33 studies, 70%), hormone analysis (in 19/33 studies, 58%) and semen analysis (in 9/33 studies, 27%). The most common method or testosterone measurement was by radioimmunoassay (in 13/19 studies, 68%). Semen analyses were usually based on World Health Organization (WHO) guidelines at the time (in 5/9 studies, 56%).In 14/33 studies (42%) athletes were followed for a period of time following AAS cessation, lasting 12-24 weeks (25^{th}-75^{th} percentile), with a median of 16 weeks.

3.2 Outcomes of included studies

Outcomes of the 33 eligible studies included the following: (a) changes of serum hormone levels (LH, FSH, testosterone) during AAS use, and following AAS discontinuation, (b) changes of semen characteristics during AAS use and following AAS cessation, and/or (c) reproductive system changes as assessed by medical history and/or clinical examination. In order to examine changes in endogenous testosterone during AAS use, we separately examined studies in which testosterone was included in the AAS regimen and studies in which the AAS used did not contain testosterone and/or AAS compounds were such that interference with the levels of serum testosterone was unlikely (methandienone, mesterolone, nandrolone). Outcome assessment is shown in Electronic Supplementary Material Table S2 and Electronic Supplementary Material Table S3.

Hormone changes during AAS use (comparison of hormone levels prior to, and during active AAS use)

Six studies, involving 65AAS users, were included in LH and FSH meta-analysis [24, 26, 34-37]. Random-effects model suggested significant reduction in both LH (WMD: -3.37 IU/l, 95%CI: -5.05 to -1.70, $p < 0.001$) and FSH concentrations

(WMD: -1.73 IU/l, 95%CI: -2.67 to -0.79, p <0.001) during the period of AAS use (Fig.**2**). There was significant high and moderate heterogeneity across these studies (I^2=88.6%, p<0.001 and I^2=55%, p=0.049, respectively). Five studies that didn't fulfill the criteria for inclusion in the meta-analysis, showed that serum gonadotropin levels decreased from baseline when AAS use was started, or alternatively a lower level compared to controls or normal values was reported [27, 31, 38-41],whereas in 3 studies no difference was found [23, 42, 43].

Data analysis from studies in which testosterone had not been used in the AAS regimen [23, 24, 26, 34], comprising 43 AAS users, revealed a decrease in endogenous blood testosterone concentration of 10.75 nmol/L (95% CI: -15.01 to -6.49, p<0.001) during the period of AAS use (Fig.**2**). High heterogeneity was found among studies (I^2=87.8%, p<0.001). Data analysis from studies in which testosterone was self-administered as part of the AAS regimen [35, 36, 44], involving 16 AAS users, revealed a marginally non-significant increase in testosterone levels (WMD: 17.55 nmol/L, 95% CI: -0.77 to 35.86, p=0.060) (Fig. **2**). There was significant high heterogeneity across studies (I^2=75.9%, p=0.016). The study by Bonetti *et al*. [37] was excluded from the analysis as testosterone was self-administered in some but not all study participants and testosterone levels were provided for the study population as a whole only (a non-significant decrease in testosterone of 1.15 nmol/L was reported).Another 2 studies which did not provide all necessary data to be included in the meta-analysis and in which testosterone was not part of the AAS regimen, reported a lower serum testosterone level during AAS use [27, 40], whereas, as expected, the opposite was reported in studies in which testosterone was included in the regimen [31, 38, 39, 42, 43]. Remarkably, in the study of Malarkey *et al*. [31], the mean serum testosterone levels were 30 times those found in the non-AAS female weightlifters or in the normal female population.

In the study of Remes *et al*. [26], results for all 3 hormones did not differ, independent of whether the AAS agent used was DHEAS or methandienone (Sensitivity analysis, Electronic Supplementary Material Fig.S1).

Hormone changes following AAS cessation (comparison of hormone levels at the end of a period of active AAS use and after a period of AAS discontinuation)

Four studies, involving 35 AAS users, were included in LH and FSH meta-analysis [34-36, 39]. LH and FSH concentrations increased during the period following AAS withdrawal (WMD: 2.68 IU/l, 95% CI: 1.59 to 3.77, p<0.001 and WMD: 1.89 IU/l, 95%CI: 1.05 to 2.74, p<0.001, respectively) (Fig. **3**). There was moderate heterogeneity across studies (I^2=70.3%, p=0.009 andI^2=54.5%, p=0.066, respectively). Consistent with these results, the two studies that were excluded from the meta-analysis due to lack of appropriate data, showed that gonadotropin levels gradually recovered when AAS were withdrawn [41, 45].

