The Complete Edge - CE6 Dietary Supplement Clinical Collateral White

As men age, their endocrinology changes, with a small and progressive decline in sex hormones – testosterone and dehydroepiandrosterone (DHT), and related increases in luteinizing hormone (LH), follicle-stimulating hormone (FSH) and sex hormone binding globulin (SHBG). This has also been termed 'Andropause'.

These biochemical changes, cause a wide range of deviations in male physiology including hypogonadism, which is when the sex glands produce little if any sex hormones, including testosterone. The age-related decrease in testosterone has direct consequences for physical and cognitive function including effecting muscle mass, sexual function, energy levels, bone formation, mood and overall quality of life.

Increasing testosterone levels is therefore favourable when it comes to aging men. Supplementation with testosterone, or testosterone therapy has been shown to increase lean mass, improve body composition, improve muscle strength and physical function. However, increasing testosterone levels via supplementation can have negative effects also.

Just as low testosterone levels can downregulate certain pathways, high testosterone levels can upregulate certain pathways, causing an imbalance in related hormones – such as estrogen and DHT. The side effects of testosterone therapy may include oily skin, increased growth of body hair, balding, breast tenderness and enlargement, hepatotoxicity and blood lipid level increases.

Additionally, there can be an increase in long-term risks of prostate and cardiovascular disorders. The higher the dose of testosterone, the greater the anabolic effect, but also the higher frequency of adverse effects. These negative effects have encouraged development of better treatments to support healthy testosterone levels and ultimately healthy aging in men.

BioGenix has set out to develop a complete formula – CE6 Rx consisting of unique complexes to support male aging including a complex to modulate and regulate hormone production at the gene level, a heart health support complex, an endocrine and antioxidant support complex for testosterone synthesis, and lastly a gut microbiome and absorption complex to ensure ingredient absorption, detoxification and a healthy balanced bacterial biome.

The following will outline the ingredients found within each complex of CE6 and outline the supporting research and mechanism of this formulation.

A Better Way to Modulate Hormone Production – CE6

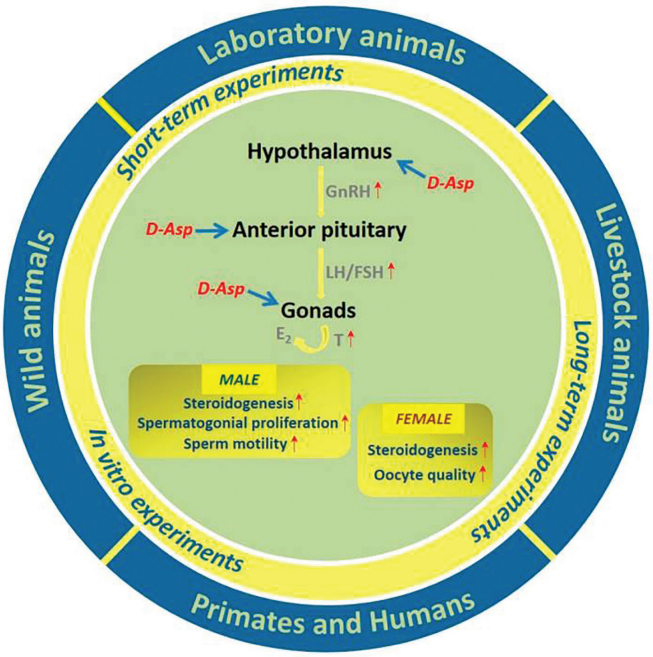
- Boost Free Testosterone
- Endocrine Support
- Improve Muscle Mass, Strength & Stamina
- Improve Energy & Focus
- Supports Heart Health
- Gut Microbiome Health

Muscle Gene Regulation Complex

D-Aspartic Malate

D-aspartate (DA) is the active form of the amino acid aspartic acid. The other form is L-aspartate. DA has been shown to play a regulatory role in endocrine and neuroendocrine function and the regulation of the reproductive system(1). DA is involved in several steps of steroidogenesis, regulating synthesis and release of sex steroid hormones including luteinizing hormone (LH), follicle-stimulating hormone (FSH) and growth hormone (GH)(1). It may also build up in the testicles, where it can influence the rate limiting step of testosterone synthesis, leading to slight increases in free testosterone levels(1).

Figure 1: The Effect of DA on the Hypothalamic Pituitary Gonad Axis

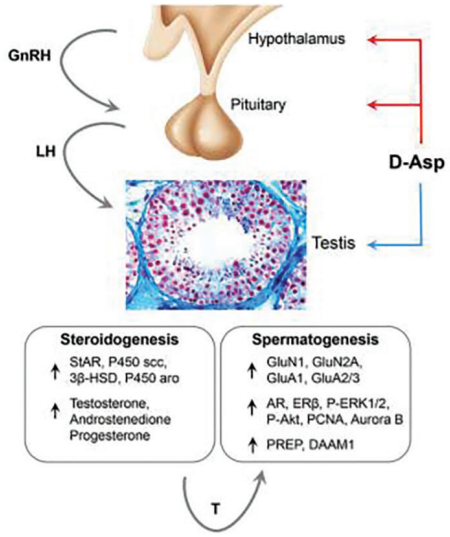


The hypothalamus–hypophysis–gonad axis is most likely the target of DA and N-methyl-D-aspartate (NMDA) as it contains the highest tissue levels of DA, has the capacity to accumulate this amino acid, and responds following its administration(1). In addition, the gonads possess enzymatic systems for the biosynthesis and degradation of DA(1).

In vivo and in vitro experiments suggest that DA acts as an excitatory molecule that induces the release of hormones including LH, GH and prolactin (PRL) by the anterior pituitary and simultaneously directly induces biosynthesis and release of testosterone by stimulating the protein expression of steroidogenic acute regulatory protein (StAR) in Leydig cells(1).

DA is also involved in the synthesis of dihydrotestosterone (DHT) and estradiol (E2) via activation of 5-alpha reductase and the P450 aromatase enzymes(1). DA's action appears to be rapid, increasing gene and protein expressions of enzymes involve in steroidogenesis, and diminishing after 24 hours(1).

Figure 2: DA Regulation of Spermatogenesis



DA stimulates the release of gonadotropin-releasing hormone (GnRH) from the hypothalamus, which, in turn, induces the release of LH from the pituitary gland ultimately resulting in increased testosterone biosynthesis(2). In the Leydig cells, DA modulates steroidogenesis by eliciting the expression of StAR, cytochrome 450 cholesterol side chain cleavage, 3β-hydroxysteroid dehydrogenase (3β-HSD) and cytrochrome P450 aromatase steroidogenic enzymes(2). In the testis, DA activated NMDA (GluN1 and GluN2A subunits) and AMPA (GluA1 and GluA2/3 subunits) receptors(2). DA enhances androgen receptor (AR) and estrogen receptor β (ERβ) expressions and induces spermatogonial proliferation by increasing expression of ERK and Akt pathways(2). Lastly, DA increases the expression of prolyl endopeptidase (PREP) and disheveled-associated activator of morphogenesis 1 (DAAM1), two proteins involved in cytoskeleton remodeling(2).

D-Aspartate, Testosterone and Fertility

In a group of 30 male patients, affected by oligo-asthenozoospermia (OAS), which consists of a decrease in concentration and percentage of motile sperm and a group of 30 male patients affected by asthenozoospermia (AS), which consists of low motility sperm, were treated with a daily dose of DA for 90-days(3).

Supplementation of DA significantly increased the concentration and the motility of spermatozoa. In the OAS group, patients increased sperm concentration was found to be 2.0-fold, and the motility increased by 1.5-fold. In the AS group, the sperm concentration was approximately 1.6-fold higher, and the rapid progressive sperm motility was increased by about 1.9-fold(3).

In conclusion, in this study demonstrated that sub-fertile men who consumed daily an oral dose of DA for 2 - 3 months, significantly improved their sperm quality in terms of number and mobility of the spermatozoa, and consequently they increased the possibility to fertilize their partner(3). It's hypothesized that this event is due to action of DA in inducing elevation of testosterone in the testis, which locally is involved in improving the spermatogenesis and in maturation of male gametes.

In another study, DA supplementation (3 g/day supplemented with folic acid and vitamins B6 and B12) in 23 men for 12 days increased the levels of LH and testosterone, respectively, to 33% and 42%(4). Three days after supplementation, LH levels returned almost to baseline, but testosterone levels remained significantly higher(4).

A possible explanation of this finding is that ingested DA remained in the testis where it continued to stimulate testosterone production(4). DA increased the release and synthesis of LH through the involvement of cGMP as a second messenger in the pituitary, whereas in the testis Leydig cells, it increases the synthesis and release of testosterone and cAMP is implicated as the second messenger(4). In the pituitary and in testes DA is synthesized by a D-aspartate racemase which convert L-Asp into DA(4). The pituitary and testes possess a high capacity to trapping circulating DA from hexogen or endogen sources.

Although DA is effective for increasing testosterone, sperm concentration and motility in infertile men, it is not shown to be effective in those with normal or high testosterone concentrations.

One study has been conducted in athletes given DA supplementation at a dose of 3g daily for 28 days, there was a failure to increase testosterone concentrations when measured at 28 days(5). This study noted a statistically significant induction of serum D-aspartate oxidase (DAO) which degrades DA to a near doubling. This suggests a possible form of negative feedback in those with higher levels. In another, 12-week double-blind randomized controlled trial in resistance-trained men, no change in total or free testosterone with 6 g/d supplementation was reported(6).

Shilajit Extract (50% Fulvic Acid)

Shilajit is a mineral pitch, found in the Himilayan and Hindukush Mountain ranges at high altitudes and has traditional uses in Ayurveda medicine as a vitality enhancer and adaptogen. Shilajit is mostly a mixture of humic acids, with plant microbial metabolites(7).

The main component of Shilajit is fulvic acid. It also contains some amino acids including glycine, glutamic acid, and aspartic acid(7). They also have di-benzo-alpha-pyrones (DBPs) and chromoproteins associated with DBPs.

Shilajit and Energy Status

It has been suggested that Shilajit supplementation may help enhance strength and energy levels. In a study on albino mice, Shilajit supplementation significantly enhanced physiological energy status in a forced swimming test (FST) model(8). The study showed a significant fall in adenosine triphosphate levels in the muscle, brain, and blood in the exercised control animals after 7 days of a swimming regime. Post exercise, shilajit retrieved loss of the energy currency (ATP), including in the muscle, brain and blood(8).

Shilajit and Muscle Adaptation

After the swimming protocol and shilajit supplementation, there was an improved model of energy related indices such as Adenylate Energy Charge (AEC) and Total Adenine Nucleotide (TAN). These augmented effects were similar and comparable to Coenzyme Q10 (CoQ10) supplementation(8).

A synergistic effect in the improvement of the energy related parameters was observed when the animals were treated with a combination of shilajit (15 mg/Kg body weight, p.o. x 4 days) and CoQ10 (7.5 mg/Kg body weight, p.o. x 4 days). Yet another improvement of shilajit treatment constituted of the CoQ10 status in muscle and blood of the treated animals(8).

The FST-induced impairment of CoQ10 status in mice was manifested by a fall of CoQ10 concentration by 75% in blood and a rise in CoQ10 by 68% in muscle in exercised control animals on the seventh day of the swimming regime8. The fall in CoQ10 concentration in blood was attenuated to 50% and its rise was arrested in muscle, when the animals were treated orally with shilajit (30 mg/Kg body weight, p.o. x 4 days)(8). Effect of shilajit on blood and muscle CoQ10 status was at par with those of orally administered CoQ10 (15 mg/Kg body weight x 4 days)(8).

Shilajit has been reported to improve physical performance and relieve fatigue with enhanced adenosine triphosphate (ATP) production. The effect of oral Shilajit supplementation on exercise training on human skeletal muscle adaptation was evaluated in a group of overweight/class I obese human subjects following 12-weeks of a study period(9).

Each subject received 250 mg of Shilajit containing at least 60% fulvic acids, twice per day for the first 8-weeks. The last 4-weeks, subjects took 250 mg of Shilajit, twice daily while also completing exercise on a treadmill performed at 70 to 75% of their max heart rate for 20 min(9).

Microarray analysis identified a cluster of 17 extracellular matrix (ECM)-related gene probe sets that were significantly upregulated in muscles following 8 weeks of oral supplementation compared with the expression at the baseline. This cluster included tenascin XB, decorin, myoferlin, collagen, elastin, fibrillin 1, and fibronectin 1(9). This increased mRNA expression of collagen, which is the major structural protein in the skeletal muscle extracellular matrix, accounts for 6% of the weight of tendinous muscle.

The differential expression of these genes was confirmed using quantitative real-time polymerase chain reaction (RT-PCR). The study provided evidence that oral Shilajit supplementation in adult overweight/class I obese human subjects promoted skeletal muscle adaptation through upregulation of ECM-related genes that control muscle mechanotransduction properties, elasticity, repair, and regeneration(9).

Shilajit and Muscular Strength

The differential expression of these genes was confirmed using quantitative real-time polymerase chain reaction (RT-PCR). The study provided evidence that oral Shilajit supplementation in adult overweight/class I obese human subjects promoted skeletal muscle adaptation through upregulation of ECM-related genes that control muscle mechanotransduction properties, elasticity, repair, and regeneration(9).

The effect of shilajit on muscle strength was evaluated in sixty-three recreationally active men. Subjects were randomly assigned to receive a high

The results demonstrated that 8 weeks of During pre-supplementation testing, the subjects performed 2 pretest maximum voluntary isometric contraction (MVICs), 2 sets of 50 maximal, bilateral, concentric isokinetic leg extensions at 180° per second separated by 2-min of rest, and 2 post-test MVICs10. Following 8 weeks of supplementation, the subjects repeated the pre-supplementation testing procedures.

The results demonstrated that 8 weeks of shilajit supplementation at 500 mg per day promoted the retention of maximal muscular strength following the fatiguing protocol and decreased baseline serum hydroxyproline (HYP), which is a biomarker of collagen degradation and integrity of connective tissue. Thus, shilajit supplementation at 500 mg per day elicited favorable muscle and connective tissue adaptations(10).

Shilajit and Testosterone

The testosterone boosting effects of Shilajit was investigated in a mice model. The effect of daily oral Shilajit (50 mg, 100 mg and 200 mg/kg body weight) was investigated for a single spermatogenic cycle (35 days) in cadium-induced infertile adult mice(11). Shilajit treatment increased weights of reproductive organs, testicular daily sperm production, activities of testicular enzymes and serum level of testosterone.

Shilajit restored spermatogenesis as reflected by a gradual augmentation in germ cell layers with increased doses of Shilajit compared to cadmium-treated mice. Further, Shilajit treatment reverted the adverse effects of cadmium on motility and concentration of spermatozoa. Secretory activities of the epididymis and seminal vesicle and libido, fertility, and the number of litters per female were also improved by Shilajit in cadmium-treated mice. Results thus suggest the potent androgenic nature of Shilajit and its role in fertility improvement against cadmium-induced infertility (11).

One study, in healthy male volunteers showed a positive effect of shilajit on testosterone levels. In a randomized, double blind, placebo-controlled study, men between 45 and 55 received 500 mg of shilajit for 90-days12. Treatment with Shilajit had a significant effect, increasing total testosterone (4.84 ± 1.54 ng/mL), free testosterone (15.36 ± 7.17 pg/mL), and dehydroepiandrosterone (DHEAS) (145.09 ± 53.17 µg/dL) by 20.45%, 19.14% and 31.35% compared with placebo. Gonadotropic hormones (LH and FSH) levels were well maintained(12).

In another human study, the safety and spermatogenic activity of Shilajit was evaluated in oligospermic, low sperm count, patients. This included men with total sperm counts below 20 million per ml semen. Shilajit was administered at 100 mg twice daily, after major meals for 90-days(13).

Twenty-eight patients who completed the treatment showed significant improvement in spermia (+37.6%), total sperm count (+61.4%), motility (12.4-17.4% after different time intervals), normal sperm count (+18.9%) with concomitant decrease in pus and epithelial cell count compared with baseline value(13).

Significant decrease of semen malondialdehyde content (-18.7%), a marker of oxidative stress was observed. Moreover, serum testosterone (+23.5%) and FSH (+9.4%) levels significantly increased. HPLC chromatogram revealed inclusion of shilajit constituents in semen(13). Unaltered hepatic and renal profiles of patients indicated that shilajit was safe at the given dose. The present findings provide further evidence of the spermatogenic nature of shilajit.

Phosphatidylserine

Phosphatidylserine (PS) is a glycerophospholipid and is a major component of the cell membrane. PS accounts for 13 to 15% of the phospholipids in the brain. It's also concentrated in organs with high metabolic activity such as the lungs, heart, liver, and skeletal muscle(14).

PS is located mainly in the internal layer of the cell membrane and has a variety of unique regulatory and structural functions. PS modulates the activity of receptors, ion channels, enzymes and signaling molecules and is involved in governing membrane fluidity PS forms part of protein docking sites necessary for the activation of several key signaling pathways(14).

Modulation of the PS level in the plasma membrane of neurons has a significant impact on these signaling processes and may help improve cognitive function and the body's ability to deal with stress(14). In recent studies, PS has been shown to enhance wellbeing during mental stress brought on by exercise, help speed up post-workout recovery, prevent muscle soreness, improve performance and accuracy for sports(14).

Blunting Exercise Induced Cortisol Response

PS has been reported to be an effective supplement for combating exercise-induced stress and preventing the physiological deterioration that accompanies too much exercise. PS has been reported to attenuate serum cortisol and adrenocorticotropic hormone (ACTH) responses to staged cycling exercise(15).

A study on eight non-physically trained healthy male volunteers (aged 24–42 years) underwent two exercise trials, composed of three distinct stages of cycle ergometry. The subjects received intravenously either placebo or 50 or 75 mg of bovine PS, 10 minutes before exercise(15). As expected, physical exercise resulted in a significant increase in ACTH and cortisol levels after administration of a placebo(15). Bovine PS supplementation significantly suppressed ACTH and cortisol responses to exercise.

PS supplementation of 800 mg for 10 days lowered cortisol response by 30%, without affecting the rise in growth hormone(16). Physical exercise significantly increased the plasma lactate concentration both after placebo and phosphatidylserine. The results suggest that supplementation of phosphatidylserine may counteract stress-induced activation of the hypothalamo-pituitary-adrenal axis in man(16).

Another double blind, study reported a 20% reduction in cortisol response to intensive resistance training in trained males given 800 mg of soy-derived PS taken daily for 2-weeks(17).

Subjects did 5 sets of 10 repetitions for 13 exercises, 4 times a week, for two 2-week periods separated by a 3-week recovery. Resting morning venous blood was sampled 6 times during each 2-week period and 15 min following the 8th training sessions(17).

ACTH did not change in PS but increased in the placebo group. Cortisol decreased in PS group after exercise, possibly by depressing ACTH and might have attenuated the negative effects of intense weight training on perception of well-being and muscle soreness(17).

Phosphatidylserine Exercise Performance and Recovery

Phosphatidylserine has been shown to influence exercise performance for both endurance and weight training protocols. In a double-blind, placebo-controlled clinical trial where fourteen active males completed staged intermittent exercise on three separate occasions, (a familiarization trial followed by two main trials that were separated by approximately 16 days)(18).

The staged intermittent protocol consisted of three 10-min stages of cycling at 45, 55 and 65% VO2max followed by a final bout at 85% VO2max that was continued until exhaustion. The exercise time to exhaustion was used as a measure of exercise capacity. After completing the first main trial the subjects consumed either 750 mg of soy PS per day or a placebo for 10 days. The main finding was that supplementation influenced exercise capacity at 85% VO2max. The group that received PS increased exercise times to exhaustion by 29 ± 8%, while the exercise times to exhaustion did not change following supplementation with placebo(18).

Another double-blind cross over study showed that PS supplementation significantly lowered creatine kinase (CK) activities in circulation, 24 hours post exercise(19). CK is an indicator of cell membrane damage and necrosis of the muscle fiber. Intense exercise and activities, where muscle damage occurs, causes an increase in CK.

Phosphatidylserine and Mental Stress Reduction

Mental and physical stress is closely linked and results in similar physiological responses in the human body. Forty-eight male undergraduate students received either 300 mg PS or placebo for 30 days. Mental stress was induced by performing a demanding mathematical task. PS supplementation resulted in improvements in feeling clear-headed, composed and confident, feeling energetic and elated in a specific sub-section of the students(20).

The effects of a three-week soy lecithin phosphatidic acid (PA) and PS supplementation to a mental and emotional stressor. The 400 mg PA resulted in a pronounced blunting of serum ACTH and cortisol levels as well as positive effects on emotional responses compared to placebo, however, higher doses (600 mg and 800 mg) did not result in the same effects(21).

In a follow-up study, the effects of supplementation with 400 mg PS and PAS per day were evaluated on the endocrine stress response to a psychosocial stressor, with emphasis on the effectiveness of supplementation in low versus high chronically stressed subjects(22).

Study supplementation was administered for 42 days for each participant. Chronic stress was measured with the Trier Inventory for Chronic Stress (TICS), and subgroups of high and low chronic stress were differentiated by median values as provided by the TICS. A six-week period of supplementation was followed by an acute stress test (Trier Social Stress Test - TSST)(22).

Acute stress was successfully induced by the TSST and resulted in a hyper-responsivity of the hypothalamus-pituitary-adrenal-axis in chronically stressed subjects. Compared to placebo, a supplementation with a daily dose of PAS 400 was effective in normalizing the ACTH, salivary and serum cortisol responses to the TSST in chronically high but not in low stressed subjects(22).

Phosphatidyl Serine and Aging

Phosphatidylserine has a long history in improving brain functions that tend to decline with age. Numerous clinical trials with PS have showed improvements in memory tasks such as name-face delayed recall, facial recognition, telephone-number recall, and misplaced-objects recall(23).

Phosphatidylserine supplementation has been reported to improve long-term memory, long-term recognition, as well as free speech and logic speaking. It has been observed that the interest in the environment and the attention span were increased while loss of motivation, socialization and initiative were reduced(23).

In a 3-month double blind, placebo-controlled study, the influence of 300 mg of PS + 240 mg of PA per day was evaluated on memory and mood in functioning, non-depressive elderly people with memory problems using the Wechsler Memory Scale(24). Additionally, a 2-month randomized, double-blind, placebo-controlled trial assessed the effect of 300 mg PS+ 240 mg of PA per day on daily functioning, mental health, emotional state, and self-reported general condition in patients with Alzheimer's disease (AD)(24).

In the elderly, PS+PA, unlike placebo, experienced a significantly improved memory and prevented "winter blues" in a pre-post comparison. In the patients with AD, daily functioning under PS+PA remained unchanged, but declined from 5.62 to 4.90 under placebo, with significant group difference(24).

The PS+PA group had 3.8% deterioration and 90.6% stability in daily functioning, compared to 17.9% and 79.5% under placebo. Forty-nine percent of the PS+PA patients reported an improved general condition, compared to 26.3% under placebo(24). No negative side effects were observed.

PS is efficiently absorbed after oral consumption. A positive influence of PS+PA on memory, mood, and cognition was demonstrated among elderly test subjects. Short-term supplementation with PS+PA in patients with AD showed a stabilizing effect on daily functioning, emotional state, and self-reported general condition.

Dehydroepiandrosterone (DHEA)

DHEA is an endogenous steroid, hormone precursor, mainly secreted by the adrenal cortex and the gonads, but also within the brain. DHEA and its sulfated metabolite, DHEAS, are the most abundant endogenous circulating steroid hormones in the body(25). It functions as metabolic intermediate in the biosynthesis of androgens including estrogens and testosterone, in the gonads and other tissues. However, DHEA and DHEAS also has a variety of potential biological effects, acting as a ligand, binding to an array of nuclear and cell surface receptors, as well as neurotrophic factor receptors. Decreased levels of DHEA are associated with aging and has been found in age-associated changes in cardiovascular tissues, female fertility, metabolism, and neuronal/CNS functions(25).

As a supplement, DHEA may help alleviate symptoms associated with a decreased DHEA pool (either aging in which DHEA declines or adrenal insufficiency where DHEA synthesis declines) or it can be used to increase the DHEA pool and downstream metabolites, such as testosterone or estrogen for a short period of time.

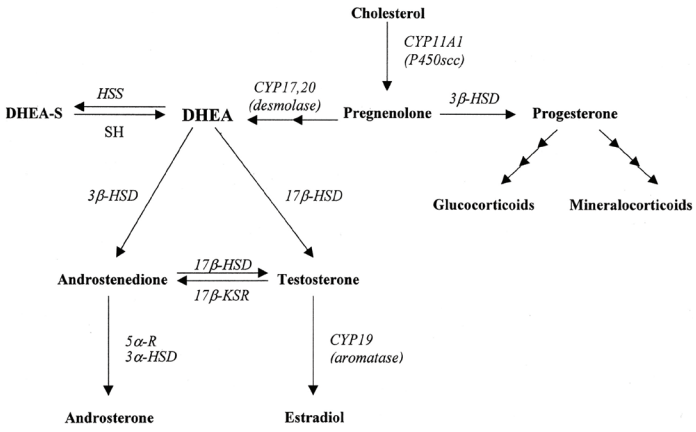
Synthesis and Metabolism of DHEA

Dietary cholesterol is converted into pregnenolone via the CYP11A1 enzyme, and then converted into DHEA via the CYP17 enzyme (P450c17)(26). DHEA converts to DHEAS via sulfotransferases and can be converted back via sulfatases, creating a large interchangeable 'pool' of DHEA:DHEAS circulating in the body for further metabolism(27).

DHEA synthesis usually occurs in the adrenal cortex due to a high localized expression of the CYP17 enzyme. However, synthesis of DHEA also occurs in the testes, ovaries, and the brain.

Starting from the DHEA: DHEAS pool, DHEA tends to be converted into androstenedione directly allowing for multiple action pathways. Androstenedione can be directed towards the most potent androgenic hormone DHT via either turning into testosterone and then becoming substrate for the 5α-reductase enzyme, or by being substrate to the 5α-reductase enzyme (to turn into 5α-Androstenedione) and being converted to DHT(27).

Figure 3: Synthesis of DHEA



If one of these androgens has not yet been substrate for the 5α-reductase enzyme, they can instead be substrate for the aromatase enzyme and be converted to estrogens. Both androstenedione and testosterone are bi-directional hormones that can turn into more potent androgens or estrogens(27).

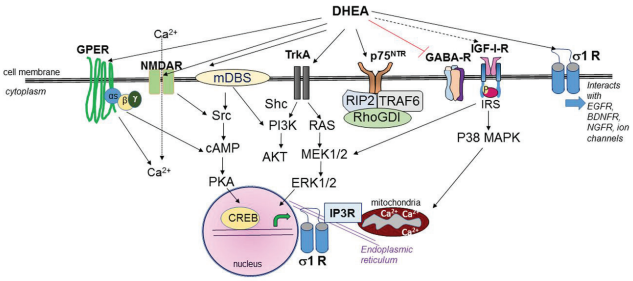
In addition, DHEA completely independent of the classical steroid pathways mentioned above, can be turned into bioactive DHEA derivatives, demonstrating another possible route for DHEA metabolism. These DHEA metabolites are more involved with the immunological and inflammation aspects of DHEA supplementation, and some neurological aspects.

Mechanism of Action of DHEA

Studies have shown that DHEA has a wide variety of effects throughout the body on liver, kidney, adipose, reproductive tissues, and central nervous system/neuronal function(27).

The mechanisms by which DHEA and DHEAS impart their physiological effects may be direct actions on plasma membrane receptors, including DHEA-specific, G-protein-coupled receptors, nuclear receptors, inflammatory receptors including PPAR, immune cell receptors including CAR, neuroreceptors including GABA, steroid receptors including androgen and estrogen receptors (ARs, ERα, or ERβ); or by their metabolism to more potent sex steroid hormones, including testosterone, dihydrotestosterone, and estradiol, which bind with higher affinity to ARs and ERs(25).

Figure 4: DHEA Activation of Membrane Receptors



DHEA, Testosterone and Training Adaptation

With advancing age, plasma testosterone levels decline, with free testosterone levels declining more significantly than total testosterone. This fall is thought to underlie the development of physical and mental weakness that occurs with advancing age. Supplementation with DHEA has been shown to help increase free testosterone levels in men.

A randomized, double-blind, placebo-controlled crossover study was conducted on 8 middle-aged participants (aged 49.3 ± 2.4 years) and an additional 8 young control participants (aged 21.4 ± 0.3 years). Each participant received DHEA (50 mg) and placebo on separate occasions one night (12 h) before a 5-session, 2-min cycling exercise performed at 100% VO_2 max(28).

While no significant age difference in total testosterone was found, middle-aged participants exhibited significantly lower free testosterone and greater luteinizing hormone (LH) levels than the young control group. Oral DHEA supplementation increased circulating DHEA-S and free testosterone levels well above baseline in the middle-aged group, with no significant effect on total testosterone levels(28).

Total testosterone and DHEA-S dropped significantly until 24 h after high intensity interval training (HIIT) for both age groups, while free testosterone of DHEA-supplemented middle-aged men remained unaffected. These results demonstrate acute oral DHEA supplementation can elevate free testosterone levels in middle-aged men and prevent it from declining during HIIT. Therefore, DHEA supplementation may have significant benefits related to HIIT adaptation(28).

DHEA, Body Composition and Muscle Strength

In another study, the effects of 100 mg of DHEA supplementation daily for 6-months were assessed on circulating sex steroids, body composition and muscle strength in healthy, non-obese advanced age men and women(29).

In both sexes, a 100 mg daily dose of DHEA restored serum DHEA levels to those of young adults and serum DS to levels at or slightly above the young adult range. Serum cortisol levels were unaltered, consequently the DS/cortisol ratio was increased to pubertal (10:1) levels. In women, but not in men, serum A, T and DHT were increased to levels above gender-specific young adult ranges. Basal SHBG levels were in the normal range for men and elevated in women, of whom 7 of 8 were on oestrogen replacement therapy.

While on DHEA, serum SHBG levels declined with a greater response in women than in men. Relative to baseline, DHEA administration resulted in an elevation of serum IGF-I levels in men (16 +/- 6%) and in women (31 +/- 12%)29. Serum levels of IGFBP-1 and IGFBP-3 were unaltered, but GHBP levels declined in women (28 +/- 6%) not in men(29).

In men, but not in women, fat body mass decreased 1.0 +/- 0.4 kg (6.1 +/- 2.6%) and knee muscle strength 15.0 +/- 3.3%, as well as lumbar back strength 13.9 +/- 5.4% increase(29). In women, but not in men, an increase in total body mass of 1.4 +/- 0.4 kg (2.1 +/- 0.7%) was noted(29). Neither gender had changes in basal metabolic rate, bone mineral density, urinary pyridinoline cross-links, fasting insulin, glucose, cortisol levels or lipid profiles. No significant adverse effects were observed.

DHEA and Aging Prevention

As discussed previously, aging in humans is accompanied by a progressive decline in the secretion of DHEA and DHEAS. At the same time, GH-insulin-like growth factor-I (GH-IGF-I) axis also declines.

The decline in DHEA may contribute to the shift from anabolism to catabolism associated with aging. A randomized placebo controlled cross over study, evaluated a 50 mg per day replacement dose of DHEA in 13 men and 17 women, between 40-70 years of age given for a 6-month duration(30).

DHEA and DHEAS serum levels were restored to those found in young adults within 2 weeks of DHEA replacement and were sustained throughout 3 months of the study. A 2-fold increase in serum levels of androgens (androstenedione, testosterone, and DHT) was observed in women, with only a small rise in androstenedione in men(30). There was no change in circulating levels of sex hormone-binding globulin, estrone, or estradiol in either gender.

Although mean 24-h GH and IGFBP-3 levels were unchanged, serum IGF-I levels increased significantly, and IGFBP-1 decreased significantly for both genders, suggesting an increased bioavailability of IGF-I to target tissues(30).

This was associated with a remarkable increase in perceived physical and psychological well-being for both men (67%) and women (84%). In conclusion, restoring DHEA and DHEAS to young adult levels in men and women of advancing age induced an increase in the bioavailability of IGF-I, as reflected by an increase in IGF-I and a decrease in IGFBP-1 levels(30).

These observations together with improvement of physical and psychological well-being in both genders and the absence of side-effects constitute the first demonstration of novel effects of DHEA replacement in age-advanced men and women.

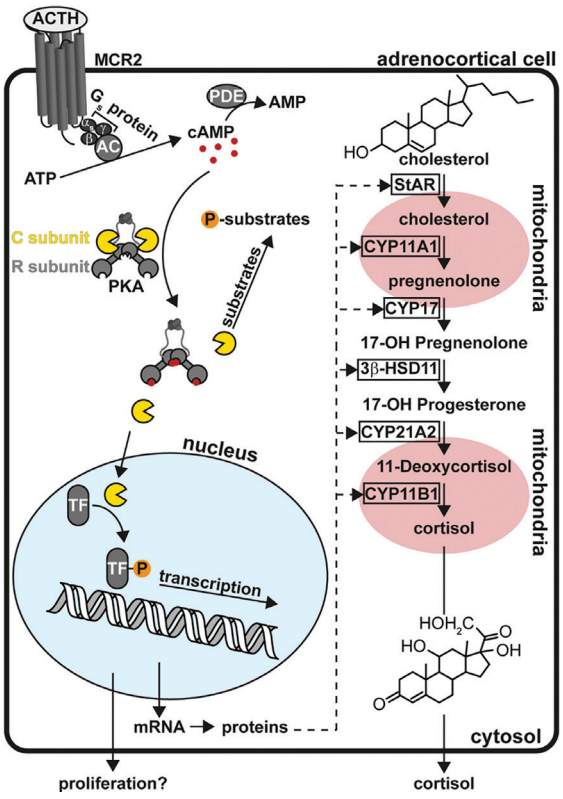
Pregnenolone - The Steroid Hormone Precursor

The second ingredient in this formulation, is pregnenolone, a naturally produced hormone in the body that is used as an anti-inflammatory and analgesic, and for supporting cognitive function including improving memory, clarity, and mood.

Pregnenolone is a steroid hormone precursor that is synthesized from cholesterol in steroidogenic tissues including the adrenal gland, gonads, and brain31. The mitochondria of these tissues are the sites for steroid hormone biosynthesis.

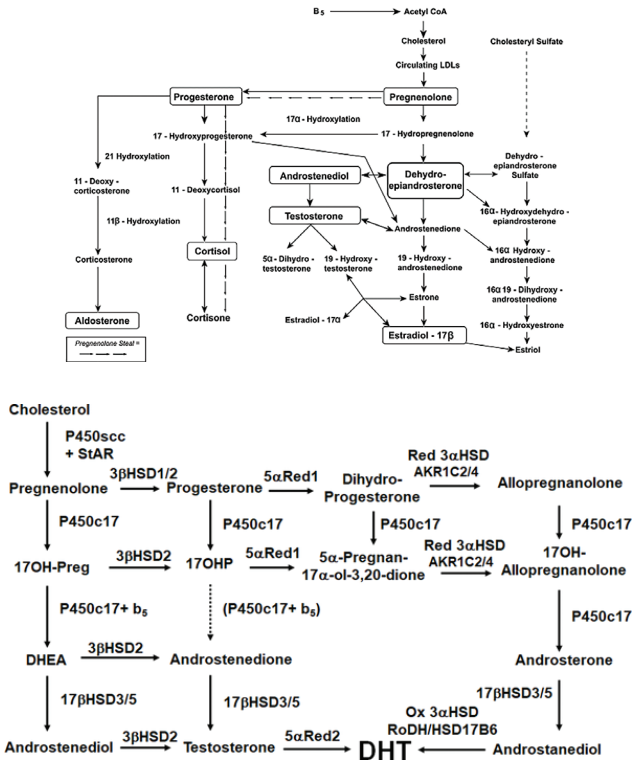
When the adrenocorticotropic hormone (ACTH) is secreted, it stimulates the adrenal glands cortical region to produce cholesterol, which binds to the steroidogenic acute regulatory protein (StAR) in the outer membrane of the mitochondri(32). Once inside the cell, the cytochrome enzyme P450 cuts the cortisol chain producing pregnenolone. This enzyme complex regulates the conversion of cholesterol to pregnenolone, regulating steroidogenesis(32).

Figure 5: ACTH cAMP/PKA Pathway Mediating Cholesterol Production



Pregnenolone can then be involved in the production of most steroid hormones including progestogens, androgens, estrogens, glucocorticoids, and mineralocorticoids. For example, pregnenolone can be converted to progesterone, which in turn can be converted to a corticosterone, and then to androstenedione, a precursor to testosterone and esterone. It can also be converted to dehydroepiandrosterone (DHEA), a precursor of androstenedione, as well as metabolized into cortisol or corticosterone. Despite its biosynthetic action, it has no progestogenic, corticosteroid, estrogenic, androgenic or antiandrogenic activity.

Figure 6: Pregnenolone and Androgen Synthesis



In the brain, pregnenolone is synthesized as a neurosteroid in the central and peripheral nervous system from glial cells and neurons(33). Neurosteroids affect synaptic functioning, are neuroprotective, neuroplastic and neurogenerative. Pregnenolone may therefore improve cognitive and memory function, mood, but also offer some benefits as a steroid precursor to help regulate hormone production, and as an anti-inflammatory, allowing for faster recovery and anti-aging benefit.

Anti-Inflammatory & Analgesic

Pregnenolone has been used for decades as an anti-inflammatory and analgesic agent. However, the mechanism by which pregnenolone exerts its exact anti-inflammatory property remains unknown. In general, steroids exhibit anti-inflammatory properties by binding to their intracellular receptors, which subsequently, translocate to the nucleus to negatively regulate genes associated with the secretion of inflammatory cytokines.

The specific action of pregnenolone, is most likely via its metabolic derivatives such as progesterone and allopregnanolone. Progesterone has been reported to exert its effects through progesterone receptors. While allopregnanolone is a positive GABA-A receptor modulator that has known analgesic and anti-inflammatory properties(33).

Clinical data suggest that neurosteroids exhibit analgesic actions, and that neurosteroids are depleted in populations with pain symptoms(34). A study of 90 male veterans of the US Military, with self-reported pain assessments, evaluated the levels of neurosteroids for allopregnanolone, pregnenolone, DHEA, progesterone, and DHEA sulfate(34).

Results suggest that allopregnanolone levels were inversely associated with low back pain, chest pain and DHEA levels were inversely associated with muscle soreness(34). DHEA sulfate levels were positively associated with chest pain. There was also a positive association between traumatic brain injury and muscle soreness. Restoring low or depleted levels of neurosteroids may therefore help reduce pain and inflammation(34).

In a randomized, double-blind placebo-controlled clinical trial of 94 US veterans between the ages of 18 to 65, with chronic low back pain resulted in a significant reduction in pain intensity ratings after 4-weeks of treatment of pregnenolone using fixed escalating doses of 100 mg for 1-week, 300 mg for 1-week and 500 mg for 2-weeks(35). Participants receiving pregnenolone reported a clinically meaningful reduction in low back pain and 2 pain interference domains compared with those receiving placebo(35). Individuals who received pregnenolone were 2.6 times more likely to report a reduction in pain intensity ratings and pain recall(35).

Neuroprotection, Memory and Cognitive Function

Pregnenolone and its metabolites have been shown to modulate neuroinflammation and promote neuroprotection. Preclinical studies have demonstrated that pregnenolone and its metabolic derivatives such as pregnenolone sulfate, allopregnanolone and DHEA exhibits antidepressant properties, improves memory and cognitive functions, control pain and stress, and relieve symptoms of mood disorder(36). Pregnenolone and its metabolites have a neuroprotective role in various neuroinflammatory diseases including Alzheimer's Disease (AD), Multiple Sclerosis (MS) and in neuropsychiatric disorder. However, the molecular mechanism behind the anti-inflammatory and neuroprotective functions of pregnenolone is not clear. In animal models, social isolation is associated with anxiety, depression, decreased GABA receptor function and pregnenolone levels, whereas pregnenolone administration is associated with improved performance on cognitive tasks(36).

In one study, the memory deficit of cognitively impaired aged rats was corrected after intracerebroventricular injection of pregnenolone, which stimulated acetylcholine (ACh) release in the hippocampus(37). It is proposed that the hippocampal content of pregnenolone plays a physiological role in preserving and enhancing cognitive abilities in older animals, possibly via interaction of the central cholinergic systems.

Central ACh neurotransmission is involved in the regulation of memory processes and is affected in normal aging and in human neurodegenerative pathologies like Alzheimer's disease(38). ACh neurotransmission is also involved in the modulation of sleep-wakefulness cycle.

Cognitive dysfunctions, particularly those observed in Alzheimer's disease, have also been related to alterations of cerebral plasticity(38). Among these mechanisms, neurogenesis has been recently studied. Preliminary data suggest that pregnenolone central infusions dramatically increase neurogenesis.

Taken together these data suggest that pregnenolone can influence cognitive processes, particularly in aging subjects, through a modulation of ACh neurotransmission associated with paradoxical sleep modifications, suggesting a role for neurosteroids in the modulation of hippocampal neurogenesis(38).

Pregnenolone and Treatment of Clinical Depression

One study showed that patients experiencing depressive episode, unipolar or bipolar type had lower cerebrospinal fluid pregnenolone levels versus healthy controls(39). A small study that included both unipolar and bipolar depressed patients suggested improvement in depressive symptoms with pregnenolone (100 mg/day) as compared with placebo(40).

In another study, the effect of the pregnenolone was evaluated on adults with depressive symptoms with bipolar disorder. Subjects were randomized to pregnenolone (titrated to 500 mg/day) or placebo, as add-on therapy, for 12 weeks(41). Depression remission rates were greater in the pregnenolone group (61%) compared with the placebo group (37%), as assessed by the Inventory of Depressive Symptomatology – Self Report (IDS-SR), but not the Hamilton Rating Scale of Depression (HRSD).

Large baseline-to-exit changes in neurosteroid levels were observed in the pregnenolone group but not in the placebo group. In the pregnenolone group, baseline-to-exit change in the Hamilton Rating Scale of Anxiety (HRSA) correlated negatively with changes in allopregnanolone and pregnanolone levels(41). The results suggest that pregnenolone may improve depressive symptoms in patients with BPD, is well tolerated and can be safely administered(41).

Heart Health Support

Policosanol – Potential Cholesterol Lowering Agent

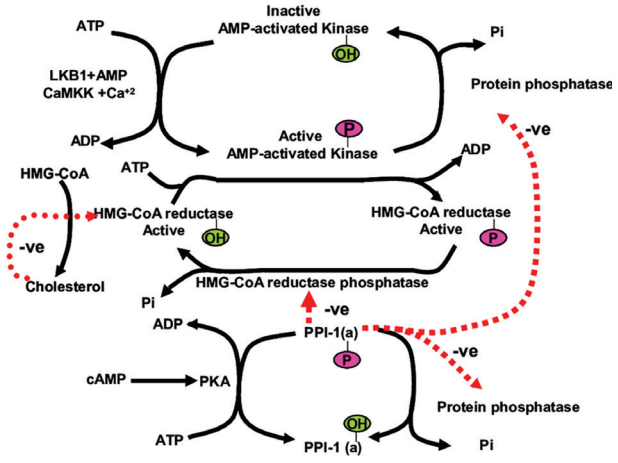
Policosanols are long-chain aliphatic alcohols found in sugar cane, beeswax, alfalfa, rice bran, fruits, nuts and wheat germ. The major phytochemical component is octacosanol. Policosanol was originally developed in Cuba, along with a significant amount of research support for its lipid lowering ability(42). Policosanol was found to reduce low density lipoprotein (LDL) cholesterol, triglycerides, platelet aggregation, endothelial damage and smooth muscle cell proliferation, as well as raise high density lipoprotein (HDL). It was also found to inhibit cholesterol synthesis and enhance cholesterol degradation(42).

Mechanism of Policosanol for Reducing Cholesterol Synthesis

Cholesterol is synthesized by 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, expression of which is regulated by the sterol regulatory element-binding protein 2, and this synthesis is deactivated by AMP-activated protein kinase (AMPK) activation(43). When cholesterol levels are high in the cells, AMPK phosphorylation deactivates phosphorylation of HMG-CoA reductase(43). Therefore, HMG-CoA reductase inhibitors, known as statins, such as atorvastatin, lovastatin, and simvastatin, have been effectively used for lowering LDL cholesterol.

Research suggest that policosanol may target the same statin pathways, inhibiting synthesis of HMG-CoA reductase or stimulating its degradation and by upregulating AMP-kinase phosphorylation, an action that suppresses HMG-CoA activity.

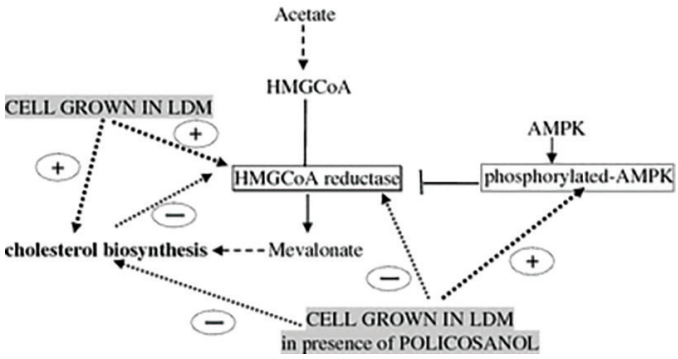
Figure 7: Cholesterol Regulation by HMG-CoA Reductase and AMPK



One study demonstrated that policosanol promotes phosphorylation of AMP-kinase and HMG-CoA reductaste in mouse hepatoma cells and liver cells after administration, providing a possible means by which policosanol might lower blood cholesterol levels(44). Treatment of hepatoma cells with policosanol produced a 2.5-fold or greater increase in the phosphorylation of AMP-kinase and HMG-CoA reductase and increased the phosphorylation of Ca++/calmodulin-dependent kinase kinase (CaMKK), an upstream AMP-kinase kinase(44).

Another study evaluated policosanol's effects against a high-fat and high cholesterol diet for inducing hypercholesterolemia in a rat model(45). The study showed that policosanol supplementation did not affect mRNA expression of HMG-CoA reductase, but it did decrease HMG-CoA reductase activity during high-fat and high-cholesterol–containing diet-induced development of hypercholesterolemia(45). In addition, policosanol supplementation stimulated AMPK phosphorylation, which effectively inhibits cholesterol levels in both the cell and blood by deactivating HMG-CoA reductase. Thus, effects of policosanol supplementation were like those of statin treatments(45).

Figure 8: Regulation of HMG-CoA Reductase by Policosanol



It's suggested that linking of long chain alcohols to activated fatty acids by policosanol in hepatoma cells causes suppression of cholesterol synthesis by increasing cellular AMP levels, causing the phosphorylation of AMPK and HMG-CoA reductase inhibition(44,45). This HMG-CoA reductase inhibition and cholesterol synthesis can trigger LDL receptor expression for uptake of circulating LDL cholesterol into the cells45. The data also show that policosanol and statin supplementation stimulated the expression of both LDL receptor mRNA and protein, indicating that policosanol suppressed the increase in cholesterol levels in the blood by AMPK-induced HMG-CoA reductase deactivation and LDL cholesterol uptake through the LDL receptor(45).

Other potential effects of policosanol that are relevant to heart health include inhibition of platelet aggregation, inhibiting bile acid absorption, blocking the effects of cholesterol on smooth muscle proliferation, inhibition of foam cell formation, inhibiting glycation and preventing lipid peroxidation.

Cholesterol Ester Transfer Protein Inhibition

Another means by which policosanol effects cholesterol production is via the inhibition of the cholesterol ester transfer protein (CETP) (46). Suppression of CETP activity contributes to enhancement of HDL functionality. Studies have shown that longevity and lower incidence of cardiovascular disease (CVD) showed a larger HDL particle size, lower CETP concentration and CETP gene mutation versus controls.

It's proposed that the long aliphatic chains in policosanol interfere with binding of CETP between LDL and HDL(46). The aliphatic chains bind to the cholesterol ester (CE)-binding site in the carboxyl terminus of CETP to form a ternary complex. The carboxyl terminus of CETP contains an active site and binding pocket for CE, forming an amphipathic α-helical region and helps in the transfer process. A component of policosanol in HDL can bind to the amphipathic α-helix and block CE/TG transfer from HDL to LDL by interfering with CETP and LDL binding.

It was showed that CETP inhibitory activity was enhanced by incorporation of policosanol into rHDL(46). Collectively, policosanol may interfere with HDL and LDL binding to CETP to form a hydrophobic channel. Enhanced LDL catabolism and reduced TGs metabolism can be accelerated by inhibition of CETP(46). The inhibition of CETP by policosanol suggests that this natural compound can lower inflammation and slow aging.

Clinical Support for Policosanol and Heart Health
Cholesterol Lowering

In a meta-analysis of 4596 patients from 52 eligible studies, the efficacy and safety of plant sterols and stanols, as well as policosanol for the treatment of heart disease, as measured by a reduction in LDL was evaluated. Weighted estimates of percent change in LDL were -11.0% for plant sterol and stanol esters 3.4 g/day (range 2-9 g/day [893 patients]) versus -2.3% for placebo (769 patients) in 23 eligible studies, compared with -23.7% for policosanol 12 mg/day (range 5-40 mg/day [1528 patients]) versus -0.11% for placebo (1406 patients) in 29 eligible studies(47).

The net LDL reduction in the treatment groups minus that in the placebo groups was greater with policosanol than plant sterols and stanols (-24% versus -10%, $p<0.0001$)47. Policosanol also affected total cholesterol, high-density lipoprotein cholesterol (HDL), and triglyceride levels more favorably than plant sterols and stanols. Policosanol caused a clinically significant decrease in the LDL:HDL ratio(47).

Although plant sterols and stanols were well tolerated, safe and effective, policosanol was found to be more effective than plant sterols and stanols for LDL level reduction and more favorably altered the lipid profile, approaching antilipemic drug efficacy(47).

In another review, the mechanism of action and pharmacology of policosanol was evaluated from placebo-controlled lipid lowering studies including animal models, healthy human models and patients with type II hypercholesterolemia(48). Doses of 10 to 20 mg of policosanol daily lowered total cholesterol by 17% to 21%, lowered LDL by 21 to 29% and raised HDL by 8 to 15%(47).

Additionally, dose of 10 mg/day was shown to be equally effective in lowering total or LDL cholesterol as the same dose of statin drugs – simvastatin and pravastatin(48). At dosages of up to 20 mg per day, policosanol was considered safe and well tolerated, as studies of greater than 3 years of therapy indicated(48).

Blood Pressure Lowering

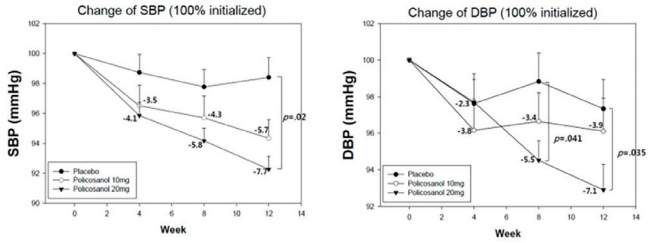
In addition to policosanol's cholesterol lowering effects, policosanol is also beneficial for the treatment of blood pressure. In a randomized, double blind, placebo-controlled trial of 84 healthy participants the long-term effects of policosanol supplementation were evaluated(49). Participants were randomly assigned to three groups receiving 10 mg, 20 mg, or a placebo for 24-weeks.

Based on an average of three measurements of brachial BP, the policosanol 20 mg group showed the most significant reduction in average systolic BP (SBP) from 138 ± 12 mmHg at week 0 to 126 ± 13 mmHg at week 2449. The policosanol 20 mg group also showed significant reductions in aortic SBP and DBP up to 9% and 8%, respectively compared with week zero(49).

Additionally, at week 24, blood renin and aldosterone levels were significantly reduced in the policosanol 20 mg group up to 63% and 42%(49). For the blood lipid profile, the policosanol 10 mg and 20 mg groups showed significant reductions in total cholesterol (TC) of around 8% and 13%, at week 24 compared with week 0(49) The study results suggest that long-term policosanol consumption simultaneously reduces peripheral BP as well as aortic BP accompanied by an elevation in HDL and % HDL versus TC in a dose-dependent manner(49).

In a follow-up study, the short-term effects of policosanol supplementation were evaluated on blood pressure in 84 healthy participants with prehypertension(50). Participants were randomly assigned to receive 10 mg policosanol, 20 mg of policosanol, or placebo daily for 12-weeks(50). Just as in the long-term group, the group that received the 20 mg of policosanol exhibited the most significant effects, including up to 7.7% reduction of average systolic BP (SBP) from 136.3 ± 6.1 mmHg (week 0) to 125.9 ± 8.6 mmHg (week 12)(50).

Figure 9: Changes in SBP and DBP After Supplementation with Policosanol



In addition to the positive effects on blood pressure, the policosanol groups showed significant reductions of TC of 9.6% and 8.6% and LDL of 21% and 18% for 10 mg and 20 mg of policosanol(50). Between group comparisons using repeated measures ANOVA showed that the policosanol (10 mg and 20 mg) groups at 12 weeks had a significant reduction of TC and LDL and elevation of %HDL(50). In conclusion, 12-week consumption of policosanol resulted in significant reductions of peripheral SBP and DBP, aortic SBP and DBP, mean arterial pressure (MAP), and serum TC and LDL with elevation of % HDL(50).

Policosanol and Fat Metabolism

Body Composition

One study showed a secondary benefit of supplementing with policosanol for heart health, reduction of visceral fat mass. In an 8-week study, the effect of policosanol analyzed serum parameters in young non-smoker (YN; n=7, 24.0±2.4 years), young smoker (YS; n=7, 26.3±1.5 years), and middle-aged subjects (MN; n=11, 52.5±9.8 years) who consumed policosanol daily (10 mg/day) for 8 weeks(51). After 8 weeks, daily consumption of policosanol for 8 weeks resulted in lowered blood pressure, reduced serum TG level and elevated HDL. In addition, the total percentage of body fat was reduced in all groups(51). The YN group showed a 30% reduction in fat mass (kg). Although the YN and YS groups showed similar levels of fat mass at week 0, the YS group showed a 1.5-fold higher visceral fat mass than the YN group at week zero(51).

However, after 8 weeks of policosanol consumption, visceral fat mass was reduced by 25% in the YS group(51). The MN group also showed 6 and 9% reduced total fat mass and visceral fat mass, respectively, at week 8 compared with week 0(51). Generally, policosanol consumption reduced blood pressure, body fat percentage, and visceral fat mass, especially in the YS and MN groups.

Brown Adipose Tissue Activation

Brown adipose tissue (BAT) activation can increase fat oxidation and help decrease body weight. BAT dissipates energy in the form of heat by uncoupling mitochondrial respiratory chain and ATP synthesis. Therefore, it's a potential therapeutic target in treatment of metabolic diseases is through its energy expenditure pathway.

Policosanol and its major component octacosanol, have been shown to elicit a positive response on BAT. In a study on mice, policosanol's effectiveness was studied against a high-fat diet (HFD) induced obesity(52). Mice were fed on chow, or HFD, with or without policosanol or octacosanol for four weeks952). HFD-fed mice showed significantly higher body weight and body fat compared with chow-fed mice(52). However, mice fed a HFD treated with octacosanol, or policosanol showed lower body weight gain, lower body fat gain, less insulin resistance and hepatic lipid content(52).

Lower body fat gain after octacosanol or policosanol was associated with increased BAT activity, reduced expression of genes involved in lipogenesis and cholesterol uptake in the liver, and amelioration of white adipose tissue (WAT) inflammation(52).

Moreover, octacosanol and policosanol significantly increased the expression of Ffar4, a gene encoding polyunsaturated fatty acid receptor, which activates BAT thermogenesis(52). Together, these results suggest that octacosanol and policosanol ameliorate diet-induced obesity and metabolic disorders by increasing BAT activity and improving hepatic lipid metabolism(52).

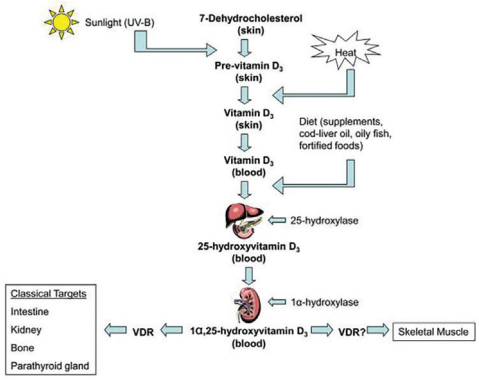
Endocrine & Antioxidant Support System

Vitamin D – Testosterone Support & Muscle Function

Vitamin D is a group of fat-soluble secosteroids, which increases the intestinal absorption of minerals calcium, magnesium, and phosphate. The most important compound in this group is vitamin D3, also known as cholecalciferol or 25-hydroxyvitamin D3 (25(OH)D). Vitamin D can be obtained from the diet or from synthesis in the lower layers of the epidermis of the skin via a chemical reaction that is dependent on sun exposure (UVB radiation)(53).

Vitamin D is inactive until activation by a two-step protein enzyme hydroxylation reaction, the first step in the liver and the second in the kidneys. Vitamin D is not considered an essential vitamin since it can be obtained in adequate amounts when exposed to sufficient sunlight(53).

Figure 10: Synthesis of Vitamin D3



With activation of vitamin D, the active form calcitriol, can then bind to the vitamin D receptor (VDR) located in various target tissues in the nuclei. The VDR is therefore a transcription factor that modulates gene expression. VDRs belong to the nuclear receptor superfamily of steroid/thyroid hormone receptors. VDR expression can have a direct effect on muscle strength, mass and function, thyroid function, bone metabolism and density, and even testosterone and androgen levels(53).

Vitamin D and Muscle Strength, Size and Function

Low levels of vitamin D status have been associated with lower testosterone levels, muscle weakness and muscle wasting, increasing muscle strength via vitamin D supplementation has been investigated. Physical power production requires the generation of force from anaerobic metabolism, produced from type II muscle fibres. Type II fibers have a limited capacity for aerobic metabolism with greater glycolytic and anerobic capability. In a small uncontrolled study, an increase in relative fiber composition and in fiber area of type II muscle fibers in muscle biopsies from elderly women after treatment with vitamin D and calcium for 3-6 months(54).

A randomized, controlled study found that treatment of 48 elderly stroke survivors with 1000 IU of vitamin D2 daily significantly increased mean type II muscle fiber diameter and percentage of type II fibers over a 2-year period(55). There was also a correlation between serum 25(OH)D level and type II muscle fiber diameter both at baseline and after two years of follow-up(55).

In a random, placebo-controlled study on indoor judo athletes, the acute effects of vitamin D3 supplementation was observed56. Subjects were randomly assigned to receive 150,000 IU of vitamin D3 or placebo. Athletes took part in regular training regimes, then blood samples were taken followed by isokinetic concentric quadriceps and hamstring muscle function test(56). The group that received the vitamin D3 demonstrated significant increase in serum 25(OH)D levels and muscle strength, while no significant differences were noticed in the placebo group(56).

In a systematic meta-analysis, muscle strength and vitamin D levels were reviewed for upper and lower body strength. Data from 310 adults, from ages 21 to 31 were evaluated57. Trials ranged from 1-month to 6-months and dosages differed from 4000 IUs per day to 60,000 IU per week. Results indicated that vitamin D supplementation significantly increased muscle strength in both upper and lower body limbs(57). A similar meta-analysis in 284 athletes, also showed vitamin D supplementation positively affected lower limb muscle strength(58).

Testosterone Support

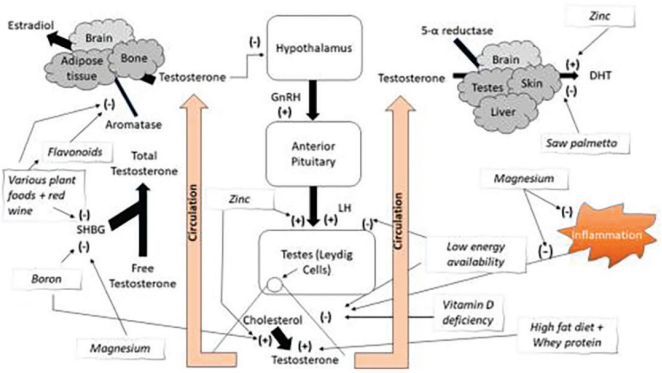
Vitamin D has been positively associated with testosterone levels. Vitamin D supplementation is a potential option to maintain vitamin D status; maintain and increase testosterone concentrations. The VDR is present in the Leydig cells on the testis, pituitary gland, and hypothalamus. Studies have shown that men with vitamin D deficiency have exhibited significantly lower testosterone levels, compared to men with normal vitamin D concentrations.

In a population study in middle-aged, older Chinese men, it was observed that lower 25(OH) D levels was significantly associated with lower testosterone, sex hormone binding globulin (SHBG), LH and FSH levels(59). This study shows an association between 25(OH)D status and hypogonadism in a large population(59).

In addition to an association between vitamin D and hypogonadism, there may also be an association with BMI, adiposity and insulin resistance. Previous studies have indicated that obesity is associated with low 25(OH)D levels, and that vitamin D may also regulate adipose tissue(60). VDR is observed in adipocytes and vitamin D deficiency can up-regulate parathyroid hormones, thereby increasing free intracellular calcium in adipocytes, blunting the lipolytic response to catecholamines and increasing lipogenesis(60).

A study evaluated if vitamin D supplementation influences testosterone levels in overweight men. Participants received either 3332 IU of vitamin D daily for 1 year or placebo, alongside a weight reduction program(61). Initially concentrations of 25(OH)D were low, and as were testosterone values in both groups. In the vitamin D supplemented group, results showed circulating levels of 25(OH)D increased by 53.5 nmol/l compared to no change in placebo group(61). The total testosterone levels, bioactive testosterone and free testosterone levels significantly increased, whereas no significant changes were observed in placebo group(61).

Figure 11: The Effect of Micronutrients on Testosterone Circulation



Zinc – Role in Testosterone Synthesis

Zinc is a mineral that interacts with many biochemical pathways in the body including the endocrine system. It also has an important role in thyroid hormone metabolism, immune function and modulating the inflammatory process. Zinc is also important in the conversion of testosterone to dihydroxy testosterone (DHT).

DHT is converted from testosterone by 5α-reductase in the cytoplasm of the cell. DHT is primarily found in peripheral tissues such as prostate, skin, hair follicles, and the liver. DHT is thought to have a stronger androgenic affect than testosterone due to its four-times greater binding affinity for the androgen receptor than testosterone and a three-times slower dissociation rate than testosterone(62). DHT has a vital role in the sexual development of males and sexual differentiation of organs and promotes prostate growth.

Zinc is also involved indirectly in testosterone production. Zinc is required for function of angiotensin converting enzyme (ACE), a zinc-dependent dicarboxypeptidase, which has a zinc binding site in its domain62. ACE increases LH production in the pituitary, impacting androgen production. Zinc deficiency can impair testosterone synthesis and is correlated with reductions in testosterone concentrations(62).

Zinc deficiency appears to be related to hypogonadism; by impairing testosterone synthesis and reducing testosterone concentrations. Several studies have shown that zinc supplementation can restore testosterone concentrations to their normal physiological range(63,64).

Zinc – Role in Testosterone Synthesis

One study examined the effect of zinc supplementation on total testosterone (TT) and free testosterone (FT) in healthy young adults before and after an exhaustive exercise protocol(81). All subjects received 3mg/kg/day oral zinc sulfate for 4-weeks. Zinc supplementation increased both TT and FT concentrations prior to and following exhaustive bicycle exercise protocol compared with pre-supplementation results(65).

In a similar study, the effects of zinc supplementation were observed in young male wrestlers after exhaustion exercise. All subjects were supplemented with 3 mg/kg/day of oral zinc sulfate for 4-weeks. Both resting and exhaustion TT and FT levels following 4-week zinc supplementation were found significantly higher than the levels (both resting and exhaustion) measured before zinc supplementation(66).

Boron – Regulation of Sex Hormones

Boron is a trace mineral that can be obtained from a diet rich in fruits, vegetables, nuts and legumes. Boron plays roles in metabolism, growth and maintenance of bones, regulates inflammation and antioxidant enzymes and influences regulation of hormones. Deficiency of boron can result in depressed growth and reduction in steroid hormone concentrations.

In fact, after only 1-week of boron supplementation of 10 mg/d in healthy males, a significant increase in FT from 11.83 pg/mL to 15.18 pg/mL and significant decrease in estrogen (E2) from 42.33 pg/ml to 25.81 pg/mL was observed(67). In addition, all inflammatory biomarkers decreased including IL-6, CRP and TNF-α, while levels of DHT, cortisol and vitamin D increased slightly(67).

The significant decrease in plasma E2 after 1 week of boron supplementation suggests a higher rate of conversion of TT to FT in the testosterone metabolic pathway. In support, the ratios of FT/T, T/E2, and FT/E2 were all significantly increased, indicating boron had an androgen amplifier effects: (1) FT/T (pg/mL/ng/mL) increased from 3.62 to 4.66; (2) T/E2 (ng/mL) rose from 91.68 to 148; and (3) FT/E2 (ng/mL) from 0.31 to 0.67(67).

It is well known that approximately 98% of testosterone molecules are bound to proteins in the blood, mostly to SHBG and are not bioavailable. It's suggested that boron may help to uncouple SHBG from testosterone, displacing hormones from their plasma carriers(68). Thus, the elevation of unbound FT seen with boron supplementation may have significant beneficial effects on testosterone regulation.

Prevention of Vitamin D Deficiency

Boron has been shown to increase serum levels of 25(OH)D in vitamin D deficient human studies. In a clinical trial, in which middle-aged men and women were placed on a low-boron diet, which was also marginal in magnesium and copper status, for 63 days (0.23 mg B/2000 kcal), 25(OH)D3 rose significantly after boron supplementation (3 mg/d as sodium borate) for an additional 49 days69. Levels of 25(OH) D3 rose from an average of 44.9 nM after the 63 days of boron deprivation to 62.4 nM after the 49 days of boron repletion, a 39% increase69. Increasing vitamin D availability may also help contribute to enhancing testosterone production.

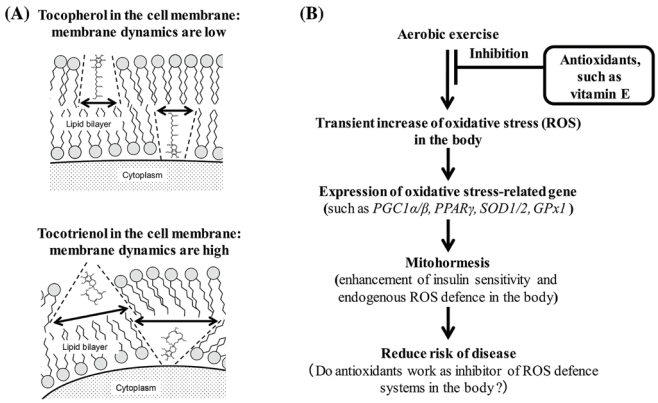
Vitamin E – Antioxidant Support

Vitamin E is a group of fat-soluble compounds including tocopherols and tocotrienols. Vitamin E cannot be synthesized in the body, so it must be obtained through the diet and or via supplementation. Vitamin E has been shown to have some benefits on human health, including cardiovascular, anti-lipidemic, anti-hypertensive, anti-inflammatory and neuroprotective.

Vitamin E is considered an antioxidant which may help protect cell membranes from reactive oxygen species (ROS), acting as a free radical scavenger and protecting against oxidative stress(70). Oxidative stress is the state of balance between oxidants/antioxidants called a redox balance. Redox signalling can result in the stress response in the presence of high concentrations of ROS or can signal second messengers that modulate receptor agonists of cells such as cytokines, growth factors and hormones to activated cells to generate ROS as a regulatory or cytotoxic event(70).

Vitamin E exists in all cell membranes and is present in the whole body at the highest concentration. Therefore, vitamin E is thought to play a significant role in the regulation of the redox interactions in the body. Vitamin E's tocopherols have weak bonds that allow them to donate hydrogen atoms to the peroxyl radicals and other free radicals, minimizing their damaging effects.

Figure 12: Effect of Vitamin E on Oxidative Stress



Vitamin E and Inflammatory Response

In addition to vitamin E's role in redox balance, it also appears to have anti-inflammatory regulation properties.

In a single-blind placebo-controlled design with randomization, young healthy men received an oral supplementation with either a combination of ascorbic acid (500 mg/day) and α-tocopherol (400 IU/day) or placebo(71). After 28 days of supplementation, the subjects performed 3 h of dynamic two-legged knee-extensor exercise at 50% of their individual maximal power output. Muscle biopsies from vastus lateralis were obtained at rest (0 h), immediately post exercise (3 h) and after 3 h of recovery (6 h)(71).

In both the control and treatment, skeletal muscle IL-6 mRNA and protein levels increased between 0 and 3 h. In contrast, the net release of IL-6 from the leg, which increased during exercise with a peak at 3.5 h in control, was completely blunted during exercise in treatment.

Arterial plasma IL-6 concentration from 3 to 4 h, when the arterial IL-6 levels peaked in both groups, was ~50% lower in the treatment group compared to control. Moreover, plasma interleukin-1 receptor antagonist (IL-1ra), CRP and cortisol levels all increased after the exercise in control, but not in treatment(71). Results show that supplementation with vitamins C and E attenuated the systemic IL-6 response to exercise primarily via inhibition of the IL-6 protein release from the contracting skeletal muscle.

In addition, to reducing exercise inflammation, vitamin E has also shown effective for reducing inflammation in patients with diabetic nephropathy (DN). In a randomized double-blind placebo-controlled clinical trial was carried out among 60 patients with DN taking vitamin E supplement(72). Patients were randomly allocated into two groups to take either 1200 IU per day of vitamin E supplements or placebo for 12 weeks. Fasting blood samples were obtained at the onset of the study and after 12-week intervention to assess biomarkers of kidney injury, inflammation, and oxidative stress(72). After 12 weeks of intervention, compared with the placebo, vitamin E supplementation resulted in a significant increase in serum vitamin E levels and a significant decrease in urine protein and protein-to-creatinine ratio. In addition, a significant reduction in serum TNF-α., matrix metalloproteinase-2, matrix metalloproteinase-9, malondialdehyde, AGE products, and insulin concentrations was seen after the administration of vitamin E supplements compared with the placebo. Overall, high-dose vitamin E supplementation for 12 weeks among DN patients had favorable effects on biomarkers of kidney injury, inflammation, and oxidative stress(72).

Gut Microbiome Absorption Complex

Chaga Mushroom Extract – Prebiotic & Detoxifier

Chaga, also known as Inonotus obliquus, is a parasitic fungus mainly of birch trees, and has numerous biological and medicinal properties, which have been commonly used in folk medicine in Northern Europe, China, Russia, and Korea, for a wide range of purposes (73).

Chaga is composed of various chemical components including polysaccharides, triterpenoids, polyphenