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Ergogenic properties, safety evaluation and regulations of selected ingredients in testosterone booster dietary supplements

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ABSTRACT

Testosterone boosters (TB) are multi-ingredient dietary supplements that have gained popularity among athletes and the general population. However, certain ingredients in TB are associated with adverse events. The aim of the present study was to analyze dietary TB supplements available for sale online on shop.bodybuilding.com and amazon.com in ten distribution area markets. We evaluated the top five best-selling TB and the most frequently used ingredients of 50 selected TB and the evidence for their efficacy and safety, as well as legislation, statements in the European Union (EU) and sports organizations such as International Olympic Committee (IOC), International Society of Sports Nutrition (ISSN), Australian Institute of Sport (AIS) and World Anti- Doping Agency (WADA). Among 50 TB, 361 components and 98 ingredients were identified. The following ingredients were included in the final review: fenugreek, D- aspartic acid (DAA), 3,3′-diindolylmethane (DIM) or indole-3-carbinol (I3C), Eurycoma longifolia, Cordyceps spp., and ecdysteroids. In EU an authorized health claims refer to few ingredients in TB, mainly minerals, vitamins. Some ingredients were recognized by international sports organizations such as the ISSN, AIS, and WADA and some were identified as unauthorized ingredients/novel food in the EU. Selected ingredients can possibly increase the risk of endocrine system dysregulation.

KEYWORDS

Testosterone booster; ergogenic aid; exercise performance; efficiency and safety; adverse events; european food safety authority (EFSA)

Introduction

Testosterone boosters (TB) are dietary supplements claimed to enhance testosterone levels. Frequently, producers report that ingredients contained in TB contribute to increase libido, sexual performance, muscle strength or support physical capacity.[1]

These dietary supplements are gaining popularity among both athletes and the general population. The athletic society may be especially interested in TB, considering the well-established relationship between testosterone levels and strength, power, and speed[2,4] as well as increased awareness of low-testosterone-associated relative energy deficiency in sports and its prevalence among both amateur and professional athletes.[5,6] However, reported many unfortunate cases of detection adulterations or contaminations with anabolic-androgenic steroids (AAS) in dietary supplements. Using of AAS remain prohibited by the World Anti-Doping Agency (WADA).[8,9,11–13]

The increasing popularity of TB dietary supplements emphasizes the need inclusion of some ingredients in selected regulations by the authorities. Some studies with ingredients contained in TB were performed on individuals with late-onset hypogonadism or infertility.[14]

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Clemesha et al. showed that on average, one TB contained 8.3 components. Only 24.8% of TB had data that supported claims regarding the impact of their components on testosterone concentration. A total of 10.1% contained components that negatively affected testosterone levels. Many individuals use supra-therapeutic doses of vitamins and minerals, occasionally over the upper tolerable limit, which have associated risks. The authors found that no studies were conducted on the effects of 67 out of 109 individual ingredients, and all performed studies confirmed an increase in the concentration of testosterone only in relation to 12 ingredients.[15]

Our search, which included the ingredient, as well as the term "testosterone," yielded 191 studies across the 10 most common ingredients in TB, of which only 19% involved human subjects, 53% animal models, 15% *in vitro* studies, and 12% other types of studies, such as case reports or review articles.[1]

A review by Lazarev et al. showed that out of 15 ingredients frequently used in TB, only 3 showed the strongest evidence: *Eurycoma longifolia* (Tongkat Ali), ashwagandha, and fenugreek.[14]

Smith et al. indicated that fenugreek and ashwagandha have positive effects on testosterone concentrations in men. Overall, four out of six studies on fenugreek demonstrated significant increases in testosterone concentrations in humans. Supplementation with ashwagandha showed positive findings in three out of four studies in terms of increases in testosterone levels.[16]

The safety of their consumption is another concern. The use of TB is associated with liver injury and nephrotoxic effects.[17,18] Certain TB components may be linked to coagulation and pancreatic disorders.[14]

Regulation-wise, a few organizations have evaluated the efficacy and safety of various ingredients present in TB supplements. Among them are the International Society of Sports Nutrition (ISSN), International Olympic Committee (IOC), and Australian Institute of Sport (AIS), as well as the European Commission (EC) and European Food Safety Authority (EFSA), which provided recommendations in their regulatory documents. The inclusion of these regulations guides athletes, as well as their personnel and the general population, regarding making decisions on dietary supplements/sports nutrition..[19,20,22,23]

In our review, we aimed to evaluate the steroidogenic or ergogenic effects of selected TB components and review safety assessment and its inclusion in the current international or government regulations.

Materials and methods

The analysis covered TB dietary supplements available for sale online on the largest and most popular sales portals aimed at the general population, such as amazon.com and shop.bodybuilding.com. In the case of amazon.com, nine different distribution area markets were analyzed: the USA, Canada, United Kingdom, Germany, France, Italy, Spain, the Netherlands, and Poland. In summary, the total number of sales markets was determined at ten (n = 10). The analysis compilation, recognize was performed from June 16, 2022, to September 16, 2022.

Dietary supplements in the TB category were identified by querying the aforementioned websites using the keyword "testosterone booster" in English and the native language: in Germany, "Testosteron-Booster"; France, "booster de testosterone"; Italy, "booster di testosterone"; Spain, "potenciador de testosterona"; Netherlands, "testosteron booster"; and Poland, "booster testosteronu."

The methodology is divided into two stages:

In stage I, market analysis and identification, we determined the top five best-selling TB on bodybuildingshop.com and amazon.com in different regions/countries. Selected 50 dietary supplements from TB category and evaluated their compositions. Excluded vitamins, minerals, Maca, *Tribulus terrestris, Panax ginseng*, and L-arginine (because these ingredients are associated with considerable scientific evidence and a large number of EU regulatory documents and statements of sports organizations). The inclusion criteria was based on the frequency of the ingredients (expressed as [%]) in the TB; a minimum of 10% prevalence was established as the threshold point.

Stage II involved the final review of the included records/ingredients. For collection and identification of the data, PubMed was searched over June 16, 2022 – September 16, 2022. The strategies were constructed on the basis of the search terms "((DAA) OR (d aspartic acid) OR (ecdysteroids) OR (ecdysten) OR (ecdysterone) OR (Rhaponticum carthamoides) OR (Eurycoma longifolia) OR (Long jack) OR (quassinoids) OR (indole 3 carbinol) OR (diindolylmethane) OR (Cruciferous vegetables) OR (Trigonella foenum graecum) OR (fenugreek) OR (saponins) OR (cordyceps) OR (cordycepin)) AND ((testosterone) OR (exercise) OR (endurance) OR (ergogenic))." Additional records, searched in the Google Scholar.

All methodologies used in stages I and II and the workflow data are presented in Fig. 1 and Fig. 2.

Results

The analysis covered 50 TB dietary supplements in ten markets in various countries. A total of 361 components were identified in the 50 selected TB (according to the labels). On average, 7.22 ingredients were present in each dietary TB supplement. Identified 98 different ingredients in the 50 TB supplements selected from the online market.

All data are shown in Table 1. According to our conducted analysis of TB dietary supplements, the most common ingredient was zinc, present in 64% of products. Subsequently, the prevalence of successive components in TB was determined as follows: Maca 52%, vitamin B6 50%, fenugreek 46%, *T. terrestris* 32%, 3,3′-diindolylmethane (DIM) or indole-3-carbinol (I3C) 32%, black pepper 30%, vitamin D_3 28%, magnesium 26%, vitamin B_{12} 22%, and DAA and *E. longifolia* 20% each.

In line with the previously adopted exclusion criteria for certain ingredients such as vitamins, minerals, Maca, *T. terrestris, P. ginseng*, and L-arginine (because of the large amount of scientific evidence and/or number of EU regulatory documents). The following ingredients were included in the final review: fenugreek, DAA, DIM (or I3C), *E. longifolia, Cordyceps* spp., and ecdysteroids.

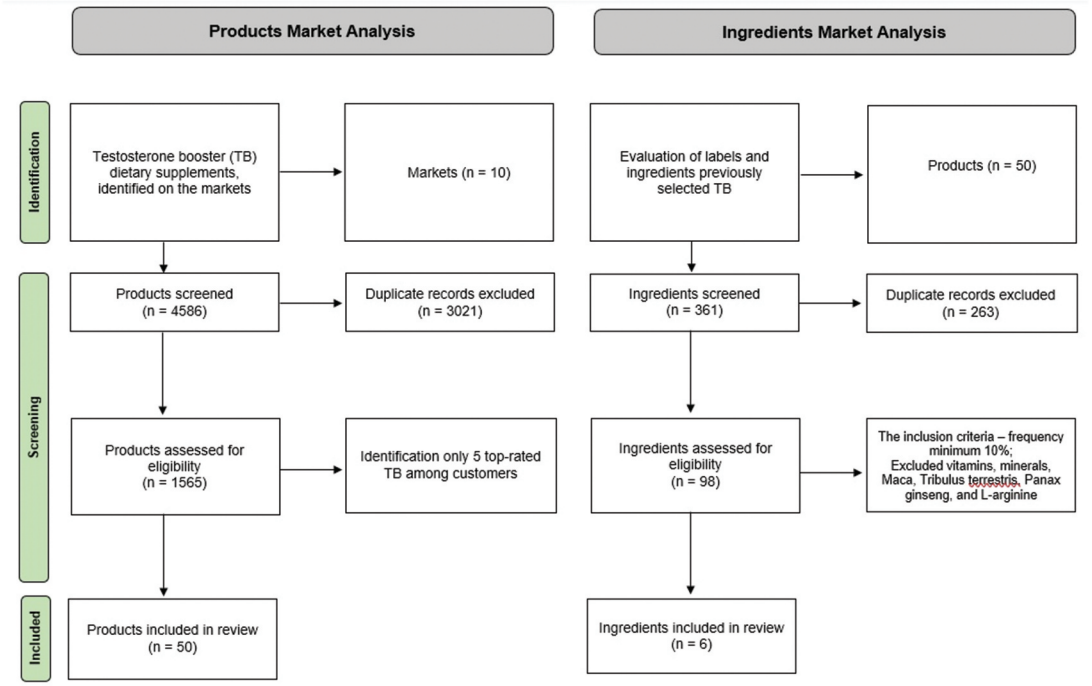

Figure 1. Flowchart of the data market analysis.

