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Review Article

Impact of anabolic steroid consumption on biochemical and hematological parameters in bodybuilders: A systematic review and evidence gap mapping

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ABSTRACT

The consumption of anabolic androgenic steroids (AAS) by competitive and non-competitive bodybuilders is relatively common, yet there is diverse and often conflicting evidence on the short- and long-term side-effects of AAS abuse. We aimed at assessing the impact of AAS use (supraphysiological doses/schedule) on adult bodybuilders by means of a broad systematic review and evidence gap mapping (CRD42023401245). Electronic searches in PubMed, Scopus, and Web of Science were performed (Apr-2024). The methodological quality of the included studies was evaluated using the Effective Public Health Practice Project tool. An evidence gap map considering the most reported parameters (e.g., liver, kidney, hematopoietic system) was built. Twenty-two studies (1,023 bodybuilders, of which 662 AAS-users) published between 1987 and 2022, mostly by North America ($n = 5$ studies; 22.7 %) and West Asia ($n = 5$; 22.7 %) and mainly designed as cross-sectional case-controls ($n = 17$; 77.3 %) were synthesized. Testosterone, nandrolone, and stanozolol were the most consumed substances. Altogether, studies reported at least 30 different parameters. Although some parameters, such as urea levels, did not significantly differ between AAS users vs. nonusers ($p > 0.05$), an increase in both serum alanine and aspartate aminotransferases and a decrease in follicle stimulating and luteinizing hormones ($p < 0.05$) were reported in AAS users. Evidence is conflicting on the effect of steroids on cholesterol, triglyceride, high density lipoprotein, and low-density lipoprotein levels. Very few studies reported data on hematological parameters. The overall methodological quality of the studies was judged as weak-to-moderate. Further larger and well-designed studies to properly inform about the benefits and risks of AAS on other outcomes are still needed.

1. Introduction

Anabolic-androgenic steroids (AAS) are a class of hormones, including endogenously produced androgens (e.g., testosterone) as well as synthetically manufactured derivatives (e.g., trenbolone, stanozolol, testosterone, nandrolone, oxymetholone, oxandrolone) whose name derives from the chemical structure (steroid nucleus) and their anabolic and androgenic effects. AAS can be administered orally, parenterally by intramuscular injection or via transdermal (Bird et al., 2016; Bordin et al., 2017). These substances are widely abused for their high strength-increasing properties and muscle-building effects, in nontherapeutic dosages, including by bodybuilders (individuals who strengthen and enlarge the muscles of their body through strenuous exercise or resistance training), given their ability to improve muscle growth for aesthetic purposes and athletes' performance, minimizing androgenic effects (Bond et al., 2022).

Nonetheless, the real epidemiology and possible consequences of AAS abuse still represent a matter of discussion given the challenges in conducting rigorous scientific research in this field (i.e., ethical concerns), obtaining accurate data on the prevalence and patterns of AAS abuse (i.e., underreporting as individuals who use AAS may not openly admit to it due to social stigma, legal implications, or concerns about judgment), modifications in AAS formulations and availability over time (i.e., new substances entering the market), and diverse practices of use

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(i.e., dosages and combinations of substances can vary widely) (Anawalt, 2019; Corona et al., 2022). These factors can make it difficult to detect and quantify these drugs in competitive athletes. Evidence shows that although a significant percentage of elite athletes declare past use of AAS, only a few tests are positive. For noncompetitive athletes, this picture is even more complex, as the consumption of AAS is not monitored nor regulated by doping control by the World Anti-Doping Agency (WADA) in most countries (Corona et al., 2022; Pope et al., 2014). A meta-analysis (187 studies) providing data from 271 lifetime prevalence rates showed a global rate of AAS use of 3.3 %, being four times higher in males than in females (6.4% vs. 1.6 %). These figures can be even higher in elite professional athletes (44 %−70 %) and noncompetitive athletes (75 % of AAS users), meaning that the prevalence of steroid use is similar to that observed for other chronic diseases (e.g., type 1 diabetes) if we consider the US American male population (Pope et al., 2014; Sagoe et al., 2014).

Chronic nonmedical high-dose abuse of AAS (frequently combined with other illicit substances) is associated with irreversible adverse effects in different organs and systems, including long-term toxicity in cardiovascular and reproductive systems and overall mortality risk, which lead to important burdens to the individual, its family/caregivers, and the healthcare system (Corona et al., 2022; Seara et al., 2020; Smoliga et al., 2023). In fact, previous systematic reviews focusing on the effects of AAS on overall male sexual and reproductive functions, demonstrated an association of substance abuse and hypogonadism (due to the capacity of exogenous AAS to suppress the hypothalamic-pituitary-testicular (HPT) function) with persistently low gonadotropin and testosterone levels, lasting for several weeks to months after AAS withdrawal (Christou et al., 2017; Corona et al., 2022; Esposito et al., 2023). The recent meta-analysis ($n = 24$ clinical studies) from Corona et al., 2022 (Corona et al., 2022) additionally showed AAS impairing sperm production and leading to side effects such as acne, hair loss, and gynecomastia, besides some alterations to the metabolic profile.

Nonetheless, despite AAS use for athletic purposes being first noted among the United States bodybuilding community in the 1950s (Kanayama & Pope, 2018), the real-world evidence on the physiological effects of substances in this specific population (i.e., more prone to chronic consumption; at higher doses) remains neglected (Pope et al., 2014), with scattered evidence published over the past years. Moreover, available studies are often limited to assessing body composition, nutritional strategies, and muscles/tendon traumas (Bauer et al., 2023; Grandperrin et al., 2021; Mitchell et al., 2017; Tidmas et al., 2022), with scarce attention given to laboratory parameters, despite their critical role in health screening and preventive medicine (e.g., risk factor identification) and for diagnostic purposes. Thus, the aim of this study was to synthesize and update the available data on the effects of AAS on biochemical and hematological parameters, enabling prompt information dissemination to researchers, practitioners, and athletes about the functional status of bodybuilders. This was achieved through a comprehensive systematic review and original evidence gap analysis.

2. Material and methods

This study was performed in accordance with the Cochrane Collaboration recommendations (i.e., methodological and editorial standards for conducting systematic reviews) and reported following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement (an evidence-based minimum set of items for reporting in reviews) (Higgins & Thomas, 2021; Page et al., 2021). The protocol of this study is registered at PROSPERO (CRD42023401245).

2.1. Search strategy and eligibility criteria

A comprehensive literature search was conducted to find relevant studies in PubMed, Scopus, and Web of Science without timeframe or language limits (last updated April 2024). A manual search in the reference lists of the included studies, conventional search engines (Google and Google Scholar), and registration databases (clinicaltrials.gov) was also performed. The complete search strategies developed using descriptors related to steroids, bodybuilding, and biochemical/hematologic parameters combined with Boolean operators AND and OR are available in Supplementary Material 1.

Registers retrieved from the databases were uploaded into Endnote X7 (reference manager), where duplicate records were removed. As recommended by the Cochrane Collaboration (Higgins & Thomas, 2021), two reviewers independently performed all the steps of studies' selection: (i) screening (i.e., title/abstract reading to check for articles' relevance), (ii) and the full-text reading phase (i.e., inclusion of studies according to the eligibility criteria) using Microsoft Excel 2013 sheets. After that, data extraction and methodological quality assessment of the included studies were performed by a single reviewer and checked by another trained reviewer using Microsoft Excel 2013 sheets. Discrepancies during all these steps were discussed with a third researcher.

This systematic review included articles meeting the following criteria (PICOS' acronym):

- Population: studies evaluating bodybuilders at any age, sex/gender;
- Interventions: studies on chronic nonmedical use of any AAS dose/regimen/schedule);
- Comparator: no use of steroids (non-exposed group or nonusers) or studies without a comparator group (i.e., single arm study);
- Outcomes: studies assessing at least one of the following outcomes: any biochemical or hematological parameters measured in blood samples (no restriction) and able to provide information about functional status (e.g., liver, kidney, hematopoietic system) were extracted (e.g., lipid profile [such as cholesterol, triglyceride, lipoprotein levels], urea, creatinine, albumin, aminotransferases, phosphatase, hormones [such as gonadotropins and testosterone levels], hemoglobin, platelets, blood count);
- Study design: primary interventional or observational analytical studies (cohort, cross-sectional, case-control).

Studies without data for extraction (unavailable information), other study designs (reviews, pharmacokinetic trials, case reports, letters), articles assessing economic outcomes, and those in non-Roman characters (e.g., Arabic, Cyrillic, Hebrew, Chinese) were excluded.

2.2. Data extraction and methodological quality assessment

A standardized form (Microsoft Excel, Redmond, WA) was used to systematically extract information on: articles' general data (including authors name, year of publication, country/continent, sample size); participants and their characteristics (age, sex whenever available); details of the intervention and controls (type of AAS and combinations, regimen); study design; clinical outcomes results.

The methodological quality of the included studies was evaluated using the Effective Public Health Practice Project tool (EPHPP) which can be applied to studies of different designs on any public health topic (Thomas et al., 2004). This tool classifies studies' quality in 'strong', 'moderate' and 'weak' by incorporating the evaluation of the following sources of bias (i.e., components of the tool): study design (randomized trials, observational studies), selection bias (i.e., representative of the target population), analysis/confounders (i.e., control of confounding factors), blinding (i.e., blinding of participants and outcome assessor), withdrawals and dropouts (i.e., follow-up rate of participants), data collection practices (i.e., use of valid and reliable tools). Overall, studies with no 'weak' rating and at least three strong ratings were considered 'strong', while those with less than three 'strong' ratings and one 'weak' rating were considered 'moderate'. Studies with two or more 'weak' ratings were judged as 'weak'. See Supplementary Material 2 for details on the component ratings of studies.

