REVIEW ARTICLE

Do "testosterone boosters" really increase serum total testosterone? A systematic review

Afonso Morgado[1]✉, Georgios Tsampoukas[2], Ioannis Sokolakis[3], Nadja Schoentgen[4], Ahmet Urkmez[5] and Selcuk Sarikaya[6]

Testosterone boosters are heavily marketed on social media and marketplaces to men with claims to significantly increase testosterone. Lax industry regulation has allowed sales of supplements to thrive in the absence of verification of their purported benefits. Our primary objective was to systematically review all data published in the last two decades on testosterone boosters and determine their efficacy. Our outcome of interest was total testosterone increase versus placebo in four different populations: male athletes, men with late-onset hypogonadism infertile men and healthy men. Following search and screening, 52 studies were included in our review, relating to 27 proposed testosterone boosters: 10 studies of cholecalciferol; 5 zinc/magnesium; 4 *Tribulus terrestris* and creatine; 3 *Eurycoma longifolia* and *Withania somnifera*; 2 betaine, D-aspartic acid, *Lepidium meyenii* and isoflavones; while the remainder were single reports. Our findings indicate that most fail to increase total testosterone. The exceptions were β-hydroxy β-methylbutyrate and betaine, which can be considered effective for male athletes. *Eurycoma longifolia*, a blend of *Punica granatum* fruit rind and *Theobroma cacao* seed extracts (Tesnor™) and purified Shilajit extract (PrimaVie™) can be considered possibly effective for men with late-onset hypogonadism; *Eurycoma longifolia* and *Withania somnifera* possibly effective for healthy men; and a non-hormonal aromatase inhibitor (Novadex XT™) possibly effective for male athletes.

IJIR: Your Sexual Medicine Journal; https://doi.org/10.1038/s41443-023-00763-9

INTRODUCTION

The term "testosterone booster" (TB) is often employed to refer to a heterogeneous group of herbal or nutrient-based supplements used for the purpose of "naturally" increasing serum testosterone levels [1]. Not all users of TBs who seek this effect have male hypogonadism or symptoms suggestive of low testosterone, some are motivated by the desire to elevate normal serum testosterone levels in order to improve libido and/or sexual performance, improve athletic performance and/or gain muscle mass [1].

Many TBs are heavily marketed on social media, men's magazines and in marketplaces using claims of strong efficacy and are sold over the counter either as pure ingredients or miscellaneous blends [2–5]. The laxity of supplement regulation, by either the European Medication Agency or the Food and Drugs Administration, has allowed the industry to thrive in the absence of verification of the claims attached to their products [6]. Moreover, their long-term history of use and the perceived "natural" origin of some supplements can cause users to believe the potential for significant adverse effects is low, which may not be the case [7, 8].

Only a limited number of systematic reviews have been performed on TBs, but none has reviewed this topic as a whole [9–13]. Some have focused on specific TBs, such as *Tribulus terrestris* [13], while others have reviewed the active ingredients contained in the most sold TB products [3].

Prior systematic reviews have omitted less used or novel TBs, and thus, our group performed this systematic review to fill this evidence gap.

MATERIAL AND METHODS

Selection criteria

Our review was intended to include all relevant literature published during the last two decades to assess if TBs increase serum total testosterone (sTT) concentration. Our group defined "testosterone booster" as a nutrient, supplement, plant-derivative, or drug, used individually or in combination, with the intent to increase sTT concentration.

The inclusion criteria of our systematic review were: prospective clinical trial studies randomized, non-randomized or non-controlled; participants aged >18 years; participants are healthy men, men with a chronic condition, infertile men or men with late-onset hypogonadism; intervention was a TB; sTT was measured before and during the intervention. Exclusion criteria were retrospective studies or reviews, non-biological male participants and use of testosterone replacement therapy in any form.

Search strategy

This systematic review was performed in accordance with the Preferred Reporting Items for Systematic Reviews and

[1]Serviço de Urologia, Centro Hospitalar Universitário São João, Porto, Portugal. [2]Department of Urology, Oxford University Hospital NHS Trust, Oxford, UK. [3]2nd Department of Urology, School of Medicine, Aristotle University of Thessaloniki, Thessaloniki, Greece. [4]Urology Department, University Hospital Center Bichat Claude Bernard, Paris, France. [5]Department of Urology, St. Elizabeth's Medical Center, Boston, MA, USA. [6]Department of Urology, Gülhane Research and Training Hospital, Ankara, Turkey.
✉email: luisafonsomorgado@gmail.com

Received: 22 March 2023 Revised: 24 August 2023 Accepted: 4 September 2023
Published online: 11 September 2023

Meta-Analyses (PRISMA) checklist and was registered with PROSPERO (ID: CRD42022353457, "Does any testosterone "booster" really increase serum total testosterone?") [14]. The search was performed on August 18, 2022, and updated on June 18, 2023, and was limited to articles with an abstract available and date of publication after January 1, 2002. Medline was the database searched. The search terms used are available as Supplement 1. After duplicates were removed, each article's title and abstract were independently reviewed by two reviewers, and discrepancies were resolved by mutual consensus. The full text was then retrieved, and manuscripts were screened once more by two independent reviewers, and excluded if the full text did not meet inclusion criteria. Data on year of publication, year of study, study design, funding, number of participants, population studied, duration of treatment, TB used and dosage, baseline and end-of-treatment sTT (and free testosterone (FT) if available), method of analysis for sTT (and FT if available), and adverse effects were extracted and collated using a spreadsheet.

Risk of bias assessment

The risk of bias of each included study was assessed by two review authors. Any disagreements were resolved by discussion. Age, body mass index and history of diabetes mellitus were preselected as confounding factors. Risk of bias was assessed using the recommended tools in the Cochrane Handbook for Systematic Reviews of Interventions.

Primary objective

Our outcome of interest was the mean percentage and/or absolute increase in sTT concentration compared to placebo at the end of treatment. Our primary objective was to review all data gathered on TBs and determine which active ingredient, if any, could be considered effective. We defined "effective" as a statistically significant increase in sTT, demonstrated in at least two independent studies, in one of the four populations of interest (healthy men, male athletes, men with late-onset hypogonadism or infertile men). The term "possibly effective" was also coined and was defined as a statistically significant increase in sTT demonstrated in just a single study in one of the four populations of interest.

RESULTS

Search results

After the removal of duplicates, the literature search yielded 3691 articles. Following title and abstract screening, the full texts of 99 articles were assessed. A total of 52 articles were included in the study and were summarized as a narrative synthesis, as data were too heterogeneous to perform meta-analysis. PRISMA flowchart is available as Supplementary Fig. 1. A total of 28 different TBs were identified, and of the 52 included articles, 10 were related to cholecalciferol ("vitamin D"); 5 to zinc and/or magnesium; 4 to *Tribulus terrestris*; 4 to creatine; 3 to *Eurycoma longifolia* ("Tongkat Ali"); 3 to *Withania somnifera* ("Ashwagandha"); 2 to betaine; 2 to D-aspartic acid (DAA); 2 to *Lepidium meyenii* ("Maca"); 2 to isoflavones; while the remaining 16 articles were single reports. Our group reported results by TB, rather than population of interest, due to our primary objective. Study and participants' basic characteristics are reported in Tables 1–4. Study characteristics for creatine, β-hydroxy β-methylbutyrate (HMB), β-alanine, zinc, magnesium, *Withania somnifera* ("Ashwagandha"), *Lepidium meyenii* ("Maca"), *Eurycoma longifolia* ("Tongkat ali") and DAA are all compiled in Table 1.

Cholecalciferol ("vitamin D"). A total of ten randomized controlled trials (RCTs) using vitamin D supplementation were identified [15–24]; however, the populations studied were heterogeneous. The conclusion from the data extraction is displayed in Table 2. Studies in male athletes without vitamin D deficiency were reported in three articles with dosages ranging from 2000 to 6000 IU daily over a short treatment duration (6–12 weeks) [18, 22, 23]. The two largest trials reported negative results when compared to both baseline and placebo [18, 23]. The smallest study was a 6-week trial in football players in Eastern Europe during winter, with the objective of preventing vitamin D deficiency due to lack of sun exposure [22]. This trial was positive both for sTT levels ($+5.3 \pm 3.2$ nmol/L vs baseline; $+1.65 \pm 1.9$ nmol/L $+ 6.2\%$ vs placebo after treatment) and for physical performance; however, these results cannot be generalized due to the specific study setting.

One author reported twice on men with concurrent vitamin D deficiency and low sTT [17, 20]. Following daily 2857 UI supplementation for 12 weeks, no improvement in sTT was detected. A trial in vitamin D-deficient men with a history of chronic heart failure or who were residents in a nursing home also showed negative results [21].

One trial in healthy, overweight men undergoing a weight reduction program showed an increase in sTT concentration following daily supplementation of 3332 IU for 1 year ($+2.7 \pm 0.8$ nmol/L vs baseline), but no significant difference was observed when compared to placebo [15]. As this was a small trial, this may be due to a lack of power to detect differences or there may have been interference from the reduction in body mass.

Other studies were RCTs on young healthy men, men with chronic conditions such as heart failure or infertile men with vitamin D deficiency but all failed to show a significant difference in sTT [16, 19, 21, 24].

Only one vitamin D trial reported adverse effects [19]. The most common adverse effect related to cholecalciferol supplementation was hypercalciuria, which was observed in 4.3% participants supplemented with cholecalciferol/placebo and 8.5% of participants supplemented with cholecalciferol/calcium. Hypercalcaemia was also reported but this effect was very rare.

Tribulus terrestris. *Tribulus terrestris* is a plant widely distributed around the world, which is native to warm temperate and tropical regions in southern Eurasia and Africa. Although it is one of the most popular and marketed TBs, only four human studies were found on *Tribulus terrestris* in the last two decades, all of which had small and heterogeneous populations [25–28]. Our findings from the data extraction are resumed in Table 3. Two studies were published by the same research group, both were single-arm trials looking at supplementation with 750 mg of *Tribulus terrestris* taken in three daily doses. One, performed in ageing men with late-onset hypogonadism, found a positive outcome after 12 weeks of treatment (sTT: 2.133 ± 0.1954 vs 2.837 ± 1.698 nmol/L) [26], while in the other, no statistically significant effect was noted in infertile men [27]. The remaining two studies were RCTs: Neychev et al. did not record any effect on healthy young men [25], and Fernández-Lázaro obtained mixed negative results in "Crossfit" athletes [28]. In the latter study, no difference in sTT was found in the 750 mg/day *Tribulus terrestris* arm following 6 weeks of treatment.

Zinc and/or magnesium aspartate. Although often sold as a combination, known as Zinc–Magnesium–Aspartate (ZMA), of the five studies, all RCTs, which were found on either zinc or magnesium only one article was found on the ZMA combination, which showed no effect on sTT in male athletes [29]. Another study looking at the effects of zinc and folic acid on fertile and infertile men was also negative [30]. Similarly, zinc and folic acid given, either in combination or separately, to on men following varicocelectomy demonstrated no significant effect at both 3 and 6 months [31]. The zinc-only arm had a positive effect on serum FT, yet results were displayed only as a figure. Selenium was tested in combination with zinc on healthy road cyclists but again, no effect was detected [32]. Magnesium was tested on taekwondo-practicing and sedentary men with no significant result when compared to placebo [33].

Table 1. Study characteristics for creatine, β-hydroxy β-methylbutyrate, β-alanine, zinc, magnesium, *Withania somnifera* ("Ashwagandha"), *Lepidium meyenii* ("Maca"), *Eurycoma longifolia* ("Tongkat ali") and D-aspartic acid.

Agent	Authors and year	Country	Objective	Type	Design	Duration of treatment	Result	Funding/support
Betain	Arazi (2022) [49]	Iran	To investigate the effect of short-term betaine supplementation on muscle endurance, plasma lactate, testosterone and cortisol levels in handball players	2-arm, double blinded, crossover, randomized placebo controlled	$N = 10$, age 16 ± 1 years Treatment group ($n = 10$): 1.25 g of betaine or placebo for 14 days followed by 30 days washout period and then crossover	4 weeks	Significant increase in serum testosterone compared to baseline and placebo conditions at resting (5.15 ± 1.5 to 10 ± 1.9 ng/ml, $p < 0.05$) and after exercise (15.2 ± 2.2 vs. 8.7 ± 1.7 ng/mL, $p < 0.05$)	None
	Nobari (2021) [48]	Iran	To investigate the effect of betaine supplementation on development-related hormones in professional youth soccer players	2-arm, unknown blinding, randomized placebo controlled	$N = 29$, age 15.45 ± 0.25 years Treatment group ($n = 14$): 2 g/day Placebo group ($n = 14$)	14 weeks	Significant increase of testosterone in the treatment group in mid-season and post-season compared to pre-season (5.4 ± 2.1 ng/mL and 5.9 ± 1.9 ng/mL vs 3.3 ± 1.4, respectively, $p < 0.05$) while a significant decrease was noticed in the placebo group in post-season compared to pre-season and mid-season (2.9 ± 0.7 ng/mL vs 4.1 ± 0.6 ng/mL and 3.6 ± 1.0 ng/mL, respectively, $p < 0.05$)	None
Creatine, β-hydroxy β-methylbutyrate and β-alanine	Samadi (2022) [37]	Iran	To investigate the effects of a 7-day creatine (C) loading protocol at the end of four weeks of β-alanine (BA) supplementation on physical performance, blood lactate, cognitive performance, and resting hormonal concentrations compared to BA alone	2-arm, double blinded, randomized placebo controlled	$N = 20$, age 21.5 ± 1.5 years Treatment group ($n = 10$): 6.4 g/day of BA for 28 days. After the third week, C (0.3 g/kg/day) Placebo ($n = 10$) 6.4 g/day of BA for 28 days, after the third week isocaloric placebo for 7 days	4 week	ANCOVA showed a significant difference between the BA + C and BA + P groups ($F_{1,17} = 9.73$, $p = 0.006$). Moreover, within-group comparisons showed a significant increase of testosterone levels in the BA + C group ($p = 0.001$) and a non-significant change in the BA + P group ($p = 0.588$)	None
	Fernández-Landa (2020) [36]	Spain	To investigate the effect of Creatine monohydrate (C) plus HMB supplementation on exercise-induced muscle damage and anabolic/catabolic hormones including testosterone in elite male traditional rowers	4-arm, double blinded, randomized placebo controlled	$N = 28$, age 30.43 ± 4.65 years Treatment group 1 ($n = 7$): 0.04 g/kg/day of C Treatment group 2 ($n = 7$): 3 g/day of HMB Treatment group 3 ($n = 7$): 0.04 g/kg/day of C plus 3 g/day of HMB Placebo ($n = 7$)	10 weeks	A significant increase in testosterone was seen in group 3 (4.91 ± 0.87 ng/dL vs 5.97 ± 1.23 ng/dL, $p < 0.05$)	None
	Durkalec-Michalski (2016) [39]	Poland	To investigate whether supplementation with HMB affects metabolism inc testosterone levels in highly trained men	2-arm, double blinded, crossover, randomized placebo controlled	$N = 58$, age 22 ± 6 years 12 weeks treatment 1000 mg/day HMB; 12 weeks of placebo	24 weeks	Significant increase in treatment group was seen (+53.1 mg/dL, $p = 0.047$)	Polish National Science Center

Table 1. continued

Agent	Authors and year	Country	Objective	Type	Design	Duration of treatment	Result	Funding/support
	Van der Merwe (2009) [35]	South African	To investigate the effect of creatine supplementation on serum androgen in male rugby players	2-arm, double blinded, randomized placebo controlled	$N = 20$, age 18.7 ± 0.53 years Treatment group 1 ($n = 8$*drop-out): Loading dose and maintenance doses of creatinine (50-5 g/d and glucose) before and after washout period of 6 weeks Placebo group ($n = 8$*drop-out): glucose only	3 weeks	No significant increase in testosterone in either groups (14.44 ± 2.95 nmol/L vs 16.69 ± 4.61 nmol/L, $p > 0.05$ in treatment group)	Not reported
	Hoffman (2006) [34]	United States of America	To investigate as a secondary purpose the effect of creatine and creatine plus β-alanine supplementation on the hormonal responses to resistance training in athletes from the college's football team with at least 2 years of resistance training experience	3-arm, double-blinded randomized placebo controlled trial	$N = 33$, age was not disclosed. The first group (CA) was provided with a daily creatine plus β-alanine supplementation (10.5 g/d of creatine monohydrate and 3.2 g/d of β-alanine), the second group (C) was provided with a daily creatine supplement only (10.5 g/d of creatine monohydrate), while the third group (P) was given a placebo (10.5 g/d of dextrose)	10 weeks	Although no significant changes were seen in resting total testosterone concentrations in P and CA during the 10-week study, a significant elevation in resting testosterone concentration was seen in C (20.0 ± 5.9 to 24.4 ± 6.4 nmol/L, $p < 0.05$).	This study was supported by a grant from EAS Inc., Golden, CO
D-Aspartic acid	Crewther (2018)	Poland	To investigate the short-term effects of D-aspartic acid on serum biomarkers of the reproductive axis in male climbers	2-arm, single blinded, randomized placebo controlled	$N = 16$, age 35.4 ± 7.3 years Treatment group ($n = 8$): 3 gr of aspartic acid daily Placebo group ($n = 8$)	2 weeks	No significant difference for treatment group (23.1 ± 4.9 to 22.8 ± 4.7 nmol/l, $p < 0.05$) after treatment	Polish Ministry for Sport and Tourism
	Willoughby (2013) [50]	United States of America	To determine the effects of resistance exercise and D-Aspartic acid supplementation serum hormones in resistance-trained men.	Randomized, double-blinded placebo-controlled trial	$N = 20$, age 22.8 ± 4.67 years, Treatment group ($n = 10$) 3 g/day of aspartic acid or placebo, and heavy resistance training.	4 weeks	In response to Aspartic acid supplementation, total testosterone ($p = 0.98$; effect size, 0.001) was not significantly changed.	Better Body Sports; Exercise and Biochemical Nutrition Laboratory at Baylor University
Eurycoma longifolia ("Tongkat ali")	Leitao (2021) [45]	Brazil	To investigate the effect of concurrent training (aerobic exercise) and supplementation with *Eurycoma longifolia* on erectile function and testosterone levels in men with symptoms of androgen deficiency and total testosterone serum levels equal to or less than 346 ng/dL	4-arm, double blinded, randomized placebo controlled	$N = 38$, age 47.38 ± 5.03 years Treatment group 1 ($n = 12$): control and placebo Treatment group 1 ($n = 9$): control and *Eurycoma longifolia* (200 mg/d) Treatment group 3 ($n = 7$): concurrent training and placebo Treatment group 4 ($n = 9$): concurrent training and *Eurycoma longifolia* (200 mg/d)	6 months	Significant improvement from baseline to 6 months for group 2 (278.2 ± 20.5 ng/dL to 400.3 ± 38.9 ng/dL, $p = 0.005$), for group 3 between 1 and 6 months (273.3 ± 40.2 ng/dL vs 370.8 ± 41,3 ng/dL, $p = 0.021$) and 4 and 6 months (286.8 ± 38.1 ng/dL vs 370.8 ± 41.3 ng/dL, $p = 0.012$) and group 4 between baseline and 6 months (253 ± 20.5 ng/dL vs 374.5 ± 38.9 ng/dL,	Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES)