Data analysis from studies in which testosterone was part of the AAS regimen [34-36, 39, 44], involving 34 AAS users, revealed, as expected, a decrease of testosterone levels following AAS cessation (WMD: -28.04nmol/L, 95% CI: -45.11 to -10.98, p=0.001) (Fig. **3**). High heterogeneity across studies was found (I^2=75.5%, p=0.003). In the study of Schurmeyer *et al*. [34], in which testosterone was not included in the AAS regimen, a statistically significant increase in testosterone of 10.64 nmol/L was reported.

Finally, in a study that didn't fulfill the criteria for inclusion in the meta-analysis and in which a control group was compared to a group of athletes during AAS use including testosterone, serum testosterone levels were lower compared to controls and did not increase significantly compared to the time point when AAS were withdrawn [45].

Hormone changes prior to AAS use and after a period of AAS discontinuation

Three studies [34-36], including 17 AAS users, were included in LH and FSH meta-analysis. LH and FSH concentrations were similar to baseline at the end of a period (range 13 to 24 weeks) of AAS discontinuation (WMD: 0.57 IU/l, 95% CI: -0.60 to 1.74, p=0.340 and WMD: 0.43 IU/l, 95%CI: -0.63 to 1.49, p=0.426, respectively) (Fig. **4**). There was non-significant low heterogeneity across studies (I^2=0%, p=0.524 andI^2=0%, p=0.639, respectively).

Data from 4 studies in which testosterone was self-administered as part of the AAS regimen [35, 36, 44, 46], involving 22 AAS users, revealed that serum testosterone levels at the end of a period of AAS discontinuation were lower compared to baseline levels (WMD: -9.40nmol/L, 95% CI: -14.38 to -4.42, p<0.001) (Fig. **4**). The period of AAS discontinuation in these 4 studies ranged from 13 to16 weeks apart from that in the study by Martikainen *et al.* [46], in which a shorter AAS discontinuation period of only 3 weeks was used, potentially explaining the larger serum testosterone level difference at the end of this study. There was non-significant moderate heterogeneity across studies (I^2=36.6%, p=0.192). In the study of Schurmeyer *et al*. [34], in which testosterone was not included in the AAS regimen, endogenous serum testosterone levels returned to normal after 6 months.

Semen changes during and following AAS use

Seven out of 8 studies showed impairment of numerous sperm characteristics, such as total number, concentration, motility and normal morphology [25, 34, 37, 39, 42, 43, 47]. However, in one study no significant change in spermatogenesis was observed [48]. All studies in which this outcome was assessed, showed persistent quantitative and qualitative sperm changes 8-30 weeks following AAS withdrawal [25, 34, 39, 42, 45, 47].

In general, in the above studies, changes were assessed and reported in a non-quantitative fashion. In the study by Holma [25] the "fertility index" was used (a score based on sperm numbers, motility, quality of motility, and morphology). This

index deteriorated during AAS use from 1.7±3.0 to 14.7±14.6 which is interpreted as "severely pathological" (mean values) [25].

Outcomes assessed by medical history and/or clinical examination

A number of studies reported testicular atrophy [32-34, 37, 47, 49-52] in male athletes. Following AAS withdrawal, one study found that former AAS users displayed smaller testicular volumes compared to non-users [53]. However, testicular atrophy was more prominent among current than past users, indicating that testicular size tends to normalize after AAS withdrawal [49], although this may take up to 16 weeks [34]. Some studies reported gynecomastia in males [32, 33, 37, 38, 50, 52, 54, 55], whereas 2 other studies stated no effects of AAS use regarding this outcome [34, 42]. Remarkably, in the study of Pope *et al.* [49], gynecomastia was equally common between current and past users, indicating that AAS-induced gynecomastia is often irreversible. In the only 6 studies which involved female athletes, clitoromegaly and menstrual irregularities were reported as the main AAS-related side effect during the period of AAS use [28-33].
Only 3 studies determined AAS effects on fertility. In the study of Karila *et al.* [39], subjects were asked about the number of children conceived and successful pregnancies, and 6 years following AAS cessation, 55.56% (10/18) of them reported having at least one child, whereas the same outcome during the period of AAS use was 27.78% (5/18). Also, Coward *et al.* [51] mentioned that 11.3% (9/80) of the subjects stated infertility/low sperm count during the period of AAS use in a self-reported questionnaire. Similarly, in the study of Korkia *et al.* [33], 6% of the male and 7% of the female athletes reported having fertility problems.

3.3 Risk of bias assessment

Based on the Cochrane Collaboration's tool for RCT, all studies [23, 26, 27] were rated as of unclear risk. Regarding randomization, allocation concealment and selective reporting, the total risk of bias was unclear. Concerning blinding of participants and personnel, blinding of outcome assessment and incomplete outcome data, the overall risk of bias was low.