2.3. Data synthesis and evidence gap mapping

Individual results of the studies were summarized as reported by the authors, including types of measures and units (narrative synthesis). Additionally, an evidence gap map structured around the most reported outcomes and the methodological quality of the included studies was built. This approach provides a visual overview of the breadth and availability of information in a given area and highlights the gaps in current evidence, which may ground further research, decision-making, and policy development (Snilstveit et al., 2016). No meta-analyses were possible given the high heterogeneity among studies in terms of design, population, comparators, and reported outcomes.

3. Results

The search strategy retrieved 367 records after duplicate removal, of which 323 were excluded during the screening process (i.e., irrelevant to the study goal). Sixteen records were excluded after full-text appraisal because they were lacking in assessing biochemical or hematological parameters of interest ($n = 14$ studies evaluating either cardiovascular risks or heart abnormalities, blood pressure, body composition, and anthropometry), did not evaluate the use of AAS ($n = 1$ study on dietary supplements), and had a different study design ($n = 1$ case-series) (see complete list of excluded studies with full reasons for exclusion in Supplementary Material 3). The remaining 28 records for data extraction and analyses refer to 22 original primary studies (i.e., the findings of some primary studies were published in more than one article) (Fig. 1) (Al-Janabi et al., 2011; Albakaa et al., 2020; Aliakbar & Vahid, 2009; Arazi, 2018; De Francesco Daher et al., 2018; Dickerman et al., 1997, 1999; Graham et al., 2006; Hartgens et al., 1996, 2004; Hassan et al., 2021; Hislop et al., 1999, 2001; Inigo et al., 2000; Keith et al., 1996; Kleiner et al., 1989; McKillop & Ballantyne, 1987; Moffatt et al., 1990; Rasmussen et al., 2017, 2016; Sader et al., 2001; Schwingel et al., 2011; Smit, Bond et al., 2022, Bond et al., 2022; Smit et al., 2021, 2020; Smit, Grefhorst et al., 2022; Torres-Calleja et al., 2001; Vahid et al., 2009). No other study was found through manual searches.

These 22 studies ($n = 1023$ bodybuilders of which $n = 662$ AAS users) were published between 1987 and 2022, being mostly conducted in Europe ($n = 7$; 31.8 % - being three in the Netherlands, two in the UK, one in Spain, and one in Denmark), followed by North America ($n = 6$; 27.3 % being five in the US and one in Mexico), West Asia ($n = 5$; 22.7 % - three in Iraq and two in Iran), and South America - Brazil ($n = 2$; 9.1 %). Australia and South Africa had one study each. Most studies ($n = 17$; 77.3 %) were designed as cross-sectional case-controls (observational comparative studies) and presented a group of 'nonusers' of AAS as controls. Four (18.2 %) studies were of single-arm design (cohorts or pre-post analyses), and one (4.5 %) was a randomized controlled trial. Males with ages varying from 15 to 45 years were the evaluated population in all studies, except in the analyses of De Francesco et al., 2018 and Moffatt et al. 1990 (De Francesco Daher et al., 2018; Moffatt et al., 1990) where females accounted for 39 % and 100 % of the cases, respectively. The duration of AAS use was variable among studies, ranging from at least 6 weeks in pre-post analyses to over ten years in cohorts; in the US, medians were 3 years of use. The most consumed substances were testosterone ($n = 13$ studies; 59.1 %), nandrolone ($n = 13$; 59.1 %), stanozolol ($n = 12$; 54.5 %), boldenone ($n = 6$; 27.3 %), oxymetholone ($n = 6$; 27.3 %) and methadienone ($n = 4$; 18.2 %). See the main characteristics of the studies in Table 1.

Altogether, studies reported at least 30 different biochemical or hematological parameters, yet using different measures, thresholds, or units (i.e., high between-study heterogeneity). Table 2 depicts the complete results according to the studies' design and for both AAS users

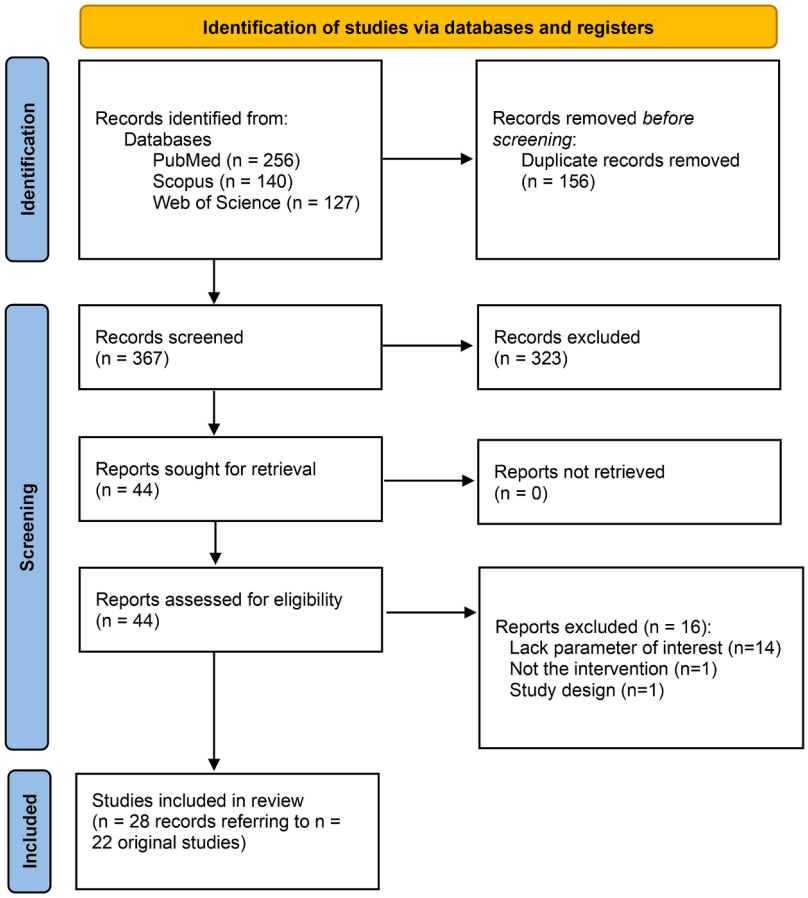

Fig. 1. Flowchart of the systematic review.

Table 1
Baseline characteristics of the included studies ($n = 22$) according to methodological design.

Authors	Year	Study design	Continent, Country	N	Age, y mean (SD)	Males, N (%)	Steroids used in AAS group	Duration of use/ cycle
Observational comparative studies (at least two groups)								
Albakaa A et al.	2020	Cross-sectional case-control	West Asia, Iraq	60 AAS users 30 nonusers	15–45	–	–	–
Al-Janabi A et al.	2011	Cross-sectional case-control	West Asia, Iraq	16 AAS users 8 nonusers	15–28	24 (100 %)	Methandienone Nandrolone Testosterone	12 weeks
Arazi H et al.	2018	Cross-sectional case-control	West Asia, Iran	20 AAS users 20 nonusers	25.0 ± 2.9 24.2 ± 3.1	40 (100 %)	Boldenone Nandrolone Oxandrolone Sustanon Stanozolol Testosterone Trenbolone	1–3 years
De Francesco E al.	2018	Cross-sectional case-control	South America, Brazil	28 AAS users 29 nonusers	26.0 ± 5.0 31.0 ± 9.0	25 (89.3 %) 16 (55.2 %)	Boldenone Stanozolol Testosterone	at least 2 months
Dickerman RD et al.	1997	Cross-sectional case-control	North America, USA	6 AAS users 6 nonusers	25.5 ± 6.0 26.2 ± 3.0	12 (100 %)	Testosterone	–
Dickerman RD et al.	1999	Cross-sectional case-control	North America, USA	15 AAS users 10 nonusers	26.9 ± 2.5 27.0 ± 1.3	25 (100 %)	–	–
Graham MR et al.	2006	Cross-sectional case-control	Europe, UK	10 AAS users 10 nonusers	42.4 ± 3.8 43.1 ± 4.6	20 (100 %)	Boldenone Methandienone Methenolone Nandrolone Oxymetholone Stanozolol Testosterone Trenbolone	>10 years
Hartgens F et al.	1996	Cross-sectional case-control	Europe, Netherlands	16 AAS users 12 nonusers	30.3 ± 7.2 28.8 ± 7.7	30 (100 %)	Boldenone Methandrostenolone Methenolone Nandrolone Oxymetholone Stanozolol Testosterone	at least 3 months
Hassan EA et al.	2021	Cross-sectional case-control	West Asia, Iraq	23 AAS users 15 nonusers	23.00 ± 1.78	38 (100 %)	–	at least 2.5 years
Hislop MS et al. Hislop MS et al.	1999 2001	Cross-sectional case-control	Africa, South Africa	9 AAS users 8 nonusers	25.0 ± 2.0 28.0 ± 11.0	17 (100 %)	Nandrolone Stanozolol Testosterone	6.5 weeks
Kleiner SM et al.	1989	Cross-sectional case-control	North America, USA	18 AAS users 17 nonusers	29.5 ± 6.5 25.6 ± 4.8	35 (100 %)	Methandrostenolone Methenolene Nandrolone Oxandrolone Oxymetholone Stanozolol Testosterone	3.0 ± 2.4 years (range 0–10)
McKillop G et al.	1987	Cross-sectional case-control	Europe, UK	8 AAS users 8 nonusers	25.0 ± 3.8 25.0 ± 2.5	16 (100 %)	Methandienone Nandrolone Stanozolol	at least 3 months
Moffatt RJ et al.	1990	Cross-sectional case-control	North America, USA	9 AAS users 8 nonusers	22.5 ± 4.9 23.8 ± 5.1	0 (all females)	Drostranolol Methandrostenolone Methenolene Nandrolone Stanozolol	at least 3 years
Rasmussen JJ et al. 2016 2017 Rasmussen JJ et al.		Cross-sectional case-control	Europe, Denmark	37 AAS users 33 former users 30 nonusers	34.8 ± 1.2 31.4 ± 1.4 31.5 ± 1.2	100 (100 %)	–	at least 3 months
Sader MA et al.	2001	Cross-sectional case-control	Oceania, Australia	10 users 10 nonusers	37.0 ± 3.1 34.0 ± 3.0	20 (100 %)	–	at least 24 months
Schwingel et al.	2011	Cross-sectional case-control	South America, Brazil	180 AAS users 85 nonusers	26.4 ± 5.9 28.1 ± 6.6	265 (100 %)	Boldenone Methenolone Nandrolone Stanozolol Testosterone	4.0 (2–10) years
Torres-Calleja J et al.	2000	Cross-sectional case-control	North America, Mexico	15 AAS users 15 nonusers	26.6 ± 64.1 26.0 ± 4.1	30 (100 %)	Methenolone Nandrolone Oxymetholone Testosterone	at least 4 weeks
Observational single-arm studies								
Aliakbar R et al. Vahid et I al.	2009 2009	Pre-post study	West Asia, Iran	10 AAS users	–	20 (100 %)	Oxymetholone	6 weeks
Inigo MA et al.	2000	Cohort (1997–1998)	Europe, Spain	39 AAS users	27.6 ± 4.8	39 (100 %)	Methandienone Nandrolone	1 year (43 cycles)

(*continued on next page*)

Table 1 (*continued*)

Authors	Year	Study design	Continent, Country	N	Age, y mean (SD)	Males, N (%)	Steroids used in AAS group	Duration of use/ cycle
Keith RE et al.	1996	Cross-sectional	North America, USA	14 AAS users	26.0 ± 6.0	14 (100 %)	Stanozolol Testosterone Methandrostenolone Nandrolone Oxandrolone Oxymetholone Stanozolol Testosterone	2–10 years
Smit DL et al. 2020 Smit DL et al. 2021 Smit DL et al. 2021 Smit DL et al. 2022		Cohort	Europe, Netherlands	100 AAS users	31.0 ± 8.4	100 (100 %)	Boldenone Stanozolol Testosterone	13 (2–52) weeks cycle
Interventional studies								
Hartgens F et al.	2004	Randomized double blind trial	Europe, Netherlands	19 AAS users 7 nonuser/ placebo	33.0 ± 8.0 31.0 ± 8.0	26 (100 %)	Nandrolone	at least 8 weeks

USA: United States of America; UK: United Kingdom.

and control group/post analyses and reference values (when reported by authors). Overall, the use of AAS did not impair some parameters, such as urea, when compared to nonusers ($p > 0.05$ in most studies). Conversely, studies demonstrate that the use of steroids led to a significant increase in both serum alanine (ALT) and aspartate aminotransferases (AST) in users vs. non users [$p < 0.05$ reported by Arazi et al. 2018 (Arazi, 2018); Dickerman et al. 1999 (Dickerman et al., 1999) and Schwingel et al. 2011 (Schwingel et al., 2011). Moreover, according to most studies, the use of steroids reduced the levels of some hormones in users compared to nonusers, including follicle stimulating hormone (FSH) and luteinizing hormone – LH ($p < 0.01$) (Hassan et al., 2021; Sader et al., 2001; Torres-Calleja et al., 2001). Rasmussen et al. 2016-2017 additionally demonstrated this significant decrease ($p < 0.01$) comparing median FSH values from users (0.3 [0.1–0.4] U/I) to nonusers and to former users (4.2 U/I and 4.4 U/I, respectively); similar results were found for LH (median 0.1 U/I for users vs. 3.1 U/I in nonusers and 3.6 U/I in former users; $p < 0.01$) (Rasmussen et al., 2017, 2016). Yet, evidence is conflicting on the effect of these substances on individuals' lipid profiles, including cholesterol, triglyceride (TG), high density lipoprotein (HDL) and low-density lipoprotein (LDL) levels, with some studies demonstrating significant changes (either higher or lower values compared to nonusers or controls) and others revealing no meaningful differences among populations. The evidence is scarce (only one to two studies) for other outcomes, especially hematological parameters such as red blood cells, leucocytes, or platelet counting. Other biochemical parameters, including glucose, bilirubin, and enzymes (alkaline phosphatase, creatine kinase) were also poorly assessed (see Table 2).

The overall methodological quality of the studies was mostly judged as weak (with 5 (27.3 %) studies presenting at least two poorly conducted and reported domains, i.e., important sources of bias) to moderate (with 13 (59.1 %) studies with some methodological concerns). Only 3 (13.6 %) studies - two cohorts and the only randomized trial were judged as having strong methodological quality (i.e., no weak rating and at least tree strong ratings on the domains of study design, selection bias, confounders, blinding, data collection, and withdrawals and dropouts reporting). Besides the potential bias arising from studies' design (most observational cross-sectional studies), the sign and balance of confounders as well as the blinding of outcome assessors were poorly acknowledged by studies – which may reduce data reliability. Conversely, the domains of data collection and reporting of withdrawals and drop-outs were associated with moderate-to-stronger quality (a complete assessment is available in Supplementary Material 3).

Table 3 summarizes the findings of this systematic review by using an evidence gap map that grouped the results of the most reported outcomes of the included comparative studies of AAS users vs. nonusers (i.e., for the purpose of data homogeneity) and additionally considered the methodological quality of the evaluated primary studies.

4. Discussion

This systematic review, with an unprecedented evidence gap mapping, synthesized, updated, and critically appraised the methodological quality of 22 primary studies published between 1987 and 2022 on the most reported effects on laboratory parameters (biochemical and hematological) of the use of AAS in bodybuilders. These clinical findings presented in one single model may better inform policy makers, practitioners, and other stakeholders about the available evidence on the real benefit-risk ratio of these substances for this specific population (i.e., provision of some insights about potential effects on functional status, e.g., liver, kidney, hematopoietic system), which can underpin updated guidance for developing (or improving) and implementing interventions for reducing harms associated with the use of AAS (including monitoring healthcare programs for routinely assessing laboratory parameters in this population). Moreover, these results can guide the development of further well-designed and standard-reporting primary studies in the field for some biochemical and hematological parameters, considering the weak-to-moderate methodological quality of most studies.

Although previous evidence from the literature highlighted that short and especially long-term use of AAS are related with several physical disorders (e.g., wide variety of cardiovascular, metabolic, endocrine, neurologic, psychiatric, infectious, hepatic, renal, and musculoskeletal disorders), psychological symptoms (e.g., depressive symptoms, dependence, antisocial and violent behaviors, suicidality) and fatal side effects (Baron et al., 2007; Pope et al., 2014), evidence is still controversial. This study overall confirms that the use of AAS in bodybuilders can affect a broad range of laboratory parameters – which should be carefully evaluated by practitioners, especially in primary care (Table 3); yet a significant heterogeneous report among studies and important confounders still exist, which should also be considered during decision-making processes. In fact, the majority of the data on the effects of supraphysiological dosages of AAS are derived from case reports and series, case-control studies, and cross-sectional studies of men, as observed in this study (Anawalt, 2019).

Moreover, it is hard to attribute the observed effects to a single AAS, especially because the use of combined substances – often a mixture of AAS, tobacco, alcohol, marijuana, or other illicit substances – in sports practitioners is commonplace. A recent study demonstrated by means of toxicological urinalysis that around 27 % of AAS users also consume amphetamine, MDMA, and cocaine, while 15 % use cannabis (Bordin et al., 2017). In addition, AAS obtained on the open market or through the internet might be counterfeit or contaminated with other substances,

Table 2
Most reported biochemical and hematological parameters by the included studies.