Table 1. continued

Agent	Authors and year	Country	Objective	Type	Design	Duration of treatment	Result	Funding/support
							$p = 0.005$) and between 4 and 6 months (308.6 ± 35.9 ng/dL vs 374.5 ± 38.9 ng/dL, $p = 0.05$)	
	Chan (2021) [46]	Malaysia	To assess the impact of *Eurycoma longifolia* on the hypothalamic-pituitary-gonadal axes in healthy young males	2-arm, double blinded, randomized placebo controlled	$N = 32$, age 24.4 ± 4.7 years Treatment group ($n = 16$): *Eurycoma longifolia* (600 mg/d) Placebo group ($n = 16$)	2 weeks	There was a significant interaction between group and time for testosterone ($F_{1, 30} = 9.039$, $p = 0.005$). Testosterone level was 15% higher in EL following two weeks of consumption	This study was partly funded by the University of Malaya postgraduate fund, PG241-2016A
Lepidium meyenii ("Maca")	Gonzales (2002) [43] Gonzales (2003) [44]	Peru	To demonstrate if effect of Maca on subjective report of sexual desire was because of effect on mood or serum testosterone levels	3-arm, double blinded, randomized placebo controlled	Treatment group 1 ($n = 30$): *Lepidium meyenii* (1.5 g/day) Treatment group 2 ($n = 15$): *Lepidium meyenii* (3 g/day) Placebo group ($n = 12$)	12 weeks	No independent effect on sexual desire was observed with score of neither depression and anxiety tests nor serum testosterone and oestradiol levels	The Laboratorios Hersil and the Universidad Peruana Cayetano Heredia supported this study
Withania somnifera ("Ashwagandha")	Chauhan (2020)	India	To investigate the effect of *Withania somnifera* on sexual performance of adult males	2-arm, double blinded, randomized placebo controlled	$N = 50$, age 34.32 ± 3.21 years Treatment group ($n = 25$): 300 mg of *Withania somnifera* twice a day for 8 weeks Placebo group ($n = 25$)	8 weeks	There was a statistically significant increase in serum testosterone levels (66.52 ng/dL; −80.70 to −52.34; $p < 0.0001$; *t*-test) when compared to placebo at 8 weeks	Did not receive any funding to carry out the study
	Lopresti (2020)	Australia	To investigate the stress-relieving and pharmacological actions of *Withania somnifera* on healthy adults	2-arm, double blinded, randomized placebo controlled	$N = 37$ men, age was not disclosed for men Treatment group ($n = 19$) of 240 mg *Withania somnifera extract* (Shoden®)	60 days	There was no difference between treatment group and placebo ($p = 0.158$), but there was a statistically significant increase in treatment group (54.01 ± 2.95 ng/dL, $p = 0.038$)	This study was funded by Arjuna Natural Ltd.
	Lopresti (2019)	Australia	To investigate the hormonal and vitality effects of *Withania somnifera* on overweight men (BMI between 25 and 35)	2-arm, double blinded, crossover, randomized placebo controlled	$N = 57$, age 51.66 ± 1.19 years 8 weeks of a *Withania somnifera* extract (Shoden®) containing 21 mg of with anolide glycosides a day	16 weeks	Treatment for 8 weeks was significantly associated with increased levels of total testosterone (45.58 ± 16.64 ng/dL, $p = 0.01$) vs placebo	This study was funded by Arjuna Natural Ltd.
Zinc and/or Magnesium	Nematollahi-Mahani (2014) [31]	Iran	To investigate the effect of folic acid and zinc sulfate administration on serum hormonal level (inc testosterone) in varicocelectomized patients	4-arm, double blinded, randomized placebo controlled	$N = 160$ men who underwent varicocelectomy for grade 3 varicocele, age n/a Treatment group 1 ($n =$ n/a): zinc sulfate 66 mg/d and folic acid 5 mg/d Treatment group 2 ($n =$ n/a): folic acid 5 mg/d	6 months	No improvement of testosterone in 6 months ($p < 0.05$)	Kerman University of Medical Sciences

Table 1. continued

Agent	Authors and year	Country	Objective	Type	Design	Duration of treatment	Result	Funding/support
					Treatment group 3 (n = n/a): zinc sulfate 66 mg/d Placebo (n = n/a)			
	Cinar (2011) [33]	Turkey	To investigate the effect of magnesium supplementation and exhaustive exercise on testosterone levels of sportsmen	Comparative, unrandomized-uncontrolled study	N = 30, age range 28–22 years Treatment group 1 (n = 10): 10 mg magnesium/kg/d Treatment group 2 (n = 10): 10 mg magnesium/kg/d with tae-kwo-do training for 90–120 min per day, 5 days a week Treatment group 3 (n = 10): tae-kwo-do training for 90–120 min per day, 5 days a week To achieve exhaustion, all groups underwent a 20-m shuttle run test prior to blood sampling	4 weeks	Total testosterone increased by exhaustion in all groups, with group 2 showing the highest increase (667.3 ± 95.2 ng/dl vs 781.4 ± 98.4 ng/dl, $p < 0.05$)	Not reported
	Neek (2011) [32]	Iran	To investigate the effect of intensive exercise on testosterone levels and plasma lactate in cyclists who were supplemented with oral zinc and selenium	4-arm, double blinded, randomized placebo controlled	N = 32, age n/a Treatment group 1 (n = 8): zinc 30 mg/d Treatment group 2 (n = 8): selenium (200 μg/day) Tretment group 3 (n = 8): zinc–selenium supplement Placebo group (n = 8)	4 weeks	Numeric differences not reported (Graphic illustration) - Significant increase in total testosterone in all groups - Significant difference between effects of exhaustion exercise on serum free testosterone in Zn group in contrast with other groups ($p < 0.05$)	Not reported
	Koehler (2009) [29]	Germany	To investigate the effect of the zinc-containing nutritional supplement ZMA on serum testosterone levels in young and healthy, regularly exercising men	2-arm, double blinded, randomized placebo controlled	N = 14, age 27.0 ± 4.2 years Treatment group (n = 7 men): 3 capsules of ZMA/d) Placebo group (n = 7 men)	56 days	Numeric differences not reported (Graphic illustration) Within groups, no statistically significant differences in total T (ZMA: $p = 0.42$; placebo: $p = 0.69$)	Not reported
	Ebisch (2006) [30]	Netherland	To investigate the underlying mechanism of increased sperm concentration after folic acid and zinc sulfate intervention in fertile and subfertile men	2-arm, double blinded, randomized placebo controlled	N = 87, age n/a Treatment group (18 subfertile, 24 fertile men): folic acid (5 mg/d) and zinc sulfate (66 mg/d) Placebo group (22 subfertile, 23 fertile men)	26 weeks	No significant differences in testosterone between pre- and post-intervention testosterone in placebo [–1.0 nmol/L (–4.1 to 5.0), $p > 0.05$] and intervention group [0.2 nmol/L (–2.9 to 2.3), $p > 0.05$] *medians (25th–75th percentile)	Zorg Onderzoek

Their study population, design and main findings are depicted on this table. For the statistical tests used for results, please consult the original articles.
C creatine, *CA* creatinine plus β-alanine, *BA* β-alanine, *HMB* β-hydroxy β-methylbutyrate, *P* placebo, *ZMA* zinc magnesium aspartate.

Creatine, β-hydroxy β-methylbutyrate (HMB) and β-alanine. Creatine is an organic compound that facilitates recycling of adenosine triphosphate, primarily in muscle and nervous system cells. It is often used to improve athletic performance. Three RCTs were found on the effect of creatine supplementation on testosterone [34–36]. As expected, all were performed on male athletes [35–37]. Two did not show any significant effect when compared to placebo [35, 36]. In one study, there was also a combination arm, where creatine was given with β-alanine, also without effect [34]. In the fourth study, creatine plus β-alanine was compared to β-alanine alone on military personnel for 4 weeks, yet no difference was found on sTT [37]. β-Alanine alone also did not show any effect on male athletes' sTT in a small RCT [38].

In Fernandez-Landa's RCT, creatine alone had no effect; however, a significant increase in sTT was recorded for both creatine in combination with HMB and HMB alone [36]. HMB has previously been tested in a single crossover RCT in male athletes, with a significant increase in sTT reported after 12 weeks (4.85 ± 1.68 vs 5.38 ± 2.1 ng/mL) [39]. Both trials of HMB used a dosage of 3000 mg/day.

Withania somnifera ("Ashwagandha"). *Withania somnifera* is a herb that grows in India and the Middle East and has been used for centuries in traditional Indian medicine to as an aphrodisiac and to enhance muscle strength among other uses. Three studies were included, two in healthy men [40, 41] and another in overweight men [42]. The former study was a 16-week crossover study that included 57 overweight men, a part of which had biochemical late-onset hypogonadism, and there was a clear increase in sTT both when compared to baseline and placebo, yet the same author could not show the same effect versus placebo on a smaller 60-day trial [40]. Adverse effects were noted on both studies and no adverse effect was reported. The third trial was a 50 healthy men RCT which reported an increase in sTT after 8 weeks of 300 mg of *Withania somnifera* twice per day both versus baseline and placebo [41]. Adverse effects were also noted, and four were reported on the treatment group (two subjects experienced sleepiness, one developed mild abdominal pain, and one low-grade joint pain).

Lepidium meyenii ("Maca"). *Lepidium meyenii* is a Peruvian hypocotyl that grows exclusively in the high altitudes of the central Andes. Its root is claimed to have aphrodisiac and fertility-enhancing properties. Two studies on *Lepidium meyenii* by the same research group were included, and reported the same data with no significant results one sTT when compared to baseline or placebo [43, 44].

Eurycoma longifolia ("Tongkat Ali"). *Eurycoma longifolia* is a flowering plant native to Indochina and Indonesia, the root of which is claimed to improve libido and treat infertility. Two RCTs, one in healthy young men (600 mg/day) [45] and the other in men with late-onset male hypogonadism (200 mg/day) [46], reported an increase in sTT when compared to both baseline and placebo. The one study by Leitão et al. was a 6-month four-arm RCT, and concurrent physical exercise was not observed to have an additive or synergistic effect to *Eurycoma longifolia* [45]. A prospective non-randomized study was also performed on *Eurycoma longifolia* for men with late-onset hypogonadism with a reported positive outcome on sTT when compared to baseline, yet a very high drop-out rate and loss-to-follow-up were observed and thus its findings can be biased [47].

Trimethylglycine ("Betaine"). Historically, the term "betaine" was reserved for trimethylglycine an amino acid derivative that occurs in plants commonly found in food, which has a role in regulating cellular hydration and maintaining cell function. Two RCTs from two different research groups in Iran were included [48, 49]. Both were performed in young athletes and showed a significant increase in sTT when compared to baseline and placebo. One of the studies used a 2500 mg/day dose for 2 weeks [49], while the other used 2000 mg/day for 14 weeks during the football season [48].

D-aspartic acid ("DAA"). DAA is an amino acid used in the biosynthesis of proteins and is currently one of the most marketed TBs. Two RCT of young male athletes were included on DAA [50, 51], both used 3 g/day and neither showed a significant increase in sTT levels when compared to baseline or placebo.

Isoflavones. Isoflavones are a class of flavonoid phenolic compounds, often referred as phytoestrogens, as they can produce biological effects via binding to estrogen receptors. They are found in leguminous plants such as soy and green beans (*Phaseolus vulgaris*). The isoflavones from a soy-protein isolate were compared with a milk protein isolate in a 57-day randomized crossover trial of healthy men [52]. No significant differences were noted in sTT concentration between treatment arms. A proprietary isoflavone blend (Trinovin®, Novogen Laboratories Pty Ltd, Australia) was tested in a small single-arm prospective trial of healthy men. There was no significant effect on sTT concentration following 3 weeks of treatment [53].