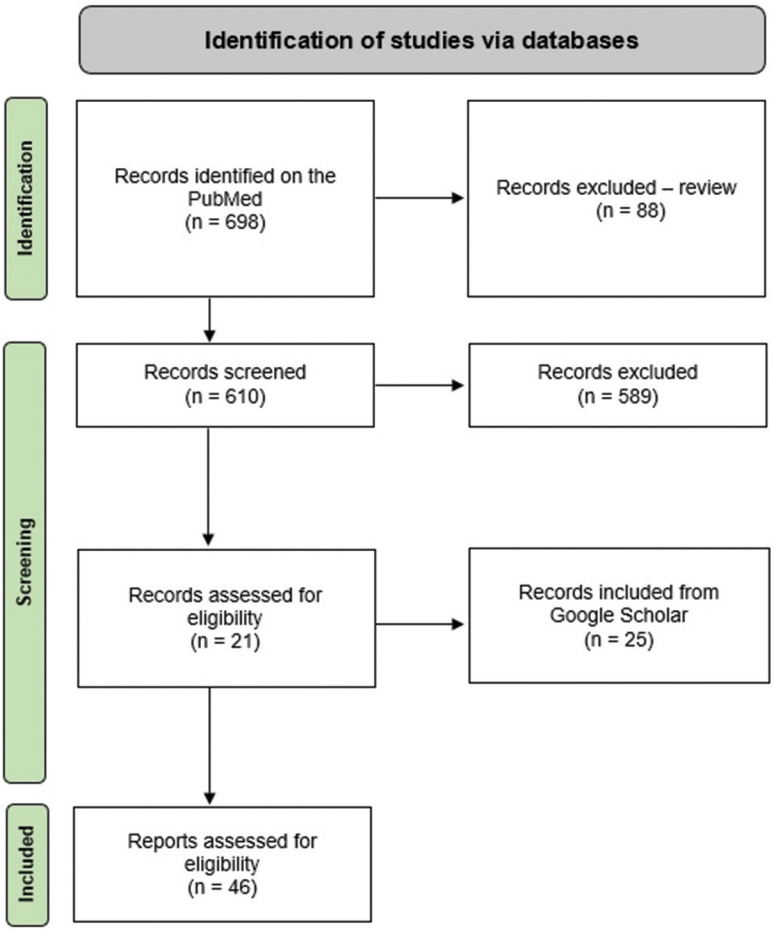

Figure 2. Flowchart of the data selection process.

D-aspartic acid

There are two isomers of aspartic acid in the human body: L-aspartic acid (L-Asp) and D-aspartic acid (D-Asp or DAA). L-Asp is an amino acid that participates in the synthesis of various proteins. In contrast, DAA, an antagonist of the NMDA receptor, acts as a neurotransmitter in the central nervous system and regulates the endocrine system. DAA is a popular ingredient in the TB supplement.[24]

DAA can influence all components of the hypothalamic–pituitary–gonadal (HPG) axis. Accumulation of DAA in HPG structures is associated with increased testosterone and luteinizing hormone production.[25] The beneficial effects of DAA on strength and exercise performance may be achieved via increased neurotransmitter availability.[26] Nonetheless, these associations are largely based on animal studies.[27–29] The number of human studies on the effect of DAA supplementation on testosterone concentration and exercise capacity is limited. Most studies were conducted on a small number of participants. DAA is most frequently administered orally at a dose of 3 g (tablet or capsule).

The positive effects of DAA on testosterone in humans were shown only in one study (43 participants). Topo et al. showed that 12-day supplementation of DAA at a dose of 3.12 g per day significantly increases the concentration of serum DAA, as well as that of testosterone and luteinizing hormone (by 42% and 33%, respectively) in healthy male non-athletes aged between 27 and 37 years.[30]

Table 1. Summarized of ingredients and their frequency of use in TB.

No.	Ingredient	Number of dietary supplements TB	[%] in total 50 TB	[%] in total 98 collected ingredients
	Zinc	32	64,00	32,65
	Lepidium meyenii (Maca)	26	52,00	26,53
	Vitamin B6	25	50,00	25,51
	Trigonella foenum-graecum (Fenugreek) [saponins]	23	46,00	23,47
	Tribulus terrestris [saponins]	16	32,00	16,33
	Cruciferous vegetables [indole-3-carbinol (I3C) or 3,3′-diindolylmethane (DIM)]	16	32,00	16,33
	Piper nigrum (Black Pepper) [piperin]	15	30,00	15,31
	Vitamin D3	14	28,00	14,29
	Magnesium	13	26,00	13,27
	Vitamin B12	11	22,00	11,22
	D-Aspartic acid (DAA)	10	20,00	10,20
	Eurycoma longifolia (Tongkat Ali) [quassinoids]	10	20,00	10,20
	Panax ginseng [ginsenosides]	9	18,00	9,18
	Boron	7	14,00	7,14
	L-Arginine	7	14,00	7,14
	Ecdysterone; sources: Leuzea/Rhaponticum carthamoides, Spinach, Cyanotis arachnoidea, Ajuga turkestanica or Fadogia agrestis [turkesterone]	6	12,00	6,12
	Vitamin C (Ascorbic acid)	6	12,00	6,12
	L-Taurine	6	12,00	6,12
	Cordyceps sinensis or Cordyceps militaris	5	10,00	5,10
	Withania somnifera (Ashwagandha) [withanolides]	4	8,00	4,08
	Urtica dioica (Nettle)	4	8,00	4,08
	Shilajit (Asphaltum punjabianum or mummio) [fulvic acid]	4	8,00	4,08
	Selenium	3	6,00	3,06
	Folate	3	6,00	3,06
	Astragalus membranaceus or AstraGin (Astragalus membranaceus and Panax notoginseng)	3	6,00	3,06
	Rhodiola rosea (Arctic root, Golden root) [rosavin; salidroside]	3	6,00	3,06
	Vitamin B3 (Niacin)	2	4,00	2,04
	Polygonum cuspidatum [resveratrol]	2	4,00	2,04
	Saw palmetto (Serenoa repens)	2	4,00	2,04
	Epimedium grandiflorum (Horny goat weed) [icariin]	2	4,00	2,04
	Chlorophytum borivilianum (Safed Musli)	2	4,00	2,04
	Quercetin	2	4,00	2,04
	Pinus pinaster [proanthocyanidins]	2	4,00	2,04
	Avena sativa	2	4,00	2,04
	Vitamin K	2	4,00	2,04
	Calcium	2	4,00	2,04
	Dioscorea villosa (Wild yam) [saponins]	1	2,00	1,02
	L-Citrulline	1	2,00	1,02
	Pantothenic acid	1	2,00	1,02
	Turnera diffusa (Damiana)	1	2,00	1,02
	Muira puama (Ptychopetalum olacoides, Marapuama, Potency Wood)	1	2,00	1,02
	Cucurbita pepo (Pumpkin)	1	2,00	1,02
	Schisandra chinensis	1	2,00	1,02
	Mucuna pruriens [L-DOPA]	1	2,00	1,02
	Griffonia simplicifolia [5-HTP]	1	2,00	1,02
	Agaricus bisporus [polysaccharides]	1	2,00	1,02
	Cissus quadrangularis [ketosterone]	1	2,00	1,02
	Ferula asafoetida [ferulic acid]	1	2,00	1,02
	Vitis vinifera (Grape seed) [proanthocyanidins]	1	2,00	1,02
	Hesperetin	1	2,00	1,02
	Apigenin	1	2,00	1,02
	Eleutherococcus senticosus (Siberian Ginseng) [eleutherosides]	1	2,00	1,02
	Punica granatum [ellagic acid]	1	2,00	1,02
	Royal Jelly	1	2,00	1,02
	Phosphatidylserine	1	2,00	1,02

(*Continued*)

Table 1. (Continued).