Authors	Year	Sample	Parameters	Results (AAS users)	Results (control group or post analyses)	p-value*	Reference values#
Observational comparative studies (at least two groups)							
Albakaa A et al.	2020	60 AAS users 30 nonusers	Urea	30.5 ± 11.69 mg/dL	28.45 ± 8.35 mg/dL	$p > 0.05$	–
			Creatinine	1.1 ± 0.31 mg/dL	0.76 ± 0.11 mg/dL	***p < 0.05***	
			Albumin	3.76 ± 0.82 g/dL	2.84 ± 0.21 g/dL	***p < 0.05***	
Al-Janabi A et al.	2011	16 AAS users 8 nonusers	FSH	5.2 mIu/ml	3.2 mIu/ml	$p > 0.05$	1.7–12 mIu/ml
			LH	3.1 mIu/ml	3.3 mIu/ml	$p > 0.05$	1.1–7 mIu/ml
			Prolactin	18.2 ng/ml	32.2 ng/ml	***p < 0.05***	1.5–19 ng/ml
			Testosterone	3.5 ng/ml	5.3 ng/ml	$p > 0.05$	3–10.6 ng/ml
Arazi H et al.	2018	20 AAS users 20 nonusers	HDL	30.7 ± 10 mg/dL	43.5 ± 15.2 mg/dL	***p = 0.02***	–
			LDL	179.2 ± 34.1 mg/dL	155.8 ± 37.7 mg/dL	***p = 0.04***	
			Cholesterol	253.2 ± 59.6 mg/dL	143.5 ± 48 mg/dL	***p = 0.01***	
			TG	166.5 ± 74.4 mg/dL	126.9 ± 48.2 mg/dL	***p = 0.04***	
			AST	53.2 ± 14.3 IU/L	34.5 ± 11.1 IU/L	***p = 0.02***	
			ALT	53.5 ± 15.1 IU/L	33.3 ± 7.8 IU/L	***p = 0.02***	
De Francesco E al.	2018	28 AAS users 29 nonusers	Creatinine	1.04 ± 0.17 mg/dL 33.0 ± 9.5 mg/dL	0.88 ± 0.14 mg/dL 29.8 ± 6.3 mg/dL	***p < 0.001***	–
			Urea			$p = 0.141$	
			Cystatin C	0.64 ± 0.46 mg/L	0.43 ± 0.36 mg/L	$p = 0.06$	
Dickerman RD et al.	1997	6 AAS users 6 nonusers	Cholesterol	107.8 ± 15.0 mg/dL	159.0 ± 41.3 mg/dL	***p < 0.05***	–
			TG	63.5 ± 19.5 mg/dL	136.7 ± 56.5 mg/dL	***p < 0.05***	
			HDL	24.2 ± 5.8 mg/dL	42.7 ± 6.1 mg/dL	***p < 0.05***	
			LDL	70.8 ± 10.2 mg/dL	89.0 ± 38.9 mg/dL	$p > 0.05$	
Dickerman RD et al.	1999	15 AAS users 10 nonusers	Testosterone	18.3 ± 1.2 ng/mL	5.7 ± 0.7 ng/mL	***p < 0.05***	3–9 ng/mL
			AST	63.5 ± 8.7 U/L	46.0 ± 5.3 U/L	***p < 0.05***	5–40 U/L
			ALT	81.1 ± 11.9 U/L	50.7 ± 4.9 U/L	***p < 0.05***	7–56 U/L
			CK	1395 ± 402 U/L	801 ± 164 U/L	***p < 0.05***	30–170 U/L
			LDH	549 ± 39 U/L	528 ± 37 U/L	***p < 0.05***	313–618 U/L
			GGT	24.1 ± 3/2 U/L	31.6 ± 2.9 U/L	***p < 0.05***	8–78 U/L
			Bilirubin	0.58 ± 0.05 mg/dL	0.55 ± 0.05 mg/dL	***p < 0.05***	0.2–1.3 mg/dL
Graham MR et al.	2006	10 AAS users 10 nonusers	Testosterone	69.4 ± 7.1 nmol/L	16.6 ± 4.9 nmol/L	***p < 0.001***	–
			Urea	5.9 ± 2.9 mmol/L	4.5 ± 1.7 mmol/L	$p > 0.05$	
			Creatinine	107 ± 14.5 mmol/L	96.1 ± 9.2 mmol/L	$p > 0.05$	
			HCT	55.7 ± 2.1%	45.6 ± 2.2%	$p > 0.05$	
Hartgens F et al.	1996	16 AAS users 12 nonusers	Cholesterol	4.94 ± 0.91 mmol/L	4.69 ± 0.98 mmol/L	$p > 0.05$	4.1–6.4 mmol/L
			HDL	1.02 ± 0.35 mmol/L	1.05 ± 0.23 mmol/L	$p > 0.05$	0.6–1.9 mmol/L
			TG	1.28 ± 0.51 mmol/L	1.18 ± 0.60 mmol/L	$p > 0.05$	0.80–1.94 mmol/L
			ALP	58.3 ± 13.1 U/L	85.9 ± 19.7 U/L	***p < 0.05***	
			GGT	19.7 ± 9.3 U/L	21.1 ± 7.5 U/L	$p > 0.05$	30–125 U/L <50 U/L
Hassan EA et al.	2021	23 AAS 15 nonusers	TSH	2.06 ± 0.20 μIU/L	2.70 ± 0.32 μIU/L	$p = 0.367$	–
			T3	1.70 ± 0.06 nmol/L	2.54 ± 0.15 nmol/L	***p < 0.001***	
			T4	90.18 ± 4.31 nmol/L	95.28 ± 6.97 nmol/L	$p = 0.570$	
			FSH	3.02 ± 0.34mIU/mL	5.85 ± 0.14 mIU/mL	***p = 0.001***	
			LH	5.96 ± 0.76mIU/mL	8.78 ± 0.57 mIU/mL	***p = 0.007***	
			Testosterone	1.03 ± 0.10 ng/mL	5.48 ± 0.23 ng/mL	***p = 0.001***	
Hislop MS et al. Hislop MS et al.	1999 2001	9 AAS users 8 nonusers	Cholesterol	4.83 ± 1.17 mmol/L	4.9 ± 0.9 mmol/L	$p > 0.05$	–
			HDL	0.7 ± 0.3 mmol/L	1.2 ± 0.1 mmol/L	$p > 0.05$	
			LDL	3.7 ± 1.3 mmol/L	3.0 ± 0.3 mmol/L	$p > 0.05$	
			Insulin	4.8 ± 1.67 mU/mL	5.15 ± 3.79 mU/mL	$p > 0.05$	
			Leptin	1.63 ± 0.37 ng/mL	2.94 ± 1.89 ng/mL	$p > 0.05$	
Kleiner SM et al.	1989	18 AAS users 17 nonusers	TG	96.6 ± 42.0 mg/dL	86.6 ± 46.3 mg/dL	$p > 0.05$	–
			Cholesterol	203.5 ± 38.7 mg/dL	178.0 ± 38.0 mg/dL	**p = 0.048**	
			LDL	141.8 ± 33.5 mg/dL	115.5 ± 32.2 mg/dL	**p = 0.029**	
			HDL	42.3 ± 9.9 mg/dL	45.3 ± 9.2 mg/dL	$p > 0.05$	
McKillop G et al.	1987	18 AAS users 17 nonusers	Cholesterol	7.45 ± 1.37 mmol/L	4.82 ± 0.98 mmol/L	***p < 0.01***	–
			TG	1.92 ± 1.32 mmol/L	1.15 ± 0.53 mmol/L	$p > 0.05$	
			LDL	6.25 ± 1.29 mmol/L	3.13 ± 0.70 mmol/L	***p < 0.0001***	
			HDL	0.41 ± 0.29 mmol/L	1.11 ± 0.31 mmol/L	***p < 0.001***	
Moffatt RJ et al.	1990	9 AAS users 8 nonusers	Cholesterol	4.70 ± 0.81 mmol/L	4.56 ± 0.21 mmol/L	$p > 0.05$	–
			TG	3.44 ± 1.44 mmol/L	1.93 ± 0.22 mmol/L	***p < 0.05***	
			LDL	3.27 ± 1.01 mmol/L	2.51 ± 0.17 mmol/L	$p > 0.05$	
			HDL	0.75 ± 0.53 mmol/L	1.70 ± 0.11 mmol/L	***p < 0.05***	
Rasmussen JJ et al. Rasmussen JJ et al.	2016 2017	37 AAS users 33 former 30 nonusers	Testosterone	98.3 (47–123) nmol/L	18.8 nmol/L (nonuser)	***p < 0.01***	–
			FSH	0.3 (0.1–0.4) U/I	14.4 nmol/L (former)	***p < 0.01***	
			LH	0.1 (0.1–0.1) U/I	4.2 U/I (nonuser) 4.4 U/I (former) 3.1 U/I (nonuser) 3.6 U/I (former)	***p < 0.01***	
Sader MA et al.	2001	10 users 10 nonusers	Cholesterol	4.9 ± 0.5 mmol/L	4.7 ± 0.3 mmol/L	$p > 0.05$	–
			TG	1.2 ± 0.2 mmol/L	1.0 ± 0.2 mmol/L	$p > 0.05$	
			HDL	0.6 ± 0.1 mmol/L	1.4 ± 0.1 mmol/L	***p < 0.001***	
			Testosterone	10.7 ± 5.9 nmol/L	22.7 ± 3.7 nmol/L	***p < 0.05***	

(*continued on next page*)

Table 2 (*continued*)