Other findings

TBs were considered "promising" if one clearly positive RCT for the outcome of interest was available, "unclear" if a positive non-controlled prospective study against baseline sTT was available, the remainder were considered as "no effect shown". TBs considered The main findings from these studies are resumed in Table 4. "Unclear" and "No effect shown" TBs' results are displayed in Supplement 2 [54–63].

Promising. A proprietary blend of *Punica granatum* fruit rind and *Theobroma cacao* seed extracts (Tesnor™, Gencor Industries Inc, United Sates of America) was tested in a three-arm RCT of Indian men with symptoms suggestive of late-onset male hypogonadism [64]. A significant increase was reported in sTT when compared to baseline and with placebo. Moreover, there may be a positive dose effect with a 400 mg/day dose resulting in a greater increase than 200 mg/day.

Shilajit is an exudate from high mountain rocks rich in mineral salts and amino acids, often used in traditional medicine. A patented purified Shilajit extract (PrimaVie™, Kolkata, West Bengal, India) was used for 90 days in an RCT of healthy middle-aged men with positive results [65]. A statistically significant increase in sTT versus baseline (4.84 ± 1.54 vs 5.83 ± 1.67 ng/mL) and placebo after treatment was recorded at 90 days, but not at 30 or 60 days. A significant decrease was observed in the placebo group which is not fully expected on 90 days.

A commercially available non-hormonal aromatase inhibitor, hydroxyandrost-4-ene-6,17-dioxo-3-THP ether and 3,17-diketo-androst-1,4,6-triene (Novadex XT™, Gaspari Nutrition, United States of America) was used in an 8-week RCT in resistance-trained men, with a mean 283% increase in sTT at 8 weeks of treatment [66]. Results were significant when compared to placebo. No adverse effects were reported by participants.

DISCUSSION

When designing the present systematic review, our aim was to scrutinize if so-called TBs could "boost" sTT concentration. Our group found a heterogeneous group of active ingredients and identified a total of 28 different TBs, most of which were the subject of only one study in the last two decades.

Our first aim was to discover if there was at least some evidence to support the bold claims made by the supplement industry regarding testosterone increase. Thus, our primary endpoint was

Table 2. Study characteristics for cholecalciferol ("Vitamin D").

Authors and year	Country	Objective	Type	Design	Duration of treatment	Result	Support
Maghsoumi-Norouzabad (2021) [24]	Iran	To investigate the effects of vitamin D3 on sperm parameters and endocrine markers in infertile men with asthenozoospermia	2-arm, triple blinded, randomized placebo controlled	$N = 86$, age 34.78 ± 5.29 years Treatment group ($n = 43$): 4000 IU VD3 OD Placebo ($n = 43$)	12 weeks	No significant effect on testosterone in treatment group (from 3.87 ± 2.08 to 3.95 ± 1.75, $p > 0.05$)	Unclear
Michalczyk (2020) [22]	Poland	To investigate the influence of natural sun exposure and 6 weeks of a high dose of vitamin D supplementation on parameters of testosterone in professional football players	2-arm, blinded, randomized placebo controlled	$N = 28$, age n/a Treatment group ($n = 15$): 2000 IU cholecalciferol TDS Placebo ($n = 13$)	6 weeks	A significant difference was noticed in treatment group (from 22.95 ± 6.4 nmol/L to 28.25 ± 3.2 nmol/L, $p < 0.05$) but not in the placebo group (from 23.64 ± 3.9 nmol/L to 26.60 ± 5.1 nmol/L, $p > 0.05$)	Institute of Sport Sciences The Jerzy Kukuczka Academy of Physical Education in Katowice
Ramezani Ahmadi (2020) [23]	Iran	To investigate the effect of vitamin D3 supplement on serum levels of anabolic hormones, cortisol, anaerobic and aerobic performance in active males	2-arm, double blinded, randomized placebo controlled	$N = 46$, age 24.22 ± 3.44 years Treatment group ($n = 20$): 2000 IU/day Placebo group ($n = 20$)	12 weeks	A significant reduction of testosterone in treatment group (6.08 ± 1.18 to 5.26 ± 1.01, $p < 0.05$)	Vice-Chancellor of Research, Ahvaz Jundishapur University of Medical Sciences
Zittermann (2018)	Germany	To investigate whether a daily vitamin D3 supplement is able to improve male sex hormone concentrations in patients with advanced HF and 25OHD concentrations <75 nmol/L	2-arm, unknown blinding, randomized placebo controlled	$N = 133$, age: intervention: 55.0 ± 9.9 years; placebo: 51.1 ± 10.5 years Treatment group ($n = 71$): 000 IU (100 µg) cholecalciferol per day Placebo group ($n = 62$)	3 years	Total testosterone declined non-significantly between baseline and follow-up in the placebo group (mean difference −1.37, −2.94 to 0.19, $p = 0.084$) and remained constant in the vitamin D group (0.29, −2.65 to 3.22, $p = 0.845$)	Heart and Diabetes Center North Rhine-Westphalia, Ruhr University Bochum, Germany Friede Springer Herz Stiftung (Berlin, Germany) Merck KGaA (Darmstadt, Germany, EMR200109-616) DiaSorin (Dietzenbach, Germany)
Mielgo-Ayuso (2018) [18]	Spain	To investigate the influence of vitamin D supplementation on the hematological and iron metabolism profile, testosterone and cortisol on elite male traditional rowers	2-arm, double blinded, randomized placebo controlled	$N = 36$, age 27 ± 6 years Treatment group ($n = 18$): 3000 IU of vitamin D3 OD Placebo group ($n = 18$)	8 weeks	No statistically significant differences in the group-by-time interaction between groups ($p = 0.852$) but there was a significant decrease in both groups (treatment: from 5.37 ± 1.5 ng/mL to 4.73 ± 1.28 ng/mL, $p < 0.05$; placebo: 5.06 ± 1.41 ng/mL to 4.37 ± 0.96 ng/mL, $p < 0.05$)	Not reported
Lerchbaum (2018)	Austria	To investigate whether vitamin D supplementation increases serum total testosterone in men with low levels at baseline	2-arm, double blinded, randomized placebo controlled	$N = 100$, median age 49 (39–56) years Treatment group ($n = 47$): 2857 IU vitamin D OD Placebo ($n = 47$)	12 weeks	No significant between-group differences (−0.188, −1.50 to 1.12, $p = 0.776$)	Austrian National Bank
Saha (2017)	India	To investigate the effect of cholecalciferol/calcium on skeletal muscle strength and serum testosterone in vitamin D-deficient young males	4-arm, double blinded, randomized placebo controlled	$N = 228$, age 20.2 ± 2.2 years Treatment group 1 ($n = 41$): calcium-carbonate (500 mg elemental calcium, BD for 6 months) and placebo Treatment group 2 ($n = 49$): cholecalciferol	6 months	At 6 months, the serum testosterone showed a significant decline from baseline in 180 subjects (21.2 ± 6.0 to 19.1 ± 5.9 nmol/L) but no significant differences between groups ($p > 0.05$)	Indian Council of Medical Research

Table 2. continued

Authors and year	Country	Objective	Type	Design	Duration of treatment	Result	Support
				(60,000 IU/week for 8 weeks followed by 60,000 IU/fortnightly for 4-months) and placebo Treatment group 3 ($n = 47$): cholecalciferol (60,000 IU/week for 8 weeks followed by 60,000 IU/fortnightly for 4-months) and calcium-carbonate (500 mg elemental calcium, BD for 6 months) Placebo ($n = 43$): double placebo			
Lerchbaum (2017) [17]	Austria	To investigate whether vitamin D supplementation increases total testosterone levels in healthy men	2-arm, double blinded, randomized placebo controlled	$N = 100$, median age 37 (27–50) Treatment group ($n = 49$): Oral dose of 20,000 IU vitamin D Placebo ($n = 49$)	12 weeks	No difference within groups after 12 weeks (treatment 0.5, −2.2 to 2.2, $p = 0.922$; placebo 0.5, −1.9 to 2.6, $p = 0.424$) and between groups ($p = 0.497$)	Austrian National Bank
Heijboer (2015) [16]	The Netherlands	To investigate a causal link between vitamin D and testosterone status in men with heart failure (study 1), male nursing home residents (study 2) and male non-Western immigrants in the Netherlands (study 3)	2-arm, double blinded, randomized placebo controlled	Study 1, $N = 92$, median age 63 [42–86] years to 6 weeks Treatment group ($n = 42$): 2000 IU cholecalciferol daily Placebo ($n = 44$) Study 2, $N = 49$, median age 82 [71–97] years – 16 weeks Treatment group ($n = 21$): Vitamin D3 600 IU/d, 4200 IU/w or 18,000 IU/month Placebo ($n = 22$) Study 3, $N = 42$, median age 53 [20–70] years – 16 weeks Treatment group ($n = 16$): 1200 IU vitamin D/d Placebo ($n = 16$)	Study 1 – 6 weeks Study – 16 weeks Study 3 – 16 weeks	Testosterone concentrations were not affected by the vitamin D supplementation in all 3 studies ($p > 0.05$)	Abbott, AstraZeneca, BG Medicine, Novartis, Pfizer, Baxter, Biomerieux and Medcon
Pilz (2011) [15]	Austria	To investigate whether vitamin D supplementation affects testosterone levels in Healthy overweight men undergoing a weight reduction	2-arm, double blinded, randomized placebo controlled	$N = 54$, age 49.4 ± 10.2 years Treatment group ($n = 31$): 83 µg vitamin D/d Placebo group ($n = 23$)	52 weeks	No significant difference in total testosterone the placebo group (11.8 ± 4.0 nmol/l vs. 12.7 ± 5.45 nmol/l, $p = 0.355$) but a significant difference in the treatment group (10.7 ± 3.9 nmol/l to 13.4 ± 4.7 nmol/l, $p < 0.001$)	Not reported

A total of 10 studies were included, their study population, design and main findings are depicted in this table. For the statistical tests used for results, please consult the original articles.

Table 3. Study characteristics for *Tribulus terrestris*.

Authors and year	Country	Objective	Type	Design	Duration of treatment	Result	Support
Fernández-Lázaro (2022) [13]	Spain	To investigate whether *Tribulus terrestris* supplementation improves body composition, hormonal response, and performance among CrossFit athletes	2-arm, single blinded, randomized placebo controlled	$N = 30$ Treatment group (n15): age 33.1 ± 5.7 years, 770 mg of TT per day Placebo ($n = 15$): age 32.9 ± 6.3	6 weeks	No significant effect on testosterone (5.76 ± 0.86 to 5.75 ± 1.24 ng/dL, $p > 0.05$) in the treatment group	Not reported
Roaiah (2016) [26]	Egypt	To investigate the effect of *Tribulus terrestris* extract on serum testosterone subfertile men	Single arm, uncontrolled interventional	$N = 30$, age n/a Treatment group (n-30): 250 mg TT	6 weeks	No significant increase in testosterone (2500 ± 0.131 to 2700 ± 0.240 nmol/L, $p > 0.05$)	Not reported
Roaiah (2015)	Egypt	To investigate the effect of *Tribulus terrestris* extract on testosterone of aging men with manifestations of partial androgen deficiency	Single arm, uncontrolled interventional	$N = 30$, age n/a Treatment group (n-30): 250 mg TT	3 months	A significant increase in testosterone (2133 ± 0.1954 to 2837 ± 1.698 nmol/L, $p < 0.05$)	Not reported
Neychev (2005) [25]	Bulgaria	to investigate the influence of *Tribulus terrestris* extract on androgen metabolism in young males	3-arm, unknown blinding, randomized placebo controlled	$N = 21$, age 20–36 years Treatment group 1($n = 7$): 20 mg/kg body weight per day of TT Treatment group 1($n = 7$): 10 mg/kg body weight per day of TT Control group ($n = 7$)	4 weeks	No significant effect of Tribulus on testosterone (15.75 ± 1.75 nmol/l for group 1, 16.32 ± 1.57 nmol/l for group 2, 17.74 ± 1.09 nmol/l for placebo, $p > 0.05$)	Not reported

A total of four studies were included, their study population, design and main findings are depicted in this table. For the statistical tests used for results, please consult the original articles.
TT Tribulus terrestris.

Table 4. Study characteristics for other testosterone boosters.

Authors and year	Country	Objective	Type	Design	Duration of treatment	Result	Support
Pandit (2022) [64]	India	To investigate the safety and efficacy of a novel combination of Punica granatum and Theobroma cocoa seed extracts in men with aging males' symptoms	3-arm, double blinded, randomized placebo controlled	$N = 120$, age 36–55 years Treatment group 1($n = 37$): 200 mg/d Treatment group 2($n = 40$): 400 mg/d Placebo group ($n = 38$)	8 weeks	Significant increase of testosterone in treatment group 1 (12.48 ± 5.47 to 15.15 ± 6.01 nmol/L, <0.0001) and treatment group 2 (13.69 ± 5.13 to 17.05 ± 4.98 nmol/L, <0.0001) from baseline at the end of the study, and significantly higher levels ($p < 0.05$) for groups 1 and 2 compared to placebo at the end of the study (12.96 ± 5.32 nmol/L)	Laila Nutraceuticals, Vijayawada, Andhra Pradesh, India
Haidari (2020)	Iran	To investigate the effect of lipoic acid on testosterone levels of infertile men with idiopathic asthenozoospermia	2-arm, triple blinded, randomized placebo controlled	$N = 44$, age 33.56 ± 5.07, years Treatment group ($n = 23$): 600 mg of lipoic acid daily Placebo group ($n = 21$)	12 weeks	No significant increase in testosterone for treatment group (14.56 ± 2.85 to 16.60 ± 3.24 nmol/L, $p = 0.081$)	
Maheshwari (2017) [61]	India	To evaluate the efficacy of Furosap, a novel *Trigonella foenum*-graecum seed extract, in enhancing testosterone level and improving sperm profile in male volunteers	Single-arm, interventional, uncontrolled study	$N = 50$, age 43.08 ± 7.35 Treatment group: 500 mg of Furosap	12 weeks	A non-significant increase in testosterone was seen at the end of the study (405.19 + 156.95 to 436.34 + 189.94, $p = 0.164$)	Cepham Inc, Piscataway, NJ, USA
Santi (2017) [62]	Italy	To investigate whether long-term, chronic treatment with vardenafil affects adrenal and testicular steroidogenesis in diabetic type 2 men	2-arm, investigator-started, double blinded, randomized placebo controlled	$N = 54$, age 56.2 ± 4.6 years Treatment group ($n = 20$): Vardenafil 10 mg twice daily Placebo group ($n = 22$)	24 weeks	No significant baseline differences in testosterone were seen and no differences were seen among visits, between the study and the control group (12.5 ± 4.5 vs 13.6 ± 4.5 nmol/L, at the end of trial, respectively)	Bayer HealthCare
Pandit (2015)	India	The investigate the effect of purified Shilajit on testosterone levels in healthy volunteers	2-arm, double blinded, randomized placebo controlled	$N = 96$, age 49.3 ± 2.6 years Treatment group ($n = 38$): 250 mg bid Placebo ($n = 37$)	3 months	A significant increase in the treatment group was seen (4.84 ± 1.54 to 5.83 ± 1.67, $p > 0.05$) in 3 months Measurement at 3 months was significantly higher in the treatment group (5.83 ± 1.67 vs 4.45 ± 1.78, $p < 0.05$)	Natreon Inc
Derouiche (2014)	Morocco	To assess the effect of virgin argan oil (VAO) and extra virgin olive oil (EVO) on the hormonal profile of androgens and anthropometric parameters among healthy adult Moroccan men	2-arms, unknown blinded, randomized, non-placebo controlled	$N = 60$, mean age of 23.42 ± 3.85 years Treatment group 1 ($n = 30$): Argan oil (dose n/a) Treatment group 2 ($n = 30$): Olive oil (dose n/a)	3 weeks	Significant improvement in testosterone in both groups (+17.37% for group 1 and +19.95% for group 2, $p < 0.05$) but no difference between groups ($p > 0.05$)	Institut Aicha Santé et Nutrition

Table 4. continued

Authors and year	Country	Objective	Type	Design	Duration of treatment	Result	Support
Giltay (2012) [59]	The Netherlands	To investigate whether the n-3 fatty acids affects (EPA-eicosapentaenoic acid, DHA-docosahexaenoic acid, ALA-alpha-linolenic acid) on testosterone levels in post-myocardial infarction patients	4-arm, double blinded, randomized placebo controlled	$N = 1850$, age 68.4 ± 5.3 years Treatment group 1 ($n = 1212$): 400 mg of EPA-DHA and 2 grams ALA daily Treatment group 2 ($n = 1192$): 400 mg of EPA-DHA daily Treatment group 3 ($n = 1197$): 2 grams of ALA daily Placebo group ($n = 1236$)	41 months	No significance in total testosterone levels in all intervention groups Borderline statistical significance for a decline in testosterone levels in the group treated with ALA compared to placebo (of −0.50 ± 0.26 nmol/L $p = 0.052$)	Netherlands Heart Foundation, US National Institutes of Health (NIH), Unilever R&D, The Netherlands Brain Foundation, Abbott Diagnostics
Goto (2011) [58]	Japan	To investigate the effects of supplementation with chicken breast meat extract (CBEX) containing carnosine and anserine on free testosterone (FT) to resistance exercise in young healthy men	2-arm, double blinded, randomization not reported placebo controlled	$N = 22$, age 25 ± 1 years Treatment group ($n = 14$): 20 g CBEX bid Placebo group ($n = 8$)	30 days	Within the treatment group, FT was significantly increased compared to pre-exercise value (numerical data not available, $p < 0.05$) but the response was similar before and after supplementation	Nippon Meat Packers, Inc., H. Maemura
Zhang (2009) [57]	China	To investigate whether the ingestion of a herbal supplement called Rhodiola–Gingko Capsule (RGC) on endurance and relevant hormones in highly trained men	2-arm, double blinded, randomized placebo controlled	$N = 70$, age 19.9 ± 1.0 years Treatment group ($n = 34$): 270 mg RGC QDS Placebo group ($n = 33$)	7 weeks	No significant change was noted in treatment group (10.3 ± 46.2 ng/dL, $p > 0.05$) or placebo (–9.7 ± 38.2 ng/dL, $p > 0.05$)	Integrated Chinese Medicine Holdings Ltd., Hong Kong, China
Cinar (2009) [56]	Turkey	To investigate the effects of calcium supplementation on serum testosterone levels in highly trained men	3-arm, interventional, non-randomized, controlled	$N = 30$, age n/a Treatment group 1: 35 mg/kg/day only Treatment group 2: 35 mg /kg/day of a calcium gluconate and training Treatment group 3: training only	4 weeks	Testosterone levels after exhaustion were significantly higher in group 2 compared to group 1 (731.20 ± 47.78 ng/dl vs 712.60 ± 63.10 ng/dl, $p < 0.05$) but the higher value was in group 3 (740.40 ± 53.30 ng/dl, $p < 0.05$)	Not reported
Hoffman (2008) [38]	United States of America	To investigate the effect of effect of b-alanine supplementation on resistance exercise performance and endocrine changes in resistance-trained men	2-arm, double blinded, crossover, randomized, placebo controlled	$N = 8$, age 19.7 ± 1.5 years Treatment group: 1.6 g TDS of alanine	12 weeks	Numeric differences not reported (Graphic illustration) —no significant difference due to supplementation	Not reported
Willoughby (2007) [66]	United States of America	To investigate the effects of an aromatase-inhibiting nutritional supplement on serum steroid hormones etc. in young, regularly exercising eugonadal men	2-arm, blinded, randomized placebo controlled	$N = 16$, age 26.11 ± 4.42 years Treatment group ($n = 8$): 72 mg/d of AI Novedex XT™ Placebo group ($n = 8$)	8 weeks	Significant increase for the Novedex group compared with placebo at the 4- and 8-week sampling period (graphic presentation)	Gaspari Nutrition, Neptune, NJ