Ganoderma lucidum	1	2,00	1,02
Velvet Antler	1	2,00	1,02
Chrysin	1	2,00	1,02
Coleus forskohlii	1	2,00	1,02
Colostrum	1	2,00	1,02
5α-hydroxylaxogenin	1	2,00	1,02
Montanoa tomentosa	1	2,00	1,02
Chenopodium album	1	2,00	1,02
Caesalpinia benthamiana	1	2,00	1,02
Curcuma longa (Turmeric) [curcumin]	1	2,00	1,02
Trichopus zeylanicus	1	2,00	1,02
Andrographis paniculata [andrographolide]	1	2,00	1,02
Borassus aethiopum (Palmyra palm)	1	2,00	1,02
Propolis	1	2,00	1,02
Paris polyphylla (herb paris) [polyphyllin D]	1	2,00	1,02
Hecogenin	1	2,00	1,02
Dicyclopentanone	1	2,00	1,02
Sphaeranthus indicus and Mangifera indica [mangiferin]	1	2,00	1,02
Cnidium monnieri	1	2,00	1,02
Pygeum africanum (Prunus africana) [beta-sitosterol]	1	2,00	1,02
Phytosterols	1	2,00	1,02
Ginkgo biloba [ginkgolides]	1	2,00	1,02
Vitamin A	1	2,00	1,02
Zingiber officinalis (Ginger)	1	2,00	1,02
Pregnenolone	1	2,00	1,02
Diosgenin	1	2,00	1,02
Dehydroepiandrosterone (DHEA)	1	2,00	1,02
Chromium	1	2,00	1,02
Corynanthe yohimbe [yohimbine]	1	2,00	1,02
Arachidonic acid	1	2,00	1,02
Grapefruit [dihydroxybergamottin]	1	2,00	1,02
L-Lysine	1	2,00	1,02
L-Glutamine	1	2,00	1,02
L-Alanyl-L-Glutamine	1	2,00	1,02
Gamma oryzanol	1	2,00	1,02
Alpha-glycerylphosphorylcholine (alpha-GPC)	1	2,00	1,02
L-Carnitine	1	2,00	1,02
Agmatine	1	2,00	1,02
Astaxanthin	1	2,00	1,02
Silybum marianum (Milk thistle) [sylimarin]	1	2,00	1,02
Alpha lipoic acid (ALA)	1	2,00	1,02
Coenzyme Q10	1	2,00	1,02
Garcinia cambogia	1	2,00	1,02

Following that study, the popularity of DAA supplements grew tremendously; however, further studies consistently showed no beneficial effects of DAA on testosterone levels.

For example, Willoughby and Leutholtz, the authors demonstrated that 4 weeks of supplementation with 3 g of DAA per day in young 20 recreationally active resistance-trained men did not increase testosterone levels and did not improve muscle strength or body composition. The serum DAA concentration did not differ significantly among the groups. In this study, reported adverse events such as irritability, nervousness, and rapid heart rate among two participants in the DAA group.[31]

In another study, Melville et al. investigated the effect of 2-week supplementation of DAA at a dose of 3 g and 6 g per day on testosterone levels in a group of 24 resistance-trained men. The administration of 3 g of DAA did not affect the testosterone concentration, whereas a higher dose of 6 g resulted in a 12.5% reduction in testosterone level.[32]

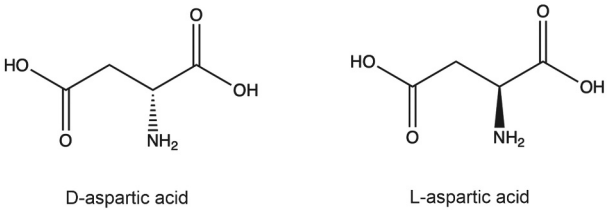

Figure 3. Chemical structures of D-aspartic acid and L-aspartic acid.

Crewther et al. conducted only on 16 climbers (aged 35.4 ± 7.3 years) who received DAA at a dose of 3 g daily for 2 weeks; no significant change was observed in testosterone levels or ergogenic benefits in exercise performance.[33]

In another trial, among 19 participants, Melville et al. demonstrated that 12-week supplementation of DAA at a dose of 6 g per day did not have any significant effect on testosterone concentration and did not increase isometric and dynamic muscle strength in healthy resistance-trained men, aged 18–36 years. The authors concluded that their study demonstrated the ineffectiveness of DAA as a testosterone-enhancing supplement.[25]

On the basis of the abovementioned studies, DAA was classified as category III as a substance with little to no evidence to support the efficacy and/or safety of the ISSN recommendations.[22] DAA is not included in the IOC consensus statement or recommendations of the AIS.[19,23]

Despite being recognized as safe by regulators, residual information on DAA is present in the Italian document Ministero della Salute "Altri nutrienti e le altre sostanze ad effetto nutritivo o fisiologico." This document states that in addition to the L-form of aspartic acid, the D form is allowed in dietary supplements [20].

More information on L-aspartic acid is available in the report from the Norwegian Scientific Committee for Food Safety (VKM) or the Report of the Scientific Committee of the Spanish Agency for Consumer Affairs, Food Safety and Nutrition (AECOSAN) on the conditions of use of certain substances in food supplements.[34,35] A comparison of the enantiomers of D-aspartic acid and L-aspartic acid is shown in Fig. 3.

The DAA has not authorized health claims by the EFSA and EC.[36] VKM performed the safety assessment only for L-aspartic acid; according to them, in adults (≥18 years) and adolescents (14 to less than 18 years), doses of 3000, 3500, 4000, 4500, 5000 and 5700 mg/day of L-aspartic acid in dietary supplements may represent a risk of adverse effects.[34] AECOSAN estimated that 490 mg of L-aspartic acid is the maximum daily acceptable quantity for human use as a dietary supplement.[35]

Ecdysteroids

Ecdysteroids are a family of steroid-like substances that include 20-hydroxyecdysone (most often abbreviated as ecdysterone or beta-ecdysone), ajugasterone, ponasterone, rubrosterone, turkesterone, viticosterone, and polypodine B. These substances are known as invertebrate hormones and are chemically different from vertebrate steroids; they retain sterane carbon skeleton (cyclopentanoperhydrophenanthrene) and possess a 14α-hydroxy-7-en-6-one chromophoric group in the B-ring and an A/B-cis-ring junction. The main differences in the chemical structure that distinguish ecdysterone from AAS are related to the 7-en-6-one chromophore group in ring B; hydroxyl group at positions C-2, C-3, and C-14; and a branched alkyl moiety with a hydroxyl group. Ecdysteroids have been found in many plant species, including *Ajuga turkestanica, Cyanotis arachnoidea*, spinach (*Spinacia oleracea*) (Table 2). In plants, phytoecdysteroids act as either antifeedants or endocrine disruptors against invertebrate predators.[49–52]

The general chemical structure of ecdysteroids is shown in Fig. 4. Turkesterone (from *A. turkestanica*), and ecdysterone from different sources e.g. *R. carthamoides* or *C. arachnoidea* are commercially available in dietary supplements.[41,53–57]

Studies have shown that ecdysteroids may exert many positive effects on human health. They are currently being investigated as potential anabolic, and neuroprotective substances. Most studies have focused on ecdysterone.[55] Interestingly, the anabolic activity of ecdysterone was comparable to that of methandienone, dihydrotestosterone, estradienedione, SARM S-1, and IGF-1.[59,] The mechanism of action of ecdysterone, appears to be completely different from that of AAS or SARM. Ecdysterone has been shown to act as an agonist of the estrogen receptor beta (ERβ) protein-coding gene.[61]

Ecdysterone is a common component of TB. It is most often found as an isolated substance or as an active ingredient in raw materials of plants such as *Rhaponticum carthamoides* or *S. oleracea. R. carthamoides* is also known as the Maral root, *Leuzea carthamoides*, or *Stemmacantha carthamoides*. Studies have shown that the actual amount of ecdysterone identified in the supplement may vary from the declared amount. In one study, 67% of supplements contained a considerably lower amount of ecdysterone than that declared by the manufacturer on the label.[53] As one of the first, Hunyadi, Attila, et al. indicates for poor quality and counterfeiting for dietary supplements with spinach available on the market as source of ecdysteroids.[62] Grucza et al. confirmed significant discrepancies in the declared ecdysterone content and the real content through analytical tests. Analysis of dietary supplements showed that ecdysterone levels ranged from 5 to 383 mg/g.[54] Also, Todorova, Velislava, et al. show that among the 11 tested dietary supplements (from different markets as Bulgaria, Czech Republic, Sweden, US), only two of the analyzed products contained the real content of ecdysteroids, similar to the concentration declared on the label. Most of dietary supplements have an actual content of ecdysteroids (determined analytically) that deviates from the concentration stated on the label.[57]

Recently, it has been shown that dietary supplements with *R. carthamoides* or *C. arachnoidea*, in addition to incorrectly amounts of ecdysteroids stated on the label, relative to the actual content determined in analytical tests, also contains undeclared on the label prohibited doping agents AAS, such as 4(5)-androstene-3,17-dione and 1,4-androstene-3,17-dione.[56]

Some studies on ecdysterone have been conducted on animals.[63–65] Few studies have been conducted on human subjects. Improvement in physical performance has been demonstrated in selected studies.[61,66–68]

Most human studies on ecdysterone reported improvement in exercise capacity in the USSR and its successor states. *R. carthamoides* root was described as a pharmaceutical raw material in the Russian Pharmacopoeia as early as 1961.[66,67,69,70] Azizov et al. showed that 20-day use of *Leuzea* tincture and Leveton was associated with improved exercise capacity in runners. In addition to the ergogenic effect, an immunostimulatory effect was confirmed, with an increase in IgA and IgG concentrations, which had previously decreased because of intense exercise.[68] The quality of these studies is often poor, raising the question of the validity of the results.[71]

Subsequent studies have shown inconsistent results. Wilborn et al., in a double-blind, placebo-controlled trial showed that 8-week supplementation of 20-hydroxyecdysone (30 mg/day) in 45 young resistance-trained men (aged 20.5 ± 3 years) did not exert a significant effect on testosterone concentration, muscle mass, body composition, training adaptations, or strength. According to the safety assessment, the participants tolerated the supplementation well and did not report any adverse events. In addition, analysis of clinical chemistry profiles, aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatine kinase (CK), lactate dehydrogenase (LDH), gamma-glutamyl transferase (GGT), and hematological parameters, confirmed no significant differences between the groups or over time. However, the real content of ecdysterone has not been confirmed in analytical tests – based only on the information from the label.[72]

One of the most thoroughly conducted studies, performed by Isenmann et al., showed that 10-week supplementation of ecdysterone at 12 mg daily and 48 mg daily in young men contributed to the improvement of anthropometric and performance parameters in the performed exercises. Significantly higher increases in muscle mass were observed in participants dosed with ecdysterone.