Authors	Year	Sample	Parameters	Results (AAS users)	Results (control group or post analyses)	p-value*	Reference values#
			Estradiol	117 ± 29 pmol/L	142 ± 30 pmol/L	$p > 0.05$	
			LH	1.2 ± 0.4 U/I	5.6 ± 1.2 U/I	***p < 0.01***	
			FSH	1.0 ± 0.3 U/I	3.7 ± 0.5 U/I	***p < 0.001***	
Schwingel et al.	2011	180 AAS users 85 nonusers	Glucose	78.9 ± 9.9 mg/dL	84.3 ± 15.3 mg/dL	***p = 0.005***	–
			Cholesterol	167.8 ± 32.2 mg/dL	171.1 ± 37.3 mg/dL	$p = 0.52$	
			HDL	44.5 ± 17.8 mg/dL	45.9 ± 12.2 mg/dL	$p = 0.55$	
			LDL	108.4 ± 30.2 mg/dL	108.2 ± 28.0 mg/dL	$p = 0.97$	
			TG	106.9 ± 85.1 mg/dL	103.4 ± 43.9 mg/dL	$p = 0.74$	
			AST	55.1 ± 68.3 U/L	32.7 ± 13.3 U/L	***p = 0.002***	
			ALT	43.1 ± 29.2 U/L	30.9 ± 12.9 U/L	***p < 0.001***	
			GGT	30.6 ± 29.2 U/L	27.6 ± 11.5 U/L	$p = 0.38$	
Torres-Calleja J et al.	2000	15 AAS users 15 nonusers	LH	4.1 ± 4.9 mIU/mL	5.0 ± 1.9 mIU/mL	$p > 0.05$	–
			FSH	1.4 ± 3.2 mIU/mL	5.0 ± 1.6 mIU/mL	***p < 0.001***	
			Testosterone	8.9 ± 7.1 ng/mL	5.0 ± 1.8 ng/mL	***p < 0.01***	
Observational single-arm studies							
Aliakbar R et al.	2009	10 AAS users	Hb	15.74 g/dL	16.61 g/dL	$p > 0.05$	–
Vahid et I al.	2009		HCT	46.35	51.12	***p < 0.05***	
			RBC	5.66×10^3	6.12×10^3	$p > 0.05$	
			WBC	6.78×10^3	8.4×10^3	$p > 0.05$	
			AST	20.75 IU/L	33.75 IU/L	***p < 0.05***	
			ALT	27.50 IU/L	43.62 IU/L	***p < 0.05***	
Keith RE et al.	1996	14 AAS users	Albumin	4.8 ± 0.4 g/dL	–	–	3.2–5.5 g/dL
			Urea	14.0 ± 3.3 mg/dL			11.0–23.0 mg/dL
			Cholesterol	198 ± 52 mg/dL			<240 mg/dL
			TG	98 ± 37 mg/dL			1–250 mg/dL
			HDL	43 ± 14 mg/dL			>35 mg/dL
			LDL	136 ± 53 mg/dL			<165 mg/dL
			Glucose	89 ± 8 mg/dL			70–115 mg/dL
Inigo MA et al.	2000	39 AAS users	AST	29.8 ± 8.6 U/L	45.0 ± 20.2 U/L	***p < 0.001***	–
			ALT	32.9 ± 11.6 U/L	51.4 ± 30.3 U/L	***p < 0.001***	
			Cholesterol	184.7 ± 42.3 mg/dL	211.0 ± 64.1 mg/dL	***p < 0.01***	
			HDL	31.4 ± 11.5 mg/dL	19.7 ± 7.1 mg/dL	***p < 0.001***	
			LDL	145.9 ± 35.3 mg/dL	173.5 ± 65.3 mg/dL	***p < 0.01***	
			FSH	3.3 ± 2.4 U/L	0.4 ± 1.0 U/L	***p < 0.001***	
			LH	2.1 ± 1.7 U/L	0.2 ± 0.6 U/L	***p < 0.001***	
			Testosterone	3.8 ± 1.7 ng/mL	3.2 ± 3.1 ng/mL	$p > 0.05$	
	2020	100 AAS users	Cholesterol	4.4 (4.2–4.6) mmol/L	4.4 (4.1–4.6) mmol/L	$p > 0.05$	–
Smit DL et al.	2021		LDL	2.9 (2.7–3.1) mmol/L	2.8 (2.6–3.1) mmol/L	$p > 0.05$	
Smit DL et al.	2021		HDL	1.2 (1.1–1.2) mmol/L	1.2 (1.1–1.3) mmol/L	$p > 0.05$	
Smit DL et al.	2022		TG	1.0 (0.8–1.1) mmol/L	1.1 (1.0–1.3) mmol/L	$p > 0.05$	
Smit DL et al.			LH	3.0 (2.7–3.4) U/I	3.1 (2.7–3.5) U/I	$p > 0.05$	
			FSH	3.9 (3.4–4.5) U/I	4.1 (3.5–4.6) U/I	$p > 0.05$	
			Testosterone	16.6 (9.3–24) nmol/L	15.6 (7–24) nmol/L	$p > 0.05$	
			Urea	6.9 (6.6–7.2) mmol/L	6.7 (6.3–7.0) nmol/L	$p > 0.05$	
			Creatinine	93.1 (90–96) µmol/l	90.0 (86.9–93) µmol/l	***p < 0.01***	
			Bilirrubin	12.9 (11.8–14) µmol/l	12.7 (11–14) µmol/l	$p > 0.05$	
			ALT	49.2 (43.6–54.7) U/I	47.2 (41–53) U/I	$p > 0.05$	
			AST	35.9 (32.4–39.5) U/I	36.8 (33–41) U/I	$p > 0.05$	
			ALP	74.0 (70.2–77.7) U/I	72.7 (68–77) U/I	$p > 0.05$	
Interventional studies							
Hartgens F et al.	2004	19 AAS users 7 nonusers placebo	Cholesterol	5.06 ± 0.57 mmol/L	5.26 ± 1.37 mmol/L	$p > 0.05$	–
			TG	1.13 ± 0.22 mmol/L	1.45 ± 0.58 mmol/L	$p > 0.05$	
			HDL	1.06 ± 0.36 mmol/L	1.22 ± 0.41 mmol/L	$p > 0.05$	
			Lipoprotein a	65 ± 44 U/L	201 ± 194 U/L	***p < 0.01***	

* Refers to the comparison between groups (AAS users vs. non-users) in comparative studies or between pre and post analyses in the study group (AAS users) in single-arm studies (reported by the authors); bold values are statistically significant. #Reference values for each parameter according to the authors/literature. Hb: hemoglobin; HCT: hematrocit; RBC: red blood cell; WBC: leucocyte counting; AST: serum aspartate aminotransferase; ALT: alanine aminotransferase; TG: triglyceride; HDL: high density lipoprotein; LDL: low density lipoprotein; CK: creatine kinase; GGT: gammaglutamyltraspeptidase; ALP: alkaline phophatase.

increasing the uncertainty about their real effects (Anawalt, 2019). Other factors contributing to between-study heterogeneity include different AAS regimens and routes of administration, use of dietary supplements and nutraceuticals, individuals' characteristics (e.g., age, sex, dehydration, excessive fatigue, comorbidities, country of origin), exercise routine/training, and analytical challenges in drug testing (Hartgens et al., 2004; Thevis et al., 2018; van Amsterdam et al., 2010).

This study found some parameters, such as urea, a product of protein metabolism, not differing among AAS users and nonusers. Previous studies demonstrated that the use of injectable AAS such as testosterone enanthate, one of the most commonly consumed substances found in the present study, has no effect on some biochemical parameters, including urea, whose concentration seems to depend only on protein intake, the body's capacity to catabolize protein, and adequate excretion of urea by the renal system (Hartgens et al., 2004).

Conversely, some oral steroids, especially active 17-α-alkylated AASs, can lead to a significant increase in aminotransferases (ALT, AST) – as confirmed in this study, since they undergo first-pass metabolism, thereby promoting marked changes, that are associated with higher risks of hepatotoxicity, adenomas, general hepatic damage, and tumors (e.g., hepatocellular adenoma and hepatocellular carcinoma) in long-term administration (Neri et al., 2011; Solimini et al., 2017). Nonetheless, it is important to consider that the increase in serum transaminases attributed to the intake of oral steroids tends to return to baseline levels

Table 3
Summary of findings: evidence gap map for the main outcomes of comparative studies.

AAS users vs. nonusers	Main outcomes											
	Urea	Creatinine	FSH	LH	Testost.	ALT	AST	GGT	Cholesterol	TG	HDL	LDL
	4 studies	3 studies	5 studies	5 studies	7 studies	3 studies	3 studies	3 studies	10 studies	9 studies	9 studies	7 studies
Studies' results	↔↔↔↔↔	↑↑ ↔	↓↓↓↓ ↔	↓↓↓ ↔↔	↑↑↑↑↓↓ ↔	↑↑↑	↑↑↑	↓ ↔↔	↑↑↑↓ ↔↔↔↔↔↔↔	↑↑↓ ↔↔↔↔↔↔↔	↓↓↓↓↓ ↔↔↔↔↔	↑↑↑↑ ↔↔↔↔
Methodological quality												

Methodological quality: the size of the circles is proportional to the number of studies (larger = 5 or more; medium = 2 to 4; small = 1)
Green circle: strong methodological quality; yellow circle: moderate methodological quality study; red circle: weak methodological quality study

Studies' results: symbols refer results from comparative interventional or observational studies (at least two arms; AAS users vs. nonusers) reporting each outcome of interest.
↔ Similar results among AAS users vs. nonusers this outcome measure (p>0.05, non-significant)
↑ AAS users presented higher levels of this outcome (p<0.05, significantly significant)
↓ AAS users presented lower levels of this outcome (p<0.05, significantly significant)

AST: serum aspartate aminotransferase; ALT: alanine aminotransferase; FSH: follicle stimulating hormone; GGT: gammaglutamyltraspeptidase; HDL: high density lipoprotein; LDL: low density lipoprotein; LH: luteinizing hormone; TG: triglyceride; Testost: testosterone

within several weeks after cessation, which may hamper the assessment of the effects of these substances in the human body and their association with clinical outcomes (Alen, 1985).

This study found a consistent reduction in endogenous production of testosterone and gonadotrophin levels (LH, FSH) in AAS users compared to nonusers. This effect is common during the period of active steroid use as a consequence of the hypothalamic-pituitary axis suppression by these substances, which leads to negative feedback (Hartgens et al., 2004). Therefore, exogenous administration of AAS will disturb the endogenous production of sexual hormones. According to the literature, reduced gonadotropin secretion results in decreased intra-testicular and peripheral testosterone levels in male individuals – as reported by part of the included studies, potentially leading to hypogonadism manifesting with oligospermia, testicular atrophy, azoospermia, and other sperm abnormalities (Fronczak et al., 2012). Conversely, in some studies, plasma levels of testosterone were considerably increased during AAS use, which could be due to exogenous testosterone administration (Alen, 1985).

The results of this systematic review also showed conflicting evidence on the effects of AAS on individuals' lipid profiles, including serum total cholesterol, triglyceride TG, high-density HDL and LDL levels – with some studies demonstrating significant changes (either higher or lower values for AAS users compared to nonusers/controls) and others revealing no meaningful differences among populations. Some authors previously mentioned that the impact of AAS on lipid metabolism is not well established (Tenório et al., 2021) and may vary considerably regarding the types of AAS used and the route of administration. Although nandrolone decanoate seems to not affect these parameters when used at therapeutic or supratherapeutic doses (Hartgens et al., 2004; Smit, Grefhorst et al., 2022), Garevik et al. 2014 (Garevik et al., 2014) hypothesized that low doses of AAS (125 mg) do not alter the lipid profile, and that changes in this outcome may be directly related to the dose. This dose-response relationship was also mentioned by Hartgens et al. 2004 (Hartgens et al., 2004) that evaluated both the use of self-prescribed drugs and dosages vs. pre-established dosages in a randomized, blinded study. Authors stated that when volunteers used drugs ad libitum (high doses), changes in HDL were found, however, triglyceride and total cholesterol did not change. Conversely, in the randomized trial, no significant changes in these parameters were reported after eight weeks of nandrolone decanoate (200 mg/week). Although therapeutic doses can vary from 50 mg to around 150 mg per day, usual doses for aesthetic purposes can exceed 1000 mg per week (Hartgens et al., 1996; Kuipers et al., 1991; Yabluchanskiy & Tsitouras, 2019). It has been suggested that AAS administration potentially induces reductions in the serum levels of lipoproteins by means of enzyme hepatic triglyceride lipase (HTGL) mediation, which regulates serum lipids (Applebaum-Bowden et al., 1987). An overexpression of hepatic lipase has a negative impact on plasma HDL and LDL values and may increase hepatic cholesterol concentrations without altering bile cholesterol secretion (Kobayashi et al., 2015; Yasuda et al., 2010); triglyceride levels seem to remain unaffected by AAS abuse in almost all analyzed studies (Hartgens et al., 2004). Considering that AAS may induce an atherogenic lipid profile and that changes in these parameters are significantly associated with increased cardiovascular risks, which can be aggravated by other factors such as diet, obesity, and physical activity, physicians and other healthcare professionals should be aware when detecting these alterations in this specific population (Albano et al., 2021; Tenório et al., 2021).

Other biochemical parameters including glucose, bilirubin, and enzymes (e.g., alkaline phosphatase, creatine kinase) were also poorly assessed by the included studies. Yet, some authors previously mentioned that glucose metabolism deregulations secondary to insulin (Cohen & Hickman, 1987) are observed in AAS users in longitudinal evaluations, although serum levels of GGT and AP remained unaffected (Alen, 1985; Kuipers et al., 1991).

Similarly, limited evidence for alteration in hematological parameters such as red blood cells, leucocytes, or platelet counting exists (only one to two studies). Even so, it is possible that these measures are increased in AAS users as these substances actively stimulate erythropoiesis and erythropoietin in the kidney and promote the differentiation of erythropoietic stem cells (Ballal et al., 1991; Berns et al., 1992). A recent study evaluating the effects of the supraphysiological dose of AAS on serum and urinary erythropoietin and blood parameters from self-reported users found a positive correlation between these parameters and testosterone levels (rs=0.46; $p = 0.01$) and reticulocyte percentage (rs=0.43; $p = 0.02$). Moreover, authors reported that individuals with AAS-induced hypogonadism had around 75 % higher serum erythropoietin and over 125 % higher reticulocyte fractions ($p < 0.001$) compared to nonusers (Heiland et al., 2023).

Altogether, this updated evidence extends the knowledge on the effects of AAS in laboratorial biomarkers of bodybuilders, especially changes in liver function and hormonal status in men, makes them aware of the importance of further health preventive strategies to minimize harm, and highlights the need for larger and well-designed studies to properly inform about the benefits and risks of these substances on clinical outcomes, namely hematological parameters.

This study has some limitations. No quantitative analyses were possible given the heterogeneity of data from different study designs and the lack of enough studies properly reporting outcome measurements and units. Moreover, the studies were not sufficiently powerful due to the relatively small number of participants. The absence of a core outcome set (COS) or standard reporting of outcomes (Kirkham et al., 2013) in the AAS use settings may contribute to selective bias, loss of information, and impair evidence gathering on the effects of these substances in different populations, including bodybuilders. Yet, although the results are only exploratory, a systematic and critical

review process was followed in this study; the data synthesized by an evidence map may support the development of further research in this field, especially about the most common biomarkers. The EPHPP was used for assessing studies' methodological quality as this is a validated and reliable tool, nonetheless, other approaches may produce equivalent results. It should be noted that studies' findings and conclusions were considered as presented by the authors, meaning that evidence may not be immediately transposed to different scenarios/settings and geographical regions.

5. Conclusion

The chronic nonmedical high-dose abuse of AAS among bodybuilders significantly increases serum alanine and aspartate aminotransferase levels while decreasing follicle stimulating and luteinizing hormones, warning for the need to monitor individuals' liver function and hormonal status. Evidence is still conflicting on the effect of steroids on lipid biomarkers and is scarce about hematological parameters in this specific population, suggesting the need for further larger and well-designed studies reporting a standardized core of outcomes.

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Data availability

All data is available within the manuscript and supplemental online material; further information may be obtained with the corresponding author upon request.

CRediT authorship contribution statement

Ana Sofia R. Tavares: Writing – review & editing, Supervision, Methodology, Conceptualization. **Márcia Vital:** Writing – review & editing, Investigation, Data curation. **Mariana Cunha:** Writing – review & editing, Investigation, Data curation. **Mário Maia Matos:** Writing – review & editing, Methodology, Conceptualization. **Fernanda S. Tonin:** Writing – original draft, Validation, Methodology, Formal analysis.

Declaration of competing interest

None

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.peh.2024.100280.

References

Al-Janabi, A. S., Kanaan, Z. A., & Al Salih, A. M. (2011). Effect of anabolic-androgenic steroids on semen parameters and serum hormonal levels in iraqi male bodybuilders. *Jordan Medical Journal, 45*(2), 159–166. https://www.scopus.com/inward/record.uri?eid=2-s2.0-82455186823&partnerID=40&md5=e5166a14a8f4de3e1f16de318138c0a4.

Albakaa, A. A. H., Ali Khudhair, H. A., & Al-Muhsen, W. R. N. (2020). Changes in blood biochemical parameters in bodybuilders using supplements, steroids and their effect on the kidneys in Iraq. *Annals of Tropical Medicine and Public Health, 23*(13 A), Article Sp231334. https://doi.org/10.36295/ASRO.2020.231334

Albano, G. D., Amico, F., Cocimano, G., Liberto, A., Maglietta, F., & Esposito, M. (2021). Adverse effects of anabolic-androgenic steroids: a literature review. *Healthcare (Basel, Switzerland), 9*(1). https://doi.org/10.3390/healthcare9010097

Alen, M. (1985). Androgenic steroid effects on liver and red cells. *British Journal of Sports Medicine, 19*(1), 15–20. https://doi.org/10.1136/bjsm.19.1.15

Aliakbar, R., & Vahid, I. (2009). Effects of oxymetholone on hematological and liver factors in the male bodybuilder's serum. *Procedia - Social and Behavioral Sciences, 1*(1), 2814–2816. https://doi.org/10.1016/j.sbspro.2009.01.500

Anawalt, B. D. (2019). Diagnosis and management of anabolic androgenic steroid use. *The Journal of Clinical Endocrinology and Metabolism, 104*(7), 2490–2500. https://doi.org/10.1210/jc.2018-01882

Applebaum-Bowden, D., Haffner, S. M., & Hazzard, W. R. (1987). The dyslipoproteinemia of anabolic steroid therapy: Increase in hepatic triglyceride lipase precedes the decrease in high density lipoprotein2 cholesterol. *Metabolism: Clinical and Experimental, 36*(10), 949–952. https://doi.org/10.1016/0026-0495(87)90130-2

Arazi, H. (2018). Effects of longitudinal abuse of anabolic steroids on liver enzymes activity and lipid profiles of male bodybuilders. *Progress in Nutrition, 20*(3), 323–328. https://doi.org/10.23751/pn.v20i3.5287

Ballal, S. H., Domoto, D. T., Polack, D. C., Marciulonis, P., & Martin, K. J. (1991). Androgens potentiate the effects of erythropoietin in the treatment of anemia of end-stage renal disease. *American Journal of Kidney Diseases : The Official Journal of the National Kidney Foundation, 17*(1), 29–33. https://doi.org/10.1016/s0272-6386(12)80246-0

Baron, D. A., Martin, D. M., & Abol Magd, S. (2007). Doping in sports and its spread to at-risk populations: An international review. *World Psychiatry : Official Journal of the World Psychiatric Association (WPA), 6*(2), 118–123. http://www.ncbi.nlm.nih.gov/pubmed/18235871.

Bauer, P., Majisik, A., Mitter, B., Csapo, R., Tschan, H., & Hume, P. (2023). Body Composition of competitive bodybuilders: a systematic review of published data and recommendations for future work. *Journal of strength and Conditioning Research / National Strength & Conditioning Association, 37*(3), 726–732. https://doi.org/10.1519/JSC.0000000000004155

Berns, J. S., Rudnick, M. R., & Cohen, R. M. (1992). A controlled trial of recombinant human erythropoietin and nandrolone decanoate in the treatment of anemia in patients on chronic hemodialysis. *Clinical nephrology, 37*(5), 264–267. http://www.ncbi.nlm.nih.gov/pubmed/1606777.

Bird, S. R., Goebel, C., Burke, L. M., & Greaves, R. F. (2016). Doping in sport and exercise: Anabolic, ergogenic, health and clinical issues. *Annals of Clinical Biochemistry, 53*(Pt 2), 196–221. https://doi.org/10.1177/0004563215609952

Bond, P., Smit, D. L., & de Ronde, W. (2022). Anabolic-androgenic steroids: How do they work and what are the risks? *Frontiers in Endocrinology, 13*, Article 1059473. https://doi.org/10.3389/fendo.2022.1059473

Bordin, D. M., Bettim, B. B., Perdona, G. C., de Campos, E. G., & De Martinis, B. S. (2017). Understanding alterations on blood and biochemical parameters in athletes that use dietary supplements, steroids and illicit drugs. *Toxicology, 376*, 75–82. https://doi.org/10.1016/j.tox.2016.05.019

Christou, M. A., Christou, P. A., Markozannes, G., Tsatsoulis, A., Mastorakos, G., & Tigas, S. (2017). Effects of anabolic androgenic steroids on the reproductive system of athletes and recreational users: a systematic review and meta-analysis. *Sports Medicine (Auckland, N.Z.), 47*(9), 1869–1883. https://doi.org/10.1007/s40279-017-0709-z

Cohen, J. C., & Hickman, R. (1987). Insulin resistance and diminished glucose tolerance in powerlifters ingesting anabolic steroids. *The Journal of Clinical Endocrinology and Metabolism, 64*(5), 960–963. https://doi.org/10.1210/jcem-64-5-960

Corona, G., Rastrelli, G., Marchiani, S., Filippi, S., Morelli, A., & Sarchielli, E. (2022). Consequences of anabolic-androgenic steroid abuse in males; sexual and reproductive perspective. *The world Journal of Men's Health, 40*(2), 165–178. https://doi.org/10.5534/wjmh.210021

De Francesco Daher, E., Duarte Fernandes, P. H. P., Meneses, G. C., Bezerra, G. F., De Souza Lima Ferreira, L., & De Almeida Viana, G. (2018). Novel kidney injury biomarkers among anabolic androgenic steroids users - Evidence of subclinical kidney disease. *Asian Journal of Sports Medicine, 9*(1), e65540. https://doi.org/10.5812/asjsm.65540

Dickerman, R. D., McConathy, W. J., & Zachariah, N. Y. (1997). Testosterone, sex hormone-binding globulin, lipoproteins, and vascular disease risk. *Journal of cardiovascular risk, 4*(5–6), 363–366.

Dickerman, R. D., Pertusi, R. M., Zachariah, N. Y., Dufour, D. R., & McConathy, W. J. (1999). Anabolic steroid-induced hepatotoxicity: Is it overstated? *Clinical Journal of Sport Medicine : Official Journal Of The Canadian Academy of Sport Medicine, 9*(1), 34–39. https://doi.org/10.1097/00042752-199901000-00007

Esposito, M., Salerno, M., Calvano, G., Agliozzo, R., Ficarra, V., & Sessa, F. (2023). Impact of anabolic androgenic steroids on male sexual and reproductive function: A systematic review. *Panminerva Medica, 65*(1), 43–50. https://doi.org/10.23736/S0031-0808.22.04677-8

Fronczak, C. M., Kim, E. D., & Barqawi, A. B. (2012). The insults of illicit drug use on male fertility. *Journal of Andrology, 33*(4), 515–528. https://doi.org/10.2164/jandrol.110.011874

Garevik, N., Rane, A., Bjorkhem-Bergman, L., & Ekstrom, L. (2014). Effects of different doses of testosterone on gonadotropins, 25-hydroxyvitamin D3, and blood lipids in healthy men. *Substance Abuse and Rehabilitation, 5*, 121–127. https://doi.org/10.2147/SAR.S71285

Graham, M. R., Grace, F. M., Boobier, W., Hullin, D., Kicman, A., & Cowan, D. (2006). Homocysteine induced cardiovascular events: A consequence of long term anabolic-androgenic steroid (AAS) abuse. *British Journal of Sports Medicine, 40*(7), 644–648. https://doi.org/10.1136/bjsm.2005.025668

Grandperrin, A., Schuster, I., Rupp, T., Izem, O., Obert, P., & Nottin, S. (2021). Left ventricular dyssynchrony and post-systolic shortening in young bodybuilders using anabolic-androgenic steroids. *American journal of physiology. Heart and Circulatory Physiology, 321*(3), H509–H517. https://doi.org/10.1152/ajpheart.00136.2021

Hartgens, F., Kuipers, H., Wijnen, J. A., & Keizer, H. A. (1996). Body composition, cardiovascular risk factors and liver function in long-term androgenic-anabolic steroids using bodybuilders three months after drug withdrawal. *International Journal of Sports Medicine, 17*(6), 429–433. https://doi.org/10.1055/s-2007-972873

Hartgens, F., Rietjens, G., Keizer, H. A., Kuipers, H., & Wolffenbuttel, B. H. (2004). Effects of androgenic-anabolic steroids on apolipoproteins and lipoprotein (a). *British Journal of Sports Medicine, 38*(3), 253–259. https://doi.org/10.1136/bjsm.2003.000199

Hassan, E. A., Hameed, R. Y., & Mezil, S. A. (2021). Impacts of androgen abuse and overtraining on endocrine profile in bodybuilders. *Iraqi Journal of Science, 62*(12), 4614–4620. https://doi.org/10.24996/ijs.2021.62.12.2

Heiland, C. E., Schickel, Y., Lehtihet, M., Borjesson, A., & Ekstrom, L. (2023). Supra-physiological doses of anabolic androgenic steroids impact erythropoietin and blood parameters. *Drug Testing and Analysis*. https://doi.org/10.1002/dta.3452

Higgins, J. P. T., & Thomas, J. (2021). *Cochrane handbook for systematic reviews of interventions version* (2 ed.„ *Version 6.2*. Wiley-Blackwell https://training.cochrane.org/handbook/current.

Hislop, M. S., Ratanjee, B. D., Soule, S. G., & Marais, A. D. (1999). Effects of anabolic-androgenic steroid use or gonadal testosterone suppression on serum leptin concentration in men. *European Journal of Endocrinology, 141*(1), 40–46. https://doi.org/10.1530/eje.0.1410040

Hislop, M. S., St Clair Gibson, A., Lambert, M. I., Noakes, T. D., & Marais, A. D. (2001). Effects of androgen manipulation on postprandial triglyceridaemia, low-density lipoprotein particle size and lipoprotein(a) in men. *Atherosclerosis, 159*(2), 425–432. https://doi.org/10.1016/s0021-9150(01)00519-6

Inigo, M. A., Arrimadas, E., & Arroyo, D. (2000). Study of 43 therapy courses with anabolic steroids among athletes: Use and secondary effects. *Revista Clinica Espanola, 200*(3), 133–138. https://doi.org/10.1016/s0014-2565(00)70586-8

Kanayama, G., & Pope, H. G. (2018). History and epidemiology of anabolic androgens in athletes and non-athletes. *Molecular and Cellular Endocrinology, 464*, 4–13. https://doi.org/10.1016/j.mce.2017.02.039

Keith, R. E., Stone, M. H., Carson, R. E., Lefavi, R. G., & Fleck, S. J. (1996). Nutritional status and lipid profiles of trained steroid-using bodybuilders. *International Journal of Sport Nutrition, 6*(3), 247–254. https://doi.org/10.1123/ijsn.6.3.247

Kirkham, J. J., Gargon, E., Clarke, M., & Williamson, P. R. (2013). Can a core outcome set improve the quality of systematic reviews?–a survey of the Co-ordinating Editors of Cochrane Review Groups. *Trials, 14*, 21. https://doi.org/10.1186/1745-6215-14-21

Kleiner, S. M., Calabrese, L. H., Fiedler, K. M., Naito, H. K., & Skibinski, C. I. (1989). Dietary influences on cardiovascular disease risk in anabolic steroid-using and nonusing bodybuilders. *Journal of the American College of Nutrition, 8*(2), 109–119. https://doi.org/10.1080/07315724.1989.10720285

Kobayashi, J., Miyashita, K., Nakajima, K., & Mabuchi, H. (2015). Hepatic Lipase: A Comprehensive View of its Role on Plasma Lipid and Lipoprotein Metabolism. *Journal of Atherosclerosis and Thrombosis, 22*(10), 1001–1011. https://doi.org/10.5551/jat.31617

Kuipers, H., Wijnen, J. A., Hartgens, F., & Willems, S. M. (1991). Influence of anabolic steroids on body composition, blood pressure, lipid profile and liver functions in body builders. *International Journal of Sports Medicine, 12*(4), 413–418. https://doi.org/10.1055/s-2007-1024704

McKillop, G., & Ballantyne, D. (1987). Lipoprotein analysis in bodybuilders. *International Journal of Cardiology, 17*(3), 281–288. https://doi.org/10.1016/0167-5273(87)90077-5

Mitchell, L., Murray, S. B., Cobley, S., Hackett, D., Gifford, J., & Capling, L. (2017). Muscle dysmorphia symptomatology and associated psychological features in bodybuilders and non-bodybuilder resistance trainers: a systematic review and meta-analysis. *Sports Medicine (Auckland, N.Z.), 47*(2), 233–259. https://doi.org/10.1007/s40279-016-0564-3

Moffatt, R. J., Wallace, M. B., & Sady, S. P. (1990). Effects of anabolic steroids on lipoprotein profiles of female weight lifters. *Physician and Sportsmedicine, 18*(9), 106–110. https://doi.org/10.1080/00913847.1990.11710139. +112+115.

Neri, M., Bello, S., Bonsignore, A., Cantatore, S., Riezzo, I., & Turillazzi, E. (2011). Anabolic androgenic steroids abuse and liver toxicity. *Mini Reviews in Medicinal Chemistry, 11*(5), 430–437. https://doi.org/10.2174/138955711795445916

Page, M. J., McKenzie, J. E., Bossuyt, P. M., Boutron, I., Hoffmann, T. C., & Mulrow, C. D. (2021). The PRISMA 2020 statement: An updated guideline for reporting systematic reviews. *BMJ (Clinical Research Ed.), 372*, n71. https://doi.org/10.1136/bmj.n71

Jr. Pope, H. G., Wood, R. I., Rogol, A., Nyberg, F., Bowers, L., & Bhasin, S (2014). Adverse health consequences of performance-enhancing drugs: An Endocrine Society scientific statement *Endocrine Reviews, 35*(3), 341–375. https://doi.org/10.1210/er.2013-1058

Rasmussen, J. J., Schou, M., Selmer, C., Johansen, M. L., Gustafsson, F., & Frystyk, J. (2017). Insulin sensitivity in relation to fat distribution and plasma adipocytokines among abusers of anabolic androgenic steroids. *Clinical Endocrinology, 87*(3), 249–256. https://doi.org/10.1111/cen.13372

Rasmussen, J. J., Selmer, C., Østergren, P. B., Pedersen, K. B., Schou, M., & Gustafsson, F. (2016). Former abusers of anabolic androgenic steroids exhibit decreased testosterone levels and hypogonadal symptoms years after cessation: a case-control study. *PloS one, 11*(8), Article e0161208. https://doi.org/10.1371/journal.pone.0161208

Sader, M. A., Griffiths, K. A., McCredie, R. J., Handelsman, D. J., & Celermajer, D. S. (2001). Androgenic anabolic steroids and arterial structure and function in male bodybuilders. *Journal of the American College of Cardiology, 37*(1), 224–230. https://doi.org/10.1016/s0735-1097(00)01083-4

Sagoe, D., Molde, H., Andreassen, C. S., Torsheim, T., & Pallesen, S. (2014). The global epidemiology of anabolic-androgenic steroid use: A meta-analysis and meta-regression analysis. *Annals of Epidemiology, 24*(5), 383–398. https://doi.org/10.1016/j.annepidem.2014.01.009

Jr. Schwingel, P. A., Cotrim, H. P., Salles, B. R., Almeida, C. E., dos Santos, C. R., Nachef, B., et al. (2011). Anabolic-androgenic steroids: A possible new risk factor of toxicant-associated fatty liver disease *Liver International : Official Journal of the International Association for the Study of the Liver, 31*(3), 348–353. https://doi.org/10.1111/j.1478-3231.2010.02346.x

Seara, F. A. C., Olivares, E. L., & Nascimento, J. H. M. (2020). Anabolic steroid excess and myocardial infarction: From ischemia to reperfusion injury. *Steroids, 161*, Article 108660. https://doi.org/10.1016/j.steroids.2020.108660

Smit, D. L., Bond, P., & De Ronde, W. (2022a). Health effects of androgen abuse: A review of the HAARLEM study [Review]. *Current Opinion in Endocrinology*. *Diabetes and Obesity, 29*(6), 560–565. https://doi.org/10.1097/MED.0000000000000759

Smit, D. L., Buijs, M. M., de Hon, O., den Heijer, M., & de Ronde, W. (2021). Positive and negative side effects of androgen abuse. The HAARLEM study: A one-year prospective cohort study in 100 men. *Scandinavian Journal of Medicine & Science in Sports, 31*(2), 427–438. https://doi.org/10.1111/sms.13843

Smit, D. L., de Hon, O., Venhuis, B. J., den Heijer, M., & de Ronde, W. (2020). Baseline characteristics of the HAARLEM study: 100 male amateur athletes using anabolic androgenic steroids. *Scandinavian Journal Of Medicine & Science in Sports, 30*(3), 531–539. https://doi.org/10.1111/sms.13592

Smit, D. L., Grefhorst, A., Buijs, M. M., de Hon, O., den Heijer, M., & de Ronde, W. (2022b). Prospective study on blood pressure, lipid metabolism and erythrocytosis during and after androgen abuse. *Andrologia, 54*(4), e14372. https://doi.org/10.1111/and.14372

Smoliga, J. M., Wilber, Z. T., & Robinson, B. T. (2023). Premature death in bodybuilders: what do we know? *Sports Medicine (Auckland, N.Z.)*, 1–16. https://doi.org/10.1007/s40279-022-01801-0

Snilstveit, B., Vojtkova, M., Bhavsar, A., Stevenson, J., & Gaarder, M. (2016). Evidence & Gap Maps: A tool for promoting evidence informed policy and strategic research agendas. *Journal of Clinical Epidemiology, 79*, 120–129. https://doi.org/10.1016/j.jclinepi.2016.05.015

Solimini, R., Rotolo, M. C., Mastrobattista, L., Mortali, C., Minutillo, A., & Pichini, S. (2017). Hepatotoxicity associated with illicit use of anabolic androgenic steroids in doping. *European Review for Medical and Pharmacological Sciences, 21*(1 Suppl), 7–16. http://www.ncbi.nlm.nih.gov/pubmed/28379599.

Tenório, M., Paz, C., Valladares, F., Guimães Junir, M., de Sá, C., & Correira, L. (2021). Effects of low-to-moderate doses of anabolic steroids on lipid profile and muscle hypertrophy in resistance training practitioners: a systematic review with meta-analysis. *International Journal of Cardiovascular Sciences, 34*(5), 531–541. https://doi.org/10.36660/ijcs.20200107

Thevis, M., Krug, O., Geyer, H., Walpurgis, K., Baume, N., & Thomas, A. (2018). Analytical challenges in sports drug testing. *Analytical and Bioanalytical Chemistry, 410*(9), 2275–2281. https://doi.org/10.1007/s00216-018-0934-9

Thomas, B. H., Ciliska, D., Dobbins, M., & Micucci, S. (2004). A process for systematically reviewing the literature: Providing the research evidence for public health nursing interventions. *Worldviews on Evidence-Based Nursing / Sigma Theta Tau International, Honor Society Of Nursing, 1*(3), 176–184. https://doi.org/10.1111/j.1524-475X.2004.04006.x

Tidmas, V., Brazier, J., Hawkins, J., Forbes, S. C., Bottoms, L., & Farrington, K. (2022). Nutritional and Non-Nutritional Strategies in Bodybuilding: Impact on Kidney Function. *International Journal Of Environmental Research And Public Health*, (7), 19. https://doi.org/10.3390/ijerph19074288

Torres-Calleja, J., González-Unzaga, M., DeCelis-Carrillo, R., Calzada-Sánchez, L., & Pedrón, N. (2001). Effect of androgenic anabolic steroids on sperm quality and serum hormone levels in adult male bodybuilders. *Life Science, 68*(15), 1769–1774. https://doi.org/10.1016/s0024-3205(01)00972-9

Vahid, I., Valiollah, S., & Mehdi, A. (2009). Effects of oxymetholone on cardiovascular risk factor in the male bodybuilders. *Procedia - Social and Behavioral Sciences, 1*(1), 2843–2846. https://doi.org/10.1016/j.sbspro.2009.01.506

van Amsterdam, J., Opperhuizen, A., & Hartgens, F. (2010). Adverse health effects of anabolic-androgenic steroids. *Regulatory toxicology and pharmacology : RTP, 57*(1), 117–123. https://doi.org/10.1016/j.yrtph.2010.02.001

Yabluchanskiy, A., & Tsitouras, P. D. (2019). Is Testosterone Replacement Therapy in Older Men Effective and Safe? *Drugs & aging, 36*(11), 981–989. https://doi.org/10.1007/s40266-019-00716-2

Yasuda, T., Ishida, T., & Rader, D. J. (2010). Update on the role of endothelial lipase in high-density lipoprotein metabolism, reverse cholesterol transport, and atherosclerosis. *Circulation Journal : Official Journal of the Japanese Circulation Society, 74*(11), 2263–2270. https://doi.org/10.1253/circj.cj-10-0934