Table 4. continued

Authors and year	Country	Objective	Type	Design	Duration of treatment	Result	Support
Che (2005) [55]	China	To investigate the efficacy and safety of Jingui Shengqi Pill in treating partial androgen deficiency in aging males	Single-arm, interventional, uncontrolled study	$N = 40$, age 60.5 ± 8.5 years Treatment group: 3 g of crude drugs	3 months	21 patients received 1 course (3 months) had serum T increased from 4.28 ± 0. 21 μg/L to 5.13 ± 0.48, $p < 0.05$ 19 patients received 2 courses (2 × 3 months) had serum T increased from 4.23 ± 0.64 μg/L to 5.68 ± 0.33, $p < 0.05$	Not reported
Abel (2005) [54]	Switzerland	To investigate whether daily intake of two different dosages of arginine aspartate during 4 weeks affects selected parameters performance, metabolic and endocrine parameters in endurance athletes	3-arm, double blinded, randomized placebo controlled	$N = 30$, age 37 ± 5.8 Treatment group 1 ($n = 10$): 5.7 g arginine and 8.7 g aspartate Treatment group 1 ($n = 10$): 2.8 g arginine and 2.2 g aspartate Placebo group ($n = 10$)	4 weeks	No significant differences before and after supplementation (37.0 ± 5.8 to 39.7 ± 7.7 for treatment group 1, 46.6 ± 12.1 to 43.8 ± 8.6 for treatment group 2, 38.2 ± 9.4 to 38.3 ± 8.2 for placebo group, $p > 0.05$)	Not reported
Dillingham (2005) [52]	United States of America	To investigate the effects of soy protein of varying isoflavone content on a wide profile of serum reproductive hormones in a sample of healthy young men	3-arm, unblinded, crossover, randomized controlled	$N = 35$, age 27.9 ± 5.7 years Treatment group 1: low-iso Soy protein (SP) ingestion Treatment group 2: high-iso Soy protein ingestion Control group: milk protein (MP) ingestion	8 weeks	Serum testosterone was decreased by the low-iso SP ingestion relative to the MP ingestion ($p = 0.023$) and the high-iso SPI ($p = 0.020$) in 1 month	Not reported
Gambelunghe (2003)	Italy	To investigate if oral supplementation with food containing the aromatase inhibitor chrysin, such as propolis and honey, could modify testosterone urinary levels in young, healthy males	2-arm, unblinded, non-randomized	$N = 20$, age 25–30 years Treatment group ($n = 10$): 1280 mg of propolis and 20 g of honey Placebo group ($n = 10$)	21 days	Small increase of testosterone from baseline to 21 days in the treatment group (48.8 ± 7.2 to 49.1 ± 6.0 ng/mL in the urine)	None reported
Lewis (2002) [53]	New Zealand	To investigate the effect of isoflavone extract ingestion, Trinovin, in hormonal status of young healthy men	Single arm, uncontrolled interventional	$N = 6$, age 40–53 years Treatment group: 40 mg of Trinovin OD	4 weeks	Reported unchanged testosterone from the basal level (expressed % of basal level) 100.0 ± 16.6	Not reported

Their study population, design and main findings are resumed on this table. For the statistical tests used for results, please consult the original articles.
ALA alpha-linolenic acid, *CBEX* chicken breast meat extract, *DHA* docosahexaenoic acid, *EPA* eicosapentaenoic acid, *FT* free testosterone, *RGC* Rhodiola–Gingko Capsule.

the ability of the intervention (TB) to increase sTT concentration when compared to placebo at end-of-treatment assessment, in at least one of the four specified populations of interest (healthy men, male athletes, men with late-onset hypogonadism and infertile men). It was clear from the outset that reporting an increase versus baseline was insufficient evidence to consider a TB effective. In the case of male athletes, participation in an exercise program can impact sTT and interfere with study results, as such, a control group was considered essential inclusion criteria. We favored sTT over serum FT as our group anticipated that, first, not all studies would measure FT, and, second, that the methods used to measure FT would be considered unreliable by current standards.

The four populations of interest, although unrelated, have in common an interest, more or less legitimate, on TBs: to inscribe sTT in order to achieve a clinical benefit. Results were not reported by population of interest, as an effect in one population was not generalizable to all. However, our group set a low evidentiary test for TB efficacy, classifying a TB "potentially effective" if its outcome was shown de facto in at least one of the four populations.

Although supplementation with cholecalciferol to increase testosterone is novel (almost all its included studies have been published in the last 5 years), cholecalciferol was the most studied TB. Our review indicates that, at present, there is no robust evidence to support a claim that cholecalciferol increases sTT concentration. Testing occurred in mostly heterogeneous scenarios and durations of treatment, with no effect shown in almost all trials. There was one study with a clearly positive finding [22]; however, it studied a very specific population (athletes without natural sun exposure during the winter season), the findings of which are not generalizable.

Tribulus terrestris is arguably the most well-known TB and is the subject of a few previous systematic reviews [9, 13]. Surprisingly, our group only found four articles, with none confirming *Tribulus terrestris* as effective for increasing sTT. Only one study was reported as positive, however it was a single-arm study in men with late-onset hypogonadism, without a control group [26]. Moreover, the sTT concentration reported at the end of treatment was still under the normal range for most participants, which is unlikely to be sufficient to manage late-onset hypogonadism. Our results are in accordance with previous systematic reviews on *Tribulus terrestris*.

Another popular but controversial TB is ZMA. Its original study from 2000 (outside our systematic review timeline) has been discredited due to funding issues disclosed during the BALCO scandal. Only one study was found on the original combination of the two active ingredients, with a clear negative result [29]. Studies of zinc and magnesium alone were also found, but all were negative. As such, there is no evidence to support a claim that ZMA has testosterone-boosting properties.

From all performance-enhancing supplements commonly used by athletes (creatine, HMB, β-alanine, DAA and betaine) that were investigated as TBs, only HMB and betaine showed promise, both with two studies each on male athletes with a positive outcome [36, 39, 48, 49]. HMB showed a mean 70 ng/mL or 14.2% increase in baseline sTT after 10–12 weeks of supplementation, while betaine showed a mean 485 ng/mL or 94.1% boost in a 2-week study. First, it is not clear if this effect is translatable to other populations of interest, or if it is the result of a synergistic effect with exercise, and second, although betaine supplementation achieved an impressive result, almost doubling sTT baseline values, both studies were performed in the same country and time frame, thus other bias cannot be excluded. External validation is recommended as betaine results appear almost "too good to be true". There were no indications that creatine, β-alanine and DAA could increase sTT. This is in accordance with a DAA systematic review in which its promise on animal studies did also not translate to human studies [10].

Withania somnifera ("Ashwagandha") and *Eurycoma longifolia* ("Tongkat Ali") were the only herbal supplements that have shown potential. *Withania somnifera* had two positive RTCs, one in overweight men and other in healthy men [41, 42], but a third one in healthy men had inconclusive results as sTT was not increased when compared to placebo [40]. Yet, this was a very small trial and there was a numerical, but not statistical, difference in baseline sTT (472.88 ± 45.06 ng/dL vs 543.47 ± 46.29 ng/dL, $p = 0.282$), which in a small trial can be attributed to a false negative. There was a clear increase in sTT in the treatment group (56.01 ± 2.95 ng/dL, 11.8%) [40]. Further studies may help clarify *Withania somnifera* role as a TB. *Eurycoma longifolia* had two positive RCTs, one in middle-aged men with late-onset hypogonadism and the other in young healthy men. The latter showed a mean increase of 122.1 ng/dL 43.8% in sTT when compared to baseline [46], while the former showed an almost identical 122 ng/mL absolute mean increase in sTT [45], yet with a lower mean relative increase (15.4%) as participants baseline sTT was higher than in the latter study. Both studies were placebo controlled but were performed on different populations of interest. As results could not be validated for the same population, Tongkat Ali could not be considered an effective TB per our categorizations, and so was classified as "promising" for both healthy men and men with late-onset hypogonadism. Our findings are in accordance with a late systematic review on *Eurycoma longifolia published* in 2017 (which postdates both RCTs) that found "convincing evidence for the prominence of *Eurycoma longifolia* in improving the male sexual health". Common herbal extract *Lepidium meyenii* ("Maca") or *Trigonella foenum-graecum* ("Fenugreek") did not show any evidence of effect. Fenugreek, at most, in its included single-arm study, demonstrated an increase in only FT, which did not meet our criteria for efficacy, yet a lack of statistical power cannot be excluded as this was a small study. A systematic review from 2019 concluded that both *Trigonella foenum-graecum* ("Fenugreek") and *Withania somnifera* ("Ashwagandha") were found to be promising TBs [12], yet the evidence supporting that claim is not that clear-cut after thorough review.

Regarding the category of "other", as these TBs were the subject of only a single study, confirmation or validation of their results using the findings of other studies would not be possible, which was an important limitation when reviewing. A proprietary blend of *Punica granatum* fruit rind and *Theobroma cacao* seed extracts (Tesnor™, Gencor Industries Inc, United States of America) [64] and a patented purified Shilajit extract (PrimaVie™, Novogen Laboratories Pty Ltd, Australia) [65] were considered possibly effective TBs, as both have shown an increase to testosterone in an RCT in middle-aged men; however, both studies were industry-funded and lack external validation. Another relevant plant-based extract was the Jingui Shenqui pill, which was also tested in middle-aged men [55], yet its study was a preliminary single-armed and lacked a placebo control; thus, in our assessment, the efficacy of the Jingui Shenqui pill is unclear according to our predefined methodology. The same was observed for the study on virgin argan oil and extra virgin olive oil [60], for which the methodology was unclear as participants were pre-treated with butter, there was a lack of a control arm and the study duration was limited to 3 weeks.

All other TBs identified and included in our systematic review that have not been mentioned in this discussion thus far failed to show any evidence of efficacy in terms of increasing sTT concentrations. A calcium gluconate study was reported by its authors as a positive result [56], yet there is no evidence in the data provided to support this claim: a statistically significant difference was not shown between exercise alone and exercise plus calcium gluconate, thus it cannot be concluded that calcium gluconate could have an additive or synergistic effect to exercise on sTT.

An aromatase inhibitor available over the counter, Novadex XT™, also showed promise with the largest reported increase in sTT of all the included trials validated against placebo (a mean 283%

increase in sTT) [66]. Although not an androgen per se, Novadex XT™ does not fit the usual profile of a TB supplement as it is neither a natural product nor it is unrelated to hormonal therapy. In accordance with our predefined methods, this study was included in the review as it did not meet any exclusion criteria.

Identifying adverse effects was a secondary objective of the systematic review. These substances are often generally regarded as safe by the public, as many are plant-based extracts with a long history of use in traditional medicine. From all included studies, only eight reported on adverse effects [19, 40–42, 56, 57, 64, 66], which is clearly underreporting. *Withania somnifera* appears to be safe in the short term as three RCTs reported no to rare minor adverse effects [40–42]. Regarding adverse effects reported, it is noteworthy that long-term vitamin D supplementation caused hypercalciuria in 4.3–8.5% of participants following 6 months of treatment [19].

Regarding the risk of bias assessment, some studies were single-armed and the lack of placebo control group conferred a high risk of bias. Moreover, some included RCT did not provide sufficient information on random sequence generation, or else a high-risk method was reported. Incomplete outcome data was also identified in a few studies as data were only reported as a figure, preventing the checking of numerical data. Looking specifically at "potential" and "possible" TBs: the two studies on HMB had an unclear risk of bias, mainly due to a lack of information regarding sequence generation; both studies on betaine had a low risk of bias; and all the studies on possible TBs (blend of *Punica granatum* fruit rind and *Theobroma cacao* seed extracts, purified Shilajit extract and the non-hormonal aromatase inhibitor) had high risk of bias, as all trials were small-scale and industry-funded. Moreover, one preselected confounder, medical history of diabetes mellitus, was not accounted for in almost any of the studies. Diabetes mellitus is a known risk factor for late-onset male hypogonadism and should have been controlled for, particularly in studies regarding middle-aged men.

Our systematic review is clearly limited by the level of available evidence. Most of the identified TBs were the subject of only one study, and of these, most had a high overall risk of bias and heterogeneous populations and methodologies that limit the ability to synthesize the evidence found. The use of medical subject headings in our search string may have narrowed the returned results, leading to the omission of studies improperly indexed. Although significant increases in sTT were noted in a few TBs, a threshold for a clinically significant increase is yet unknown and thus it may not have the desired clinical effect. Although some studies have assessed if an increase in sTT did translate in relevant clinical outcomes, whether through male hypogonadism symptoms questionnaires or physical performance tests, these reports were far inconsistent and heterogeneous; our group did not at this point in time decide to take them into account in our systematic review.

A key strength is that this is the first systematic review on TBs collectively that was properly designed and conducted. All identified abstracts and full texts were reviewed twice before being selected for inclusion by a group of well-trained reviewers.

CONCLUSIONS

Our extensive systematic review has concluded that most TBs fail to demonstrate their ability to increase sTT under RCT conditions. Notable exceptions were HMB and betaine, which can be considered effective TBs in male athletes. *Eurycoma longifolia* ("Tongkat Ali"), a proprietary blend of *Punica* granatum fruit rind and *Theobroma cacao* seed extracts (Tesnor™) and a patented purified Shilajit extract (PrimaVie™) can be considered as possibly effective TBs for men with late-onset hypogonadism; *Withania somnifera* ("Ashwagandha") and "Tongkat Ali" possibly effective for healthy men; and a non-hormonal aromatase inhibitor (Novadex XT™) possibly effective for male athletes. Adverse effects were rarely reported. Many studies presented a high risk of bias, while known confounders were not accounted for, as such our conclusions should be interpreted with caution.

DATA AVAILABILITY

The data that support the findings of this study are available from the corresponding author, AM, upon reasonable request.

REFERENCES

1. Cui T, Kovell RC, Brooks DC, Terlecki RP. A urologist's guide to ingredients found in top-selling nutraceuticals for men's sexual health. J Sex Med. 2015;12:2105–17.
2. Herriman M, Fletcher L, Tchaconas A, Adesman A, Milanaik R. Dietary supplements and young teens: misinformation and access provided by retailers. Pediatrics. 2017;139:e20161257.
3. Balasubramanian A, Thirumavalavan N, Srivatsav A, Yu J, Lipshultz LI, Pastuszak AW. Testosterone imposters: an analysis of popular online testosterone boosting supplements. J Sex Med. 2019;16:203–12.
4. Clemesha CG, Thaker H, Samplaski MK. 'Testosterone boosting' supplements composition and claims are not supported by the academic literature. World J Mens Health. 2020;38:115–22.
5. de Lange RW. Testosterone boosters: a report of a supplement's misleading labelling claims. S Afr J Sports Med. 2020;32:v32i1a7426.
6. Regan KS, Wambogo EA, Haggans CJ. NIH and USDA funding of dietary supplement research, 1999-2007. J Nutr. 2011;141:1–3.
7. Rahnema CD, Crosnoe LE, Kim ED. Designer steroids – over-the-counter supplements and their androgenic component: review of an increasing problem. Andrology. 2015;3:150–5.
8. Jedrejko K, Lazur J, Muszynska B. Risk associated with the use of selected ingredients in food supplements. Chem Biodivers. 2021;18:e2000686.
9. Qureshi A, Naughton DP, Petroczi A. A systematic review on the herbal extract *Tribulus terrestris* and the roots of its putative aphrodisiac and performance enhancing effect. J Diet Suppl. 2014;11:64–79.
10. Roshanzamir F, Safavi SM. The putative effects of D-Aspartic acid on blood testosterone levels: a systematic review. Int J Reprod Biomed. 2017;15:1–10.
11. Thu HE, Mohamed IN, Hussain Z, Jayusman PA, Shuid AN. *Eurycoma longifolia* as a potential adoptogen of male sexual health: a systematic review on clinical studies. Chin J Nat Med. 2017;15:71–80.
12. Santos HO, Howell S, Teixeira FJ. Beyond tribulus (*Tribulus terrestris* L.): the effects of phytotherapics on testosterone, sperm and prostate parameters. J Ethnopharmacol. 2019;235:392–405.
13. Fernandez-Lazaro D, Fernandez-Lazaro CI, Seco-Calvo J, Garrosa E, Adams DP, Mielgo-Ayuso J. Effects of *Tribulus terrestris* L. on sport and health biomarkers in physically active adult males: a systematic review. Int J Environ Res Public Health. 2022;19:9533.
14. Page MJ, Moher D, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. PRISMA 2020 explanation and elaboration: updated guidance and exemplars for reporting systematic reviews. BMJ. 2021;372:n160.
15. Pilz S, Frisch S, Koertke H, Kuhn J, Dreier J, Obermayer-Pietsch B, et al. Effect of vitamin D supplementation on testosterone levels in men. Horm Metab Res. 2011;43:223–5.
16. Heijboer AC, Oosterwerff M, Schroten NF, Eekhoff EM, Chel VG, de Boer RA, et al. Vitamin D supplementation and testosterone concentrations in male human subjects. Clin Endocrinol (Oxf). 2015;83:105–10.
17. Lerchbaum E, Pilz S, Trummer C, Schwetz V, Pachernegg O, Heijboer AC, et al. Vitamin D and testosterone in healthy men: a randomized controlled trial. J Clin Endocrinol Metab. 2017;102:4292–302.
18. Mielgo-Ayuso J, Calleja-Gonzalez J, Urdampilleta A, Leon-Guereno P, Cordova A, Caballero-Garcia A, et al. Effects of vitamin D supplementation on haematological values and muscle recovery in elite male traditional Rowers. Nutrients. 2018;10:1968.
19. Saha S, Goswami R, Ramakrishnan L, Vishnubhatla S, Mahtab S, Kar P, et al. Vitamin D and calcium supplementation, skeletal muscle strength and serum testosterone in young healthy adult males: randomized control trial. Clin Endocrinol (Oxf). 2018;88:217–26.
20. Lerchbaum E, Trummer C, Theiler-Schwetz V, Kollmann M, Wolfler M, Heijboer AC, et al. Effects of vitamin D supplementation on androgens in men with low testosterone levels: a randomized controlled trial. Eur J Nutr. 2019;58:3135–46.
21. Zittermann A, Ernst JB, Prokop S, Fuchs U, Dreier J, Kuhn J, et al. Vitamin D supplementation does not prevent the testosterone decline in males with advanced heart failure: the EVITA trial. Eur J Nutr. 2019;58:673–80.

22. Michalczyk MM, Golas A, Maszczyk A, Kaczka P, Zajac A. Influence of sunlight and oral D(3) supplementation on serum 25(OH)D concentration and exercise performance in elite soccer players. Nutrients. 2020;12:1311.
23. Ramezani Ahmadi A, Mohammadshahi M, Alizadeh A, Ahmadi Angali K, Jahanshahi A. Effects of vitamin D3 supplementation for 12 weeks on serum levels of anabolic hormones, anaerobic power, and aerobic performance in active male subjects: a randomized, double-blind, placebo-controlled trial. Eur J Sport Sci. 2020;20:1355–67.
24. Maghsoumi-Norouzabad L, Zare Javid A, Mansoori A, Dadfar M, Serajian A. The effects of Vitamin D3 supplementation on Spermatogram and endocrine factors in asthenozoospermia infertile men: a randomized, triple blind, placebo-controlled clinical trial. Reprod Biol Endocrinol. 2021;19:102.
25. Neychev VK, Mitev VI. The aphrodisiac herb *Tribulus terrestris* does not influence the androgen production in young men. J Ethnopharmacol. 2005;101:319–23.
26. Roaiah MF, El Khayat YI, GamalEl Din SF, Abd El Salam MA. Pilot study on the effect of botanical medicine (*Tribulus terrestris*) on serum testosterone level and erectile function in aging males with partial androgen deficiency (PADAM). J Sex Marital Ther. 2016;42:297–301.
27. Roaiah MF, Elkhayat YI, Abd El Salam MA, Din SFG. Prospective analysis on the effect of botanical medicine (*Tribulus terrestris*) on serum testosterone level and semen parameters in males with unexplained infertility. J Diet Suppl. 2017;14:25–31.
28. Fernandez-Lazaro D, Mielgo-Ayuso J, Del Valle Soto M, Adams DP, Gonzalez-Bernal JJ, Seco-Calvo J. The effects of 6 weeks of *Tribulus terrestris* L. supplementation on body composition, hormonal response, perceived exertion, and CrossFit((R)) performance: a randomized, single-blind, placebo-controlled study. Nutrients. 2021;13:3969.
29. Koehler K, Parr MK, Geyer H, Mester J, Schanzer W. Serum testosterone and urinary excretion of steroid hormone metabolites after administration of a high-dose zinc supplement. Eur J Clin Nutr. 2009;63:65–70.
30. Ebisch IM, Pierik FH, FH DEJ, Thomas CM, Steegers-Theunissen RP. Does folic acid and zinc sulphate intervention affect endocrine parameters and sperm characteristics in men? Int J Androl. 2006;29:339–45.
31. Nematollahi-Mahani SN, Azizollahi GH, Baneshi MR, Safari Z, Azizollahi S. Effect of folic acid and zinc sulphate on endocrine parameters and seminal antioxidant level after varicocelectomy. Andrologia. 2014;46:240–5.
32. Shafiei Neek L, Gaeini AA, Choobineh S. Effect of zinc and selenium supplementation on serum testosterone and plasma lactate in cyclist after an exhaustive exercise bout. Biol Trace Elem Res. 2011;144:454–62.
33. Cinar V, Polat Y, Baltaci AK, Mogulkoc R. Effects of magnesium supplementation on testosterone levels of athletes and sedentary subjects at rest and after exhaustion. Biol Trace Elem Res. 2011;140:18–23.
34. Hoffman J, Ratamess N, Kang J, Mangine G, Faigenbaum A, Stout J. Effect of creatine and beta-alanine supplementation on performance and endocrine responses in strength/power athletes. Int J Sport Nutr Exerc Metab. 2006;16:430–46.
35. van der Merwe J, Brooks NE, Myburgh KH. Three weeks of creatine monohydrate supplementation affects dihydrotestosterone to testosterone ratio in college-aged rugby players. Clin J Sport Med. 2009;19:399–404.
36. Fernandez-Landa J, Fernandez-Lazaro D, Calleja-Gonzalez J, Caballero-Garcia A, Cordova A, Leon-Guereno P, et al. Long-term effect of combination of creatine monohydrate plus beta-hydroxy beta-methylbutyrate (HMB) on exercise-induced muscle damage and anabolic/catabolic hormones in elite male endurance athletes. Biomolecules. 2020;10:140.
37. Samadi M, Askarian A, Shirvani H, Shamsoddini A, Shakibaee A, Forbes SC, et al. Effects of four weeks of beta-alanine supplementation combined with one week of creatine loading on physical and cognitive performance in military personnel. Int J Environ Res Public Health. 2022;19:7992.
38. Hoffman J, Ratamess NA, Ross R, Kang J, Magrelli J, Neese K, et al. Beta-alanine and the hormonal response to exercise. Int J Sports Med. 2008;29:952–8.
39. Durkalec-Michalski K, Jeszka J. The effect of beta-hydroxy-beta-methylbutyrate on aerobic capacity and body composition in trained athletes. J Strength Cond Res. 2016;30:2617–26.
40. Lopresti AL, Smith SJ, Malvi H, Kodgule R. An investigation into the stress-relieving and pharmacological actions of an ashwagandha (*Withania somnifera*) extract: a randomized, double-blind, placebo-controlled study. Medicine (Baltimore). 2019;98:e17186.
41. Chauhan S, Srivastava MK, Pathak AK. Effect of standardized root extract of ashwagandha (*Withania somnifera*) on well-being and sexual performance in adult males: a randomized controlled trial. Health Sci Rep. 2022;5:e741.
42. Lopresti AL, Drummond PD, Smith SJ. A randomized, double-blind, placebo-controlled, crossover study examining the hormonal and vitality effects of ashwagandha (*Withania somnifera*) in aging, overweight males. Am J Mens Health. 2019;13:1557988319835985.
43. Gonzales GF, Cordova A, Vega K, Chung A, Villena A, Gonez C, et al. Effect of *Lepidium meyenii* (MACA) on sexual desire and its absent relationship with serum testosterone levels in adult healthy men. Andrologia. 2002;34:367–72.
44. Gonzales GF, Cordova A, Vega K, Chung A, Villena A, Gonez C. Effect of *Lepidium meyenii* (Maca), a root with aphrodisiac and fertility-enhancing properties, on serum reproductive hormone levels in adult healthy men. J Endocrinol. 2003;176:163–8.
45. Leitao AE, Vieira MCS, Pelegrini A, da Silva EL, Guimaraes ACA. A 6-month, double-blind, placebo-controlled, randomized trial to evaluate the effect of *Eurycoma longifolia* (Tongkat Ali) and concurrent training on erectile function and testosterone levels in androgen deficiency of aging males (ADAM). Maturitas. 2021;145:78–85.
46. Chan KQ, Stewart C, Chester N, Hamzah SH, Yusof A. The effect of *Eurycoma longifolia* on the regulation of reproductive hormones in young males. Andrologia. 2021;53:e14001.
47. Tambi MI, Imran MK, Henkel RR. Standardised water-soluble extract of *Eurycoma longifolia*, Tongkat ali, as testosterone booster for managing men with late-onset hypogonadism? Andrologia. 2012;44:226–30.
48. Nobari H, Kargarfard M, Minasian V, Cholewa JM, Perez-Gomez J. The effects of 14-week betaine supplementation on endocrine markers, body composition and anthropometrics in professional youth soccer players: a double blind, randomized, placebo-controlled trial. J Int Soc Sports Nutr. 2021;18:20.
49. Arazi H, Aboutalebi S, Taati B, Cholewa JM, Candow DG. Effects of short-term betaine supplementation on muscle endurance and indices of endocrine function following acute high-intensity resistance exercise in young athletes. J Int Soc Sports Nutr. 2022;19:1–16.
50. Willoughby DS, Leutholtz B. D-aspartic acid supplementation combined with 28 days of heavy resistance training has no effect on body composition, muscle strength, and serum hormones associated with the hypothalamo-pituitary-gonadal axis in resistance-trained men. Nutr Res. 2013;33:803–10.
51. Crewther B, Witek K, Draga P, Zmijewski P, Obminski Z. Short-term d-aspartic acid supplementation does not affect serum biomarkers associated with the hypothalamic-pituitary-gonadal axis in male climbers. Int J Sport Nutr Exerc Metab. 2019;29:259–64.
52. Dillingham BL, McVeigh BL, Lampe JW, Duncan AM. Soy protein isolates of varying isoflavone content exert minor effects on serum reproductive hormones in healthy young men. J Nutr. 2005;135:584–91.
53. Lewis JG, Morris JC, Clark BM, Elder PA. The effect of isoflavone extract ingestion, as Trinovin, on plasma steroids in normal men. Steroids. 2002;67:25–9.
54. Abel T, Knechtle B, Perret C, Eser P, von Arx P, Knecht H. Influence of chronic supplementation of arginine aspartate in endurance athletes on performance and substrate metabolism – a randomized, double-blind, placebo-controlled study. Int J Sports Med. 2005;26:344–9.
55. Che WJ, He XZ, Jiang JP, Cai WY, Xie SJ. Preliminary study on treatment of partial androgen deficiency in aging males with Jingui Shenqi Pill. Chin J Integr Med. 2005;11:300–2.
56. Cinar V, Baltaci AK, Mogulkoc R, Kilic M. Testosterone levels in athletes at rest and exhaustion: effects of calcium supplementation. Biol Trace Elem Res. 2009;129:65–9.
57. Zhang ZJ, Tong Y, Zou J, Chen PJ, Yu DH. Dietary supplement with a combination of *Rhodiola crenulata* and *Ginkgo biloba* enhances the endurance performance in healthy volunteers. Chin J Integr Med. 2009;15:177–83.
58. Goto K, Maemura H, Takamatsu K, Ishii N. Hormonal responses to resistance exercise after ingestion of carnosine and anserine. J Strength Cond Res. 2011; 25:398–405.
59. Giltay EJ, Geleijnse JM, Heijboer AC, de Goede J, Oude Griep LM, Blankenstein MA, et al. No effects of n-3 fatty acid supplementation on serum total testosterone levels in older men: the Alpha Omega Trial. Int J Androl. 2012;35: 680–7.
60. Derouiche A, Jafri A, Driouch I, El Khasmi M, Adlouni A, Benajiba N, et al. Effect of argan and olive oil consumption on the hormonal profile of androgens among healthy adult Moroccan men. Nat Prod Commun. 2013;8:51–3.
61. Maheshwari A, Verma N, Swaroop A, Bagchi M, Preuss HG, Tiwari K, et al. Efficacy of Furosap(TM), a novel *Trigonella foenum*-graecum seed extract, in enhancing testosterone level and improving sperm profile in male volunteers. Int J Med Sci. 2017;14:58–66.
62. Santi D, Granata AR, Pignatti E, Trenti T, Roli L, Bozic R, et al. Effects of chronic administration of the phosphodiesterase inhibitor vardenafil on serum levels of adrenal and testicular steroids in men with type 2 diabetes mellitus. Endocrine. 2017;56:426–37.
63. Haidari F, Mohammadi-Asl J, Kavianpour M, Dadfar M, Haghighian HK. Effect of lipoic acid supplementation on gene expression and activity of glutathione S-transferase enzyme in infertile men. Hum Fertil (Camb). 2021;24:276–83.
64. Pandit SL, Yaligar D, Halemane M, Bhat A. A proprietary blend of standardized Punica granatum fruit rind and *Theobroma cocoa* seed extracts mitigates aging

males' symptoms: a randomized, double-blind, placebo-controlled study. Int J Med Sci. 2022;19:1290–9.
65. Pandit S, Biswas S, Jana U, De RK, Mukhopadhyay SC, Biswas TK. Clinical evaluation of purified Shilajit on testosterone levels in healthy volunteers. Andrologia. 2016;48:570–5.
66. Willoughby DS, Wilborn C, Taylor L, Campbell W. Eight weeks of aromatase inhibition using the nutritional supplement Novedex XT: effects in young, eugonadal men. Int J Sport Nutr Exerc Metab. 2007;17:92–108.

ACKNOWLEDGEMENTS

The author would like to acknowledge the support from the other members of the Young Academic Urologist (YAU) Male Sexual and Reproductive Health group who are not cited as authors.

AUTHOR CONTRIBUTIONS

The authors confirm their contribution to the paper as follows: study conception and design: AM; data collection: AM, GT, IS, NS, AU, SS; analysis and interpretation of results: AM, GT, IS, NS, AU, SS; draft manuscript preparation: AM, GT, IS, NS, AU, SS. All authors reviewed the results and approved the final version of the manuscript.

COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41443-023-00763-9.

Correspondence and requests for materials should be addressed to Afonso Morgado.

Reprints and permission information is available at http://www.nature.com/reprints

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.