Based on the RTI Item Bank tool, 9 cohort studies (82%) were rated as having an unclear risk of bias [24, 35-37, 41, 44-47], 1 study (9%) as having a high risk [39] and 1 more study (9%) as having a low risk of bias [34]. Moreover, of low risk were the following items: sample definition and selection (Question 1), soundness of information (Question 6), follow-up (Question 7) and interpretation of results (Question 11). All the other questions were rated as of high or unclear risk of bias. Seven cross-sectional studies (39%) were of unclear risk of bias [29, 31, 38, 40, 42, 43, 49], 10 studies (55%) were rated as high risk [28, 30, 32, 33, 50-52, 54-56] and 1 more study (6%) was of low risk [53]. Furthermore, the creation of treatment group (Question 3) and interpretation of results were rated as low risk (Question 11). All the other

questions were rated as having a high or unclear risk of bias. The results for the assessment of risk of bias are shown in the Electronic Supplementary Material Table S4.

4 Discussion

4.1 Main findings

To our knowledge this is the first comprehensive systematic review and meta-analysis, examining the effects of AAS use on the reproductive system of athletes. As few studies focused on female subjects, the meta-analysis involved exclusively studies on male athletes and recreational users. AAS use results in a state of prolonged hypogonadotropic hypogonadism in males; gonadotropin levels recover 13-24 weeks after discontinuation of AAS whereas serum testosterone remains reduced for up to 16 weeks. Moreover, systematic review of available evidence revealed that chronic AAS use results in prolonged hypogonadotropic hypogonadism in both sexes, changes of sperm characteristics, reduction of testicular volume and gynecomastia in men, as well as clitoromegaly and menstrual irregularities in females.

In almost all studies included in the meta-analysis there was reduction of serum LH and FSH levels during the period of active AAS use [24, 26, 34-37]. Specifically, AAS suppress gonadotropin release from the pituitary through negative feedback mechanisms, either directly on the pituitary gland or indirectly through suppression of the hypothalamic GnRH release. The lack of a clear effect on gonadotropin levels in some studies, may be explained by the low dosage and/or short period of AAS use, or by the self-administration of synthetic AAS with weak androgenicity compared to testosterone [23, 42, 43].

In addition, during AAS use, meta-analysis revealed a reduction of basal serum testosterone levels [23, 24, 26, 34]. In addition to causing a drop in levels of endogenous testosterone by inhibiting gonadotropin secretion, AAS use might also accelerate the metabolic clearance rate of testosterone or inhibit its biosynthesis by direct action on the gonads[24]. As expected, in studies in which exogenous testosterone was included in the AAS regimen, plasma levels of testosterone were considerably increased during AAS use [35, 36, 44].

In 2 studies, evaluation of gonadal function was assessed by the human chorionic gonadotropin (hCG) or luteinizing hormone releasing hormone (LHRH) stimulation test. Notably, in subjects that had been using high AAS doses for a period of 3 months, the testicular responsiveness to a single injection of hCG was similar to that in prepubertal boys [46]. Moreover, Holma and Adlercreutz [24] showed that in well-trained athletes who had been taking 15 mg of methandienone daily for 8 weeks, post-stimulation testosterone values after LHRH administration were lower compared

to those prior to AAS use. HCG has also been used as a secondary supplement alongside AAS use or following AAS discontinuation, in an attempt to trigger the production and release of endogenous testosterone, by mimicking LH action. In this respect, Karila *et al.*[39] suggested that when AAS use was combined with hCG, spermatogenesis was maintained, regardless of the AAS-induced suppression of gonadotropin secretion, although some structural and functional sperm changes occurred.

In theory, cyclical AAS use may allow recovery of HPG axis, resulting in less pronounced effects on the reproductive system. In the majority of the studies included in the meta-analysis, AAS were used in a continuous way. Since only 2 studies examined cyclical AAS use [37, 39], the available data were insufficient to allow an assessment of the impact of the method of AAS use (continuous or cyclical) on the reproductive system.

Following AAS withdrawal, LH and FSH levels increased to reach the levels prior to AAS use after a period of 13 to 24 weeks [34-36]. Interestingly, despite normalization of gonadotropin levels in some studies, serum testosterone remained lower compared to baseline (mean difference of 9.40 nmol/L) even up to 16 weeks after AAS withdrawal [35, 36, 44, 46], indicating prolonged impairment of the hypothalamus-pituitary-testicular axis and/or testicular atrophy. In all these studies, testosterone was included in the AAS regimen. It is possible that shorter duration of AAS use, lower AAS doses, younger age of the athletes and higher testosterone levels at baseline, are associated with a more "elastic axis", capable of recovering GnRH pulsation and gonadotropin secretion faster and more completely [57]. In these studies, in which subjects used AAS not affecting the measurement of serum testosterone, endogenous serum testosterone levels were reduced compared to basal values during AAS use (mean difference of 10.75nmol/L) [34] and returned to normal after 6 months [34].

In men, testosterone is synthesized in the testes under the regulation of LH, whereas FSH is mainly responsible for initiation of spermatogenesis. Full maturation of the spermatozoa requires the effect of both FSH and testosterone. Therefore, suppression of gonadotropins by AAS use, results in reduced endogenous testosterone production as well as a decrease in spermatogenesis and sperm production, atrophy of the seminiferous tubules and eventually testicular atrophy [9]. In most studies included in the systematic review that assessed semen changes during AAS use, a number of sperm parameters were found to be impaired [25, 34, 37, 39, 42, 43, 47] and in the 3 studies that examined these changes following AAS cessation, recovery of semen characteristics to normal, occurred within varying periods ranging from 8 to 30 weeks [25, 34, 47].

Studies that assessed AAS effects by medical history and/or clinical examination found reduced testicular volume in male athletes [32-34, 37, 47, 49-52], which tends to normalize following AAS withdrawal. Gynecomastia was reported in

several studies [32, 33, 37, 38, 49, 50, 52, 54, 55]; this may occur as a result of peripheral conversion of AAS to estrogen in those men abusing aromatisable AAS [9]. Moreover, clitoromegaly and menstrual irregularities were reported in females [28-33]. Notably, Gruber and Pope[29] attributed amenorrhea to a direct effect of AAS use, or alternatively, to the low body fat attained through the consumed low calorie diet. AAS use also seems to have a negative impact on the fertility of AAS users, as has been stated in questionnaires [33, 39, 51]. A number of factors, such as type of sport, exercise intensity with high energy expenditure, energy balance, fat composition, disordered eating behavior, physical and emotional stress, may contribute to a state of hypogonadotropic hypogonadism, evidenced clinically by menstrual irregularity in female athletes, even without AAS abuse [58, 59]. AAS use associated with prolonged periods of hypogonadism may have a number of adverse health consequences such as effects on mood and memory, reduced libido, fatigue, lipid abnormalities, low bone mineral density/osteoporosis, atherosclerosis, and increased cardiovascular risk [7, 60].

4.2 Strengths and limitations

An important strength of this study is that it is the first systematic review and meta-analysis which assesses the relationship between use of AAS and effects on the reproductive system of athletes. Furthermore, this paper includes both systematic review, as well as meta-analysis of the hormones LH, FSH and testosterone which are considered to be the main regulators of the reproductive system.

Research in the field of AAS use in sports is limited by problems and restrictions, i.e. the sample size is small due to the secret nature of drug use; self-selection may produce subjects who are not representative of the overall population of AAS users; diversity in the type, dose, duration and route of AAS use; most studies have not used urine testing to confirm AAS used; AAS may be hidden in food supplements and are not easily accessible; false positive responses on anonymous questionnaires regarding “steroids”; contemporary use of more than one AAS at high doses; different methodology of hormone measurements in serum; and possible interactions with other commonly used drugs. Additionally, the stated AAS doses in studies may have been considerably lower than the actual self-administered doses and different methods of AAS use were employed (e.g. cyclical use, stacking, pyramiding).

However, two main limitations include the fact that the majority of included studies were rated as having unclear risk of bias and also many studies were not considered eligible to be included in the meta-analysis, thus limiting the possibility of achieving greater power and more robust associations. Moreover, eligible studies did not control for potential confounding factors, such as age, gender, type of exercise and different AAS characteristics, which can be responsible for a spurious relationship between AAS use and the observed effects on the reproductive system of athletes.

Notably, there were a few studies conducted in female athletes, and as a result limited data were available concerning AAS effects on the female reproductive system, as well as on fertility. For the same reason, meta-analysis involved exclusively studies on male athletes.

4.3 Conclusion

In conclusion, the present meta-analysis showed that serum gonadotropin and endogenous testosterone levels decreased during a period of active AAS use in male athletes. Hormone levels gradually returned to normal, although serum testosterone remained lower compared to baseline several weeks following AAS cessation. Moreover, systematic review of the literature revealed that the effects of chronic AAS use include testicular atrophy, gynecomastia and impairment of sperm characteristics in men, as well as clitoromegaly and menstrual irregularities in women, potentially affecting fertility in both sexes. AAS abuse has negative, potentially serious long-terms effects in the reproductive system and general health of users; further action is necessary to manage this global public health issue, together with education of the public, athletes, trainers and health care providers.

Compliance with ethical standards

Funding No funding was obtained.

Conflict of interest (COI) statement

Maria A. Christou, Panagiota A. Christou, Georgios Markozannes, Agathocles Tsatsoulis, George Mastorakos and Stelios Tigas declare that they have no conflict of interest.

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Figure captions

Fig. 1 PRISMA flow diagram

Fig. 2 Hormone changes during AAS use

Fig. 3 Hormone changes following AAS cessation

Fig. 4 Hormone changes during the overall period

Electronic Supplementary Material Fig. S1 Sensitivity analysis