Table 2. Specify of chemical, nutritional and sensory properties among selected natural ingredients in TB.

Natural ingredient found in TB	Main part(s) of material	Phytochemical classification of main bioactive compound(s)	Concentration	Nutritional values[a] [%w/w]	Sensory properties	References
Asparagus officinalis	shoots, stem	ecdysteroids amino acids	20-hydroxyecdysone (20E) 0.4–2.5% L-aspartic acid 0.5–3.11%	protein 2.2% carbohydrate 3.9% fat 0.12% fiber 2.1%	green colour; urinary odour; slightly nutty to earthy flavor	[37, 38]
Ajuga turkestanica	roots	ecdysteroids	20E; predominant turkesterone 0.2–0.4%	–	–	[39]
Cyanotis arachnoidea	roots	ecdysteroids	20E 2.0–3.0%	–	–	(Tóth, Gábor 2021)
Rhaponticum carthamoides	roots	ecdysteroids	20E 0.04–1.51%	–	brown colour; earthy odour; slightly bitter to earthy flavor	[40]
Spinacia oleracea	leaves	ecdysteroids	20E 50–90 µg/g fresh weight; 17–885 µg/g dry mass	protein 2.86% carbohydrate 3.63% fat 0.39% fiber 2.2%	green colour; earthy odour (related to bicyclic compound geosmin); slightly bitter to earthy flavor	[41,42]
Eurycoma longifolia	roots	quassinoids saponins	eurycomanon 0 non 0.8–1.5% 40–65%	protein 22% – 45% carbohydrate 30% – 55% fat – negligible level	light brown to brown colour; characteristic odour; bitter flavor	[43]
Brassicaceae family, Brussels sprouts, cauliflower, broccoli, cabbage	leaves	glucosinolates and sulforaphane	indole-3-carbinol (I3C), precursor of 3,3′-diindolylmethane (DIM) I3C 77–117 mg/100 g fresh weight sulforaphane 72–304 mg/100 g fresh weight	protein 1.28% – 3.38% carbohydrate 5.8% – 8.95% fat 0.1% – 0.3% fiber 2% – 3.8%	green colour; sulfurous odour; bitter flavor	[44]
Trigonella foenum-graecum	seeds	steroidal saponins	Total amount of saponins 0.6–1.7% diosgenin 0.1–0.3%	protein 23.0% – 30.0% carbohydrate 58.5% fat 6.4% – 7.0% fiber (galactomannans) 24.5% – 45.0%	brownish-yellow colour; maple syrup-like odour (related to aromatic compound sotolon); bitter-spicy flavor	[3]
Cordyceps militaris	fruiting bodies, mycelium	nucleosides amino acids polysaccharides	cordycepin 1.10–8.37 mg/g; 25.9–57.5 mg/100 g ergothioneine, γ-aminobutyric acid (GABA) beta-glucan derivatives	protein 29.7% carbohydrate 29.1% fat 2.9% fiber 26.1%	yellowish-orange, to brown colour; earthy odour (related to bicyclic compound geosmin); nutty-like flavor	[45, 46, 47]

[a]data comes from the U.S. Department of Agriculture [.48

The authors suggested that ecdysterone should be included in the WADA Prohibited List as "other anabolic agent." Interestingly, the actual amount of ecdysterone in the capsule was only 6 mg, whereas the declared amount was 100 mg. Additionally, these experiments indicated falsification in the declared ecdysterone dose and the presence of counterfeits among the dietary supplements. Safety evaluation was performed on the basis of the serum concentrations of selected biomarkers of kidney and liver function. In this study, the above-mentioned markers did not significantly change during the 10-week intervention period.[61]

Ecdysterone is covered by the WADA Monitoring Program.[73] The possibility of the prohibition of ecdysterone in the future led to an increase in interest in its pharmacokinetics. The parent compound and products of its biotransformation – deoxyecdysone were determined analytically in the excreted urine of healthy volunteers who administered orally 20 mg of ecdysterone. The main product of ecdysterone biotransformation in urine is deoxyecdysone, detectable up to 21 hours after application.-[74] Current studies indicate that after a single oral administration of 51.5 mg of ecdysterone, the parent compound can be identified in the urine up to 48 h after ingestion.[75]

A later study by Ambrosio et al. allowed the identification and quantitation of ecdysterone and its metabolites, 14-deoxy-ecdysterone and 14-deoxy-poststerone, after intake of 50 mg of ecdysterone.[76] The metabolism, pharmacokinetics, and pharmacodynamics parameters of ecdysterone continue to be evaluated in humans.[55,77,78] The International Federation of Horseracing Authorities (IFHA) was one of the first organizations to limit the use of ecdysterone in equine sporting events in the past, but currently is not appear on the IFHA.[79]

According to the ISSN 2018 recommendations, ecdysterone is included among category III ingredients, which have "Little to No Evidence to Support Efficacy and/or Safety".[22] Ecdysterone was not included in IOC or AIS statements.[19,23]

R. carthamoides (a source of ecdysterone) is included in the EFSA novel food catalogue. According to the status, this product was on the market as a food or food ingredient and was consumed before 1997. Thus, its access to the market is not subject to Novel Food Regulation (EU) 2015/2283, and it is an authorized and approved ingredient. Nonetheless, other specific legislation may restrict placing *R. carthamoides* on the market as a food or food ingredient in some Member States.[20] The root of *R. carthamoides* is authorized in food supplements in Belgium and Italy. In the Czech Republic, the entire plant is authorized. In France, the root is authorized, but the substances to be monitored are ecdysterones.[20,80]

A monograph for *R. carthamoides* is absent in the Ayurvedic Pharmacopoeia of India, European Medicines Agency (EMA), the WHO monographs, the British Pharmacopoeia, The Korean Pharmacopoeia, the Japanese Pharmacopoeia, and the monograph of European Scientific Cooperative on Phytotherapy (ESCOP).[80]

Eurycoma longifolia (quassinoids)

Quassinoids are active compounds in the root of *E. longifolia*, which is also known as longjack, tongkat ali, or Malaysian ginseng. In Southeast Asia, this plant is widely used in folk medicine to improve male libido, fertility, and sexual function. *E. longifolia* has also been used as an adaptogen and in the treatment of diabetes, fever, or high blood pressure. The roots of *E. longifolia* contain specific biologically active molecules (classified as nortriterpenoids), such as eurycomalactone, eurycomanone, and eurycomanol, which are generally known as quassinoids; their general structure is shown in Fig. 5.[14,81,82]

Quassinoids may inhibit the conversion of testosterone to estradiol by the aromatase enzyme, as well as increase the release rate of "free" testosterone from sex-hormone-binding-globulin (SHBG), thereby increasing testosterone levels.[14,83] Substances that increase testosterone levels.[14,84]

Additionally, *E. longifolia* has been studied in patients with hypogonadism. For example, Tambi et al. showed that in patients with late-onset hypogonadism, daily intake of 200 mg *E. longifolia* extract for 4 weeks increased testosterone levels.[85] Another study showed that 200 mg/day of *E. longifolia* improved erectile function and increased total testosterone levels in subjects with hypogonadism.[86]

Table 3. Regulation via EU and sport organizations.

Ingredients \ Organization	ISSN	IOC	AIS	EFSA/EC	RASFF notifications/ warnings from 2020	WADA/USADA
D-aspartic acid (DAA]	III	–	–	authorized ingredient, unauthorized health claims	–	–
Leuzea/Rhaponticum carthamoides	III	–	–	authorized ingredient, unauthorized health claims	–	–
Ecdysteroids	III	–	–	not include on Novel food catalogue, unauthorized health claims	–	Ecdysterone covered by WADA Monitoring Program
Eurycoma longifolia	–	–	–	unauthorized novel food	–	–
Cruciferous vegetables	–	–	–	authorized ingredient, unauthorized health claims	–	–
I3C or DIM	–	–	–	unauthorized novel food	DIM 5 notifications	–
Trigonella foenum-graecum	III	–	–	authorized ingredient, unauthorized health claims	–	–
Cordyceps spp.	–	–	–	*C. sinensis* authorized ingredient, unauthorized health claims *C. militaris* unauthorized novel food	*C. militaris* 4 notifications	–
Tribulus terrestris	III	–	Group D	authorized ingredient, unauthorized health claims, unauthorized in Denmark	2 notifications in Denmark	–
Lepidium meyenii (Maca)	III	–	Group D	authorized ingredient, unauthorized health claims	3 notifications in Denmark	–
Garcinia cambogia	–	–	–	authorized ingredient, unauthorized health claims	3 notifications in Denmark	–
Withania somnifera (Ashwagandha)	–	–	–	authorized ingredient, unauthorized health claims	3 notifications in Denmark	–
Griffonia simplicifolia (5-HTP)	–	–	–	unauthorized novel food	9 notifications	–
Mucuna Pruriens (L-DOPA)	–	–	–	unauthorized novel food	2 notifications	–
Epimedium grandiflorum (icariin)	–	–	–	unauthorized novel food	11 notifications	–
Agmatine	III	–	–	unauthorized ingredient	2 notifications in Sweden	–
Corynanthe yohimbe/ Yohimbine	–	–	–	unauthorized ingredient	39 notifications	Not recommend
Colostrum	–	–	Group D	authorized in Italy unauthorized in Ireland	1 notification in Ireland	Not recommend
Pregnenolone	–	–	–	–	–	Not recommend
5α-hydroxylaxogenin	–	–	–	–	–	Not recommend
Dehydroepiandrosterone (DHEA)	III (prohormones)	–	Group D	In EU registered as medicines	–	Anabolic-androgenic steroids (AAS). Class S1

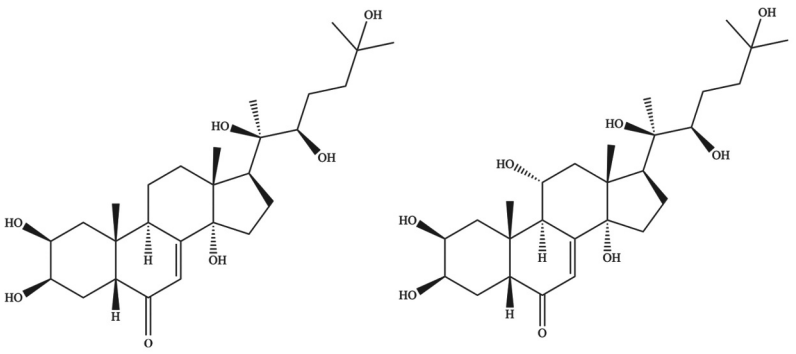

Figure 4. Chemical structures of selected ecdysteroids.

Talbott et al. showed that supplementation with *E. longifolia* root extract (quantified content of eurypeptides) at a dose of 200 mg/day for 4 weeks reduced the level of cortisol and increased the concentration of testosterone in the saliva in volunteers.[83]

The application of 600 mg of *E. longifolia* extract (standard is eurycomanone 1.45%) each day for 2 weeks contributed to increased testosterone, free testosterone, and estradiol levels among young men (age 24.4 ± 4.7 years). An increase in estradiol may indicate that the active substances in *E. longifolia* do not inhibit aromatase. The increase in testosterone has been found to be associated with an effect on the HPA.[87]

Interestingly, *E. longifolia* may not affect the urinary testosterone/epitestosterone (T/E) ratio. Chen et al. showed that the application of *E. longifolia* extract at a dose of 200 mg per day for 8 weeks did not affect the urinary testosterone/epitestosterone (T/E) ratio, which is widely used to prove AAS abuse in athletes. This creates opportunity for the misuse of quassinoids among competitive athletes.[88,89]

E. longifolia is not included in ISSN, IOC, or AIS recommendations.[19,22,23] In the EU, *E. longifolia* is an unauthorized novel food.[20] The EFSA Panel concluded that *E. longifolia* has the potential to induce DNA damage, and its safety has not been established under any condition of use.[43]

Cruciferous vegetables (indole-3-carbinol or 3,3′-diindolylmethane)

Vegetables from the *Brassicaceae* family, including broccoli, Brussels sprouts, and cauliflower, contain glucosinolates. Indole-3-carbinol (I3C), a glucosinolate, is a precursor of 3,3′-diindolylmethane (DIM). DIM is the primary metabolite of I3C. Both, DIM and I3C are ones of the most common components in TB. Studies have shown that DIM is one of the most popular ingredients in TB. A comparison of the chemical structures of DIM and I3C is presented in Fig. 6.[1,15,44,90]

The initial enthusiasm for the use of DIM as a TB component was apparently caused by evidence of the anti-cancer and anti-estrogen properties of this substance.[91–94] *In vitro* experiments have shown that DIM can activate the estrogen receptor beta (ERβ). This mechanism is similar to that of ecdysterone. Theoretically, this effect could lead to anabolic.[58,95]

DIM was measured in the urine of volunteers who consumed vegetables from the *Brassicaceae* family. It indicated that dietary I3C condenses into DIM through metabolism in the human body. Urinary DIM is a marker of I3C exposure.[96,97]

Clinical studies have been conducted for analyzing the effect of DIM administration or nutritional intervention in the form of natural raw materials (cruciferous vegetables) providing DIM (or I3C) in men with prostate cancer, women with breast cancer, or postmenopausal women with a history of early-stage breast cancer. In postmenopausal women with a history of early-stage breast cancer,

supplementation with DIM at a dose of 108 mg/day for 30 days significantly increased the levels of 2-hydroxyestrone.[98,99] In postmenopausal women, oral administration of DIM at 100 mg daily in the last 1 year was related to decreased estradiol level.[100]

Studies have demonstrated that DIM exerts anti-androgenic activity, as well activates estrogen receptor alpha (ERα). This effect may lead to a pro-estrogenic response, rendering the use of this substance irrational in terms of increasing testosterone levels. Therefore, some authors they warn about administration of DIM and its precursors until further studies show their effectiveness and safety.[94,101–103]

I3C and DIM were included in the CFSAN Adverse Event Reporting System (CAERS). CAERS is a database related to the collection and archiving of information on potential/suspected adverse events associated with products other than medicines, such as foods or dietary supplements.[104]

In a randomized crossover trial in healthy men and women, consumption of cruciferous vegetables was proven to contribute to decreased levels of IL-6; nonetheless, it had no impact on other inflammatory markers, such as C-reactive protein (CRP) or tumor necrosis factor α (TNF-α).[105]

I3C and DIM were not included in the ISSN, IOC, or AIS recommendations.[19,22,23] In the EU, health claims associated with I3C or DIM have not been authorized by the EFSA and EC. In the past, health claims have been reported for I3C (150–450 mg daily), such as "support to balance the level of sex hormones in human body needed for correct function of reproductive organs" or "induction of apoptosis of transformed and damaged cells"; however, they have not been approved in EU.[36] Isolated I3C, and synthetic form of DIM, is considered as novel food ingredient in the EU.[20]

Trigonella foenum-graecum (sapogenins)

T. foenum-graecum (fenugreek) is a well-known pharmacopeial plant that is registered as a traditional herbal medicine in Poland, France, and Spain. Fenugreek has traditionally been used in folk medicine to increase libido, maintain erection, regulate blood sugar levels, and increase lactation in women. Traditionally, fenugreek is used both as an herb (leaves) and as a spice (seeds).[3]

The pharmacopeial raw material is fenugreek seeds, which contains phytochemicals such as steroidal saponins (diosgenin, jamogenin, sarsasapogenin, tigogenin, gitogenin, yuccagenin, protodioscin), polysaccharides (galactomannans), amino acids (4-hydroxyisoleucine), and alkaloids (trigonelline). The general chemical structures of steroidal saponins and triterpene saponins are shown in Fig. 7.[3] In particular, diosgenin may be an important precursor for testosterone synthesis.[106] Additionally, steroidal saponins from fenugreek has been suggested to increase testosterone levels through aromatase and 5α reductase inhibition.[107]

Fenugreek is considered one of the most effective TB components. A relatively large evidence base exists regarding testosterone levels, body composition, and muscle strength.[14]

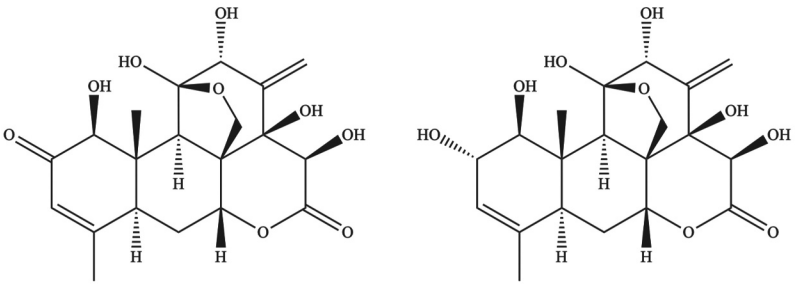

Figure 5. Chemical structures of selected quassinoids.

The beneficial effects of fenugreek on human body composition were shown in a study by Poole et al., in which adipose tissue was reduced and testosterone concentration was increased in a group of 30 resistance-trained men (aged 18–24 years) were supplemented for 8 weeks with fenugreek seed extract at 500 mg/day. An improvement was observed in the bench press and the leg press. The study showed no significant changes in the parameters describing muscle strength and endurance.[108]

In a group of 30 trained young men, supplementation with 500 mg daily of fenugreek (standardization graecunin) for 8 weeks has been proved to contribute to an increase in testosterone levels, lower DHT, and lower body fat content. This study showed no increase in muscle strength or lean body mass. Partial inhibition of aromatase and 5α-reductase was confirmed.[109] Improvement in sexual condition was demonstrated in healthy men aged 25–52 years who used 600 mg per day of standardized fenugreek extract (50% saponins) for 6 weeks. However, fenugreek extract supplementation had no significant effect on testosterone levels in volunteers.[110]

Rao et al., reported increase in blood testosterone concentration, improvement in sexual function, and alleviation of andropause symptoms in men (43–75 years) with age-related androgen deficiency, as a result of 12 weeks of supplementation with fenugreek seed extract at 600 mg per day.[111]

Improvement in muscle strength and endurance, as well as an increase in testosterone concentration, was demonstrated in a group of young, healthy men who were administered 600 mg of a standardized fenugreek seed extract daily for 8 weeks. This study additionally showed a reduction in body fat percentage.[107]

Swaroop et al. and Maheshwari et al. assessed the effect of a 12-week daily supplementation with 500 mg of fenugreek seed extract (20% protodioscin) on testosterone levels and sperm quality in men. They showed an increase in the concentration of free testosterone, an increase in the amount of sperm in semen, and an improvement in sexual condition.[112,113]

In physically active men aged 20–28 years, the impact of 12-week supplementation with fenugreek seed extract (20% protodioscin) on the hormone profile, body composition, and exercise capacity was assessed. The study showed an increase in testosterone concentration and lean body mass. Fenugreek supplementation did not improve aerobic endurance, upper and lower body strength, or grip strength.[114]

Rao et al. investigated the effect of 8-week daily supplementation with 300 mg and 600 mg of fenugreek seed extract (50% saponins) on exercise capacity in young to middle-aged men in calisthenic training. Fenugreek seed extract at 600 mg/day improved muscle strength and endurance, increased lean body mass, and increased testosterone levels.[115] 12-week of daily supplementation with 500 mg of fenugreek seeds (20% protodioscin) improved testosterone levels in healthy male volunteers with no adverse events reported.[116] In contrast, Bushey et al. proved that 8 weeks of daily supplementation with 500 mg of fenugreek extract did not affect testosterone concentration and showed no anabolic effect among young resistance-trained men. A decrease in DHT levels was observed in the group receiving fenugreek.[117]

Among young healthy men, supplementation with fenugreek improved grip strength.[118]

According to the ISSN recommendations from 2018, fenugreek was placed in category III of dietary supplements, which have insufficient scientific evidence confirming the effectiveness or safety of

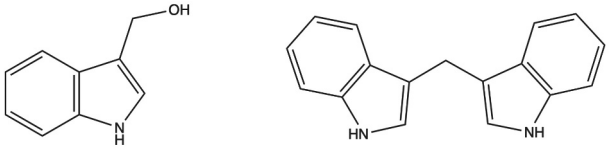

Figure 6. Chemical structures of indole-3-carbinol and 3,3′-diindolylmethane.

use.[22] The IOC and AIS did not include *T. foenum-graecum* in any group of dietary supplements or ingredients that can support or enhance sports performance.[19,23]

T. foenum-graecum is an authorized ingredient in food and dietary supplements in EU. Only the seeds of fenugreek were used as the source or raw material for food or dietary supplements within the EU before 1997.[20] The health claims for fenugreek have not been approved by the EFSA and EC, because the cause-and-effect relationship between consumption and corresponding health benefits has not yet been established.[36]

Cordyceps spp. (cordycepin)

Entomopathogenic fungi *Cordyceps* spp. (classified as *Ascomycota* group) have a long tradition of use in ethnomedicine in Asian countries owing to their anti-fatigue effect and stimulation of the immune system in humans. Cordycepin (3'-deoxyadenosine) is a natural biometabolite produced by *Cordyceps* spp. that parasitizes the larvae of certain moth. Cordycepin was isolated for the first time from the fruiting bodies of *Cordyceps militaris* (caterpillar mushroom).[119,120,121]

Cordycepin is a nucleoside, structurally analogous to adenosine (Fig. 8). Animal and human studies have shown that cordycepin or *Cordyceps* spp. interacts via adenosine receptors, 5′AMP-activated protein kinase (AMPK), and the adenosine-5'-triphosphate (ATP) signaling pathway, exerting significant anti-inflammatory activity. Thus, they may decrease inflammation process in response to physical effort.[123–125]

The cordycepin concentration in the dry fruiting bodies of *C. militaris* was determined to be 1.10 mg/g d.w..[45] In contrast, cordycepin was estimated at 2.33 mg/g in fresh fruiting bodies.[123] The concentration of cordycepin was determined to be approximately 8.37 mg/g in ethanol extract and 5.28 mg/g in water extract obtained from fruiting bodies.[46] The latest research has demonstrated that

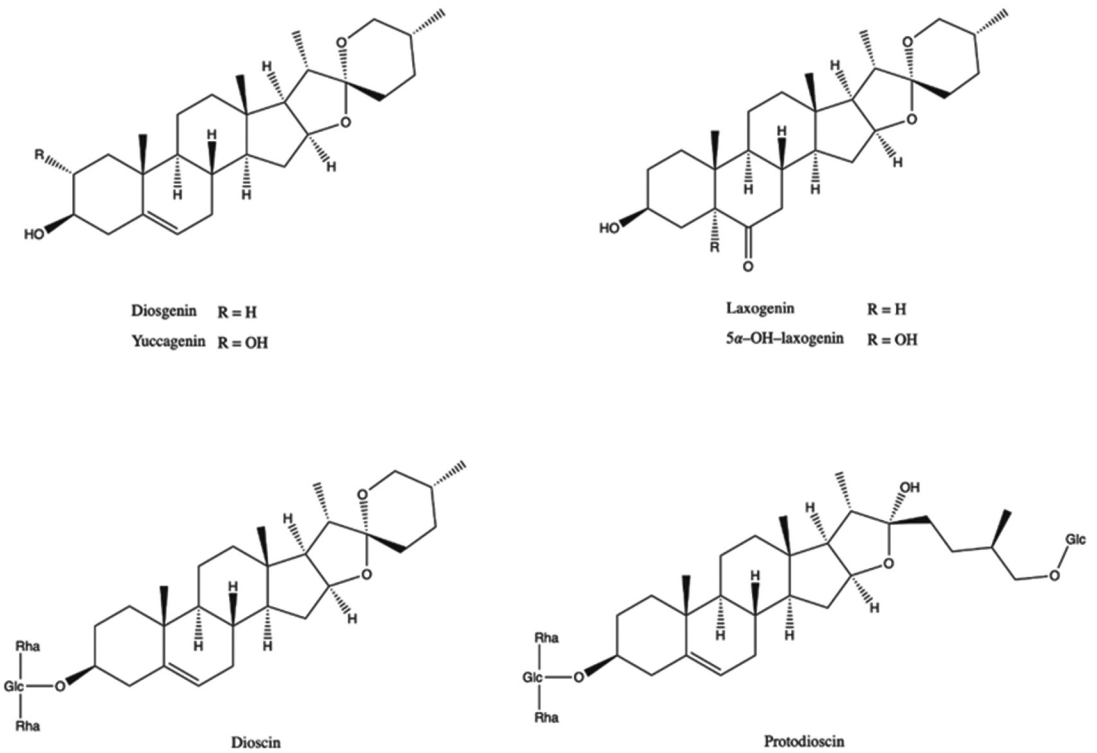

Figure 7. Chemical structures of selected saponins.

C. militaris is a source of cordycepin, present in high amounts in commercially available and self-cultivated fruiting bodies at 57.5 mg/100 g d.w. and 25.9 mg/100 g d.w., respectively.[47]

The fame and popularity of *Cordyceps* spp. as an "ergogenic" agent in other regions of the world (mainly US and Europe) was associated with the sport event at 1993, where Chinese female runners broke records. These achievements were associated with consumption of *Cordyceps* spp. mushrooms by runners.[126,127]

There is increasing interest in the correlation between consumption/supplementation of selected mushrooms (including *Cordyceps* spp.) and the effect on exercise capacity [129,121,124,126]

In many experiments using animal models, *C. sinensis* or *C. militaris*, as well as cordycepin, showed an effect on the endocrine system, mouse Leydig cell steroidogenesis, and increase/stimulation of testosterone production.[130–135]

In terms of effect on testosterone levels, limited trials have been conducted in humans. Till date, studies on volunteers have not shown results similar to animal experiments. Hsu et al., showed that 8 weeks of supplementation with *C. sinensis* containing adenosine (5.92 μmol/g), cordycepin (1.23 μmol/g), and ergosterol (8.81 μmol/g) did not improve exercise capacity and did not affect testosterone concentration in healthy young men.[136]

Meanwhile, in a small number of participants, 3 months of supplementation with *Ophiocordyceps sinensis* and *Ganoderma lucidum* influenced salivary testosterone and cortisol levels in only seven healthy men (amateur cyclists, aged 30–40 years).[137]

No study has been conducted in humans for evaluating the effect of *C. militaris* on testosterone concentration in men. Most reported animal and human studies have focused on the effects of *C. sinensis* and *C. militaris* on exercise capacity.

Mice fed with *C. militaris* extract have shown slightly increased grip strength; these results were similar to those for the comparison group treated with red ginseng). ELISA showed that *C. militaris*/cordycepin increased the concentrations of ATP, AMPK, and phosphocreatine.[123]

C. sinensis has shown mixed results in human studies. In healthy older subjects, consumption of *C. sinensis* for 12 weeks improved exercise parameters. In this study, the *C. sinensis* supplement used was CordyMax™ Cs-4, which contains adenosine (0.14%), mannitol (5.0%), and polysaccharides (0.5%). The cordycepin concentration was not determined.[138]

A another study demonstrated that 5 weeks of daily supplementation with *C. sinensis* 3.15 g per day (as CordyMax™ Cs-4) did not improve aerobic capacity or exercise performance in endurance-trained male cyclists.[139]

Likewise, the results of subsequent studies indicated that the consumption of *C. sinensis* in combination with *Rhodiola rosea* had no significant effect on exercise capacity in physically active subjects.[140–142] In contrast, supplementation with a mixture of *Rhodiola crenulata* and *C. sinensis* was shown to support exercise capacity.[143,144]

Savioli et al., in a recent randomized, double-blind, placebo-controlled trial, showed that supplementation with 2 g/day *C. sinensis* (CordyMax CS-4®) for 12 weeks improved aerobic performance in amateur marathoners.[145] In terms of *C. militaris*, three studies verified the impact on exercise performance of a mushroom mixture/blend containing *C. militaris* as the primary ingredient, as well as other mushrooms such as *Hericium erinaceus, Ganoderma lucidum, Pleurotus eryngii, Lentinula edodes*, and *Trametes versicolor*.

In young recreationally active subjects, 3-week daily supplementation with 4 g of these mushroom blends contributed to improvement of maximum oxygen consumption (VO_2max), ventilation threshold (VT), and increase in time to exhaustion (TTE).[146,147]

A similar result was observed in terms of improvement in selected exercise capacity parameters in young adult subjects who consumed dietary supplement containing a mixture of these mushrooms at low doses (1.0–2.0 g/day) for 28 days and at higher doses (12 g/day) for 7 days.[148]

Gastrointestinal adverse events were reported in only a single subject following supplementation with a mushroom blend containing *C. militaris*.[148] No significant changes were reported, compared to the placebo group, in markers such as ALT, AST, after 10-week supplementation with *C.*

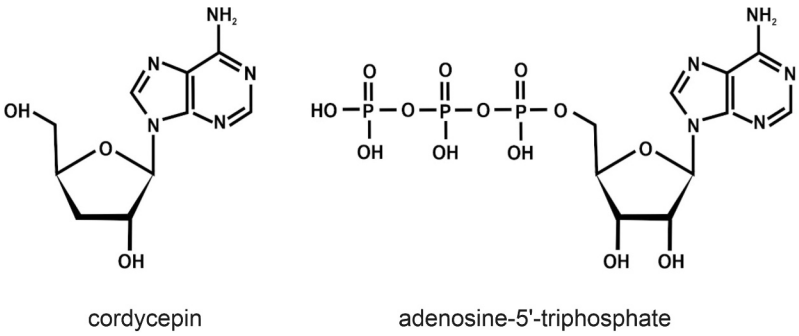

Figure 8. Chemical structures of cordycepin and adenosine-5′-triphosphate.

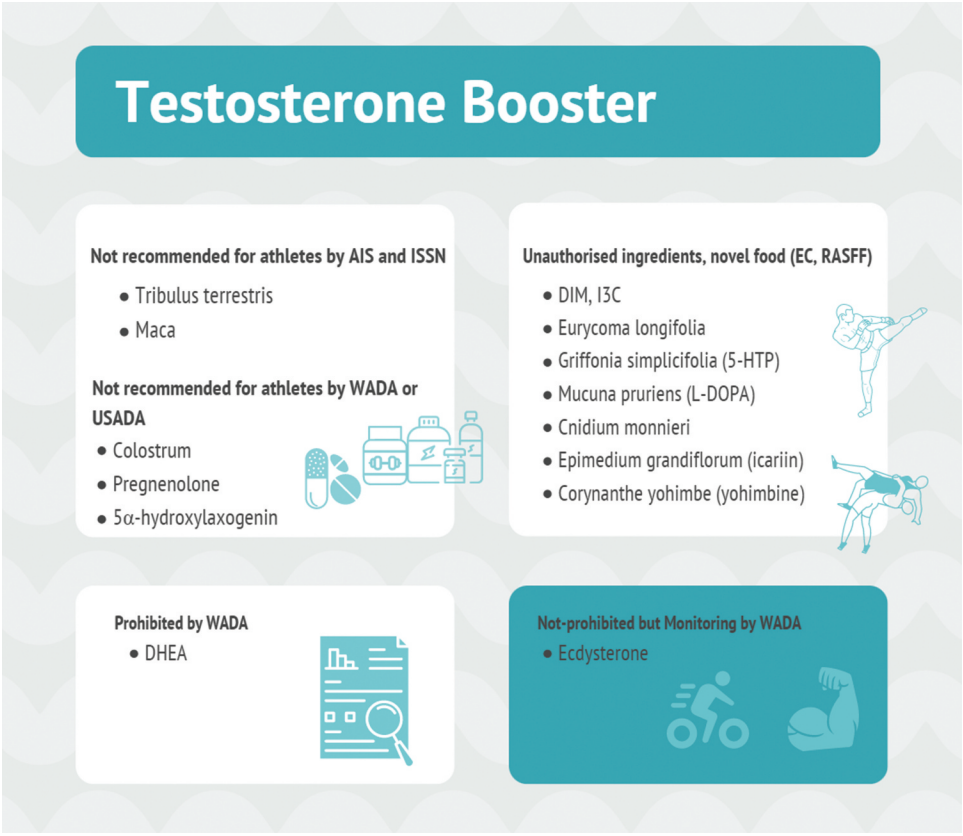

Figure 9. Graphical abstract (created by Prezi Inc. Oakland, CA).

sinensis.[136] No adverse events were reported by Savioli et al..[145] Adverse events such as headache, dry mouth, and sleep disturbance have been reported following supplementation with *C. sinensis* in combination with Ashwagandha, *Astragalus membranaceus, Rhodiola rosea*, and green tea (leaf) extract.[142] Additionally, the Italian Phyto Vigilance database reported adverse events as dizziness and disorientation related to the use of a dietary supplement containing a mixture of *Cordyceps* sp., *Bacopa* sp., Ashwagandha, and Holy Basil.[149]

Although ISSN mentioned *C. sinensis* in a previous study, they never included this mushroom in their recommendations through the years 2004, 2010, and 2018.[22,150] *C. sinensis* has also been mentioned in reports published in The American Journal of Clinical Nutrition.[151]

IOC, similar to AIS, did not recognize *Cordyceps* spp. in their statements.[19,23] According to EU regulations, only *C. sinensis* is an authorized ingredient in foods and food supplements. *C. militaris* is not authorized ingredient in dietary supplements or foods.[20,80,152]

The EFSA and EC did not approve the following health claims for *C. sinensis*: antioxidant properties (400–800 mg/day), stimulation of the immune system, and increasing performance during exercise (3 g of dried powder or equivalent extracts).[36]

Discussion

One of the most popular components of TB is *T. terrestris*, which supplementation had been associated with case reports of adverse events such as gynecomastia, which indicates its impact on the hormonal system of the body and its dysregulation.[153] Talasaz et al. described the nephrotoxic and hepatotoxic effects attributed to supplementation with *T. terrestris*.[18] Acute kidney failure and hyperbilirubinemia associated with the use of *T. terrestris*.[154] In addition, a case of cerebral venous thrombosis in a man was reported to be related to *T. terrestris* supplementation.[155]

Our analysis of ingredients present in TB supplements has shown that ingredients such as *Mucuna* spp. can affect not only testosterone levels but also other hormones. Shukla et al. showed an increase in testosterone concentration and a decrease in prolactin levels in infertile men after supplementation with *Mucuna pruriens*.[156] Another study proved that in physically active men, acute supplementation with *M. pruriens* and *Chlorophytum borivilianum* caused an increase in the lostrum was not specifically prohibited. Ho of growth hormones in the blood.[157]

Some of these ingredients have been mentioned by international sports organizations such as AIS, ISSN, likewise by anti-doping authorities WADA or U.S. Anti-Doping Agency (USADA). According to AIS, *T. terrestris* and Maca are included in category D, which means that the use of these dietary supplements is not recommended in athletes because of the association of these ingredients with the contamination of banned substances that could lead to a positive doping test.[19]

Generally, in the professional sports community, *T. terrestris* does not have a good reputation, and it is not recommended or even discouraged for elite athletes. The safe intake limit for *T. terrestris* has not been determined in Denmark. Therefore, dietary supplements containing *T. terrestris* are classified as unauthorized ingredient on the market in Denmark, and notified in the RASFF panel.[152,158,159]

Ecdysterone and colostrum are mentioned in the WADA documents. The Monitoring Program covered ecdysterone. Colostrum was not specifically prohibited. However, it can contain IGF-1 and other growth factors that are prohibited and can influence the outcome of anti-doping tests. Therefore, the WADA does not recommend the ingestion of this product by elite athletes.[73]

Among all identified ingredients in TB, only dehydroepiandrosterone (DHEA) was mentioned in the WADA Prohibited List as anabolic agent.[73] DHEA is an unauthorized ingredient in dietary supplements in the EU because it is a component of OTC or RX medications. In various dietary supplements, the contents of DHEA marked on the label are 5 mg, 10 mg, 25 mg, 50 mg, and even 100 mg. In comparison, in the past in Poland, approved doses of DHEA in the range of 5–25 mg were registered as medicines by the regulatory authority.[160]

Pregnenolone is the major precursor of all steroid hormones, in the first line it is a direct precursor of progesterone and an indirect (in further lines) precursor of testosterone. Similar to DHEA, pregnenolone is referred to as a prohormone. Pregnenolone is not prohibited as a doping agent by the WADA. However the USADA, in relation to pregnenolone, generally considers that the use of dietary supplements that claim to provide or "boost" hormones is especially risky for athletes. The U.S. Food and Drug Administration (FDA) in Warning Letters have been published regarding this substance; they consider pregnenolone an unapproved pharmaceutical (Pregnenolone: What You Need to Know | USADA 2022).[161] Pregnenolone was identified in the CAERS database. A 29-year-

old woman reported headache, nausea, and tremor. Another case was a 41-year-old man with increased blood pressure, dizziness, increased heart rate, nausea, and blurred vision.[104]

The 5α-hydroxylaxogenin is not included directly on the WADA Prohibited List, but some warnings regarding this substance are reported by the USADA and are being evaluated as potential anabolic or androgenic activity.[161,162**]

As per notifications included in the Italian Phyto Vigilance system, one product (Epistane) contains an unauthorized ingredient undeclared on the label – desoxymethyltestosterone.[149,163,164]

Corynanthe yohimbe bark extract (isolated active substance yohimbine) and agmatine are unauthorized ingredients in dietary supplements in the EU.[20,152]

Agmatine, according to ISSN recommendations, was placed in category III of dietary supplements.-[22] Till date, little is known about agmatine in relation to supporting exercise capacity or improving the focus/motivation to workout in athletes.[121]

The extracts of 5-HTP from *Griffonia simplicifolia* seeds and *M. pruriens* are considered unauthorized novel foods. In addition, *Agaricus bisporus, Cnidium monnieri*, and *Epimedium grandiflorum* have been recognized as novel foods in the EU.[20]

Additionally, doubts may exist about icariin, the active ingredient in *Epimedium grandiflorum* (horny goat weed). Some TB not have specific standardization of plant extracts. In other cases, TB contains a blend of active ingredients without exact content. Only the summary amount of mixture/blend is provided. Another interesting observation is that TB supplements from the same manufacturer, signed with the same brand name, have a different qualitative composition of active ingredients, depending on the country's market.

The daily dose of saponins delivered by TB remains debatable. TB has been reported to contain a combination of various plant materials standardized for saponins. In many cases, daily levels of saponins are exceeded. According to AECOSAN, the daily intake of *T. terrestris* in food supplements should not exceed the dose used in registered herbal medicinal products. The fruit of *T. terrestris* is included in the British Pharmacopoeia. Herbs of *T. terrestris* are under assessment by the EMA as part of the establishment of EU herbal monographs.[3,35]

In Poland, the Chief Sanitary Inspectorate (GIS) issued a resolution establishing that *T. terrestris* fruit can be used as raw material at up to 3 g per day, the maximum content of saponins may not exceed 200 mg in the recommended daily portion, and the manufacturer should attach on the label a quantitative specification confirming the total content of saponins in the daily recommended amount of dietary supplement.[165]

Nonetheless, fenugreek extracts are available on the market, with a high standardization for total content of saponins on the range 50%, or enriched with 20% protodioscin.[113,115] Reported single case of liver injury in women after consumption of dietary supplements with fenugreek.[166] Likewise various of adverse events, notified in the CAERS database.[104]

The another moot point is related to the daily dose of ecdysterone in TB. The safety of ecdysterone supplementation has been assessed up to a dose of 48 mg/day. Most TB analyses of the label indicate 100–250 mg ecdysterone. Additionally, analytical tests confirmed a high concentration of ecdysterone in the selected dietary supplements.[54] Ecdysterone (or other ecdysteroids) as isolated substance was not mentioned in the RASFF panel, likewise in EU Novel Food Catalogue. Some information about ecdysteroids such as ecdysone, polypodine B, inokosterone, pterosterone, abutasterone was found in the scientific report EFSA Compendium of botanicals reported to contain naturally occuring substances of possible concern for human health, however is related to rhizome/root of *Polypodium vulgare* but not for *R. carthamoides* [20, 152 EFSA Scientific Report. Compendium of botanicals 2022]. On the last time, EFSA Emerging Risks Exchange Network (EREN) briefed a note on possible emerging health risks, concerns the use of ecdysterone in food supplements for anabolic purposes.[167] A summary of TB ingredients regulations in EU and sport organizations is presented in Table 3 and supplementary graphical abstract (Fig. 9).

Selected TB herbal components our multi-ingredient products associated with herb-induced liver injury (HILI) include *T. terrestris, E. longifolia, Lepidium meyeni* (Maca), *T. foenum-graecum, Avena sativa, P. nigrum, Serenoa repens, Withania somnifera* (Ashwagandha),*Garcinia cambogia*.[149,168–170]

Acute hepatitis requiring hospitalization was reported after the intake of dietary supplements containing Maca root extract and 150 mg of beta-ecdysone.[149]

The RUCAM system is the primary tool used for assessing causality in cases of drug- or herb-induced liver injury (DILI or HILI, respectively). Not all adverse events reported as HILI were evaluated using the RUCAM algorithm. Among the aforementioned plants, only a few have been assessed using the RUCAM system, such as liver injury associated with *S. repens* or *G. cambogia*.[171,172]

– Not specified

In our results, the health claims authorized and approved by the EFSA and EC refer to ingredients found in TB, mainly minerals such as zinc, magnesium, selenium, calcium, chromium, and vitamins – vitamin B_3, vitamin B_6, vitamin B_{12}, vitamin C, vitamin D_3, vitamin A, vitamin K, folate, and pantothenic acid.[36]

Four ingredients, zinc, selenium, vitamin B_6, and pantothenic acid, have health claims related to their effects on the hormonal system and reproductive functions. Zinc contributes to the maintenance of normal blood testosterone levels;. selenium, normal spermatogenesis; vitamin B_6, regulation of hormonal activity; and pantothenic acid, normal synthesis and metabolism of steroid hormones.[36]

Other ingredients do not have health claims directly related to the influence of the endocrine system. However, they also have potential benefits in terms of exercise capacity. Magnesium contributes to normal muscle function and protein synthesis; calcium, normal muscle function; vitamin B_{12}, normal red blood cell formation; vitamin D_3, maintenance of normal muscle function; folate, normal amino acid synthesis; vitamin B_3 (niacin), reduction of fatigue; And vitamin C, maintenance of normal function of the immune system during and after intense physical exercise. Health claims for chromium (maintenance of normal blood glucose levels) or vitamin K (maintenance of normal bones) are not directly or indirectly related to their impact on the endocrine system or exercise capacity.[36]

Some ingredients in dietary TB supplements, such as *T. terrestris* or *E. longifolia*, were covered and expanded by the specialty educational program IOC Certificate in Drugs in Sport.[173,174]

Conclusions

The most prevalent individual TB components are zinc, Maca, vitamin B_6, fenugreek, *T. terrestris*, DIM (or I3C), black pepper, vitamin D_3, magnesium, vitamin B_{12}, DAA, and *E. longifolia*. Currently, insufficient evidence is available regarding the inclusion of TB in the diets of professional athletes. Selected ingredients are unauthorized novel food in EU such as I3C and DIM, *E. longifolia, E. grandiflorum* (icariin) and *C. yohimbe* (yohimbine). Ingredients such as *T. terrestris, L. meyenii* (Maca), ecdysteroids, DAA are not recommended based on the current statements of ISSN or AIS. Few ingredients not found in any of reviewed databases – *Cissus quadrangularis* (ketosterone), hecogenin and dicyclopentanone. Further studies are needed to evaluate the efficacy and safety of *Cordyceps* spp. (or cordycepin). Fenugreek and *E. longifolia* have the most promising results based on previous studies, but the purity and safety of TB are still of concern. TB can pose significant risks of adulteration with prohibited substances.

Some TB, according to the label, contain ingredients that may have a lowering effect on testosterone levels, contrary to consumer expectations. Nevertheless, they are combined with other ingredients that have been proven to stimulate the production of testosterone. Certain TB contain a mixture of several plant materials standardized for saponins, often providing high single (or daily) doses of saponins. Hypothetically, all cases pose a possible risk of endocrine system dysregulation or imbalance. Some ingredients are backed by low scientific evidence, primary animal studies. Many ingredients in TB have been used in ethnomedicine in several countries as traditional aphrodisiac agents that support erections, sex drive, and potency in men. Most ingredients in TB do not have authorized health claims.

In analysis of TB only one ingredient – DHEA included in the WADA Prohibited List. Ecdysterone is covering by Monitoring Program. Nonetheless ecdysterone not include in the EFSA Novel food catalogue. Three ingredients, colostrum, pregnenolone and 5α-hydroxylaxogenin are not recommended by WADA or USADA for elite athletes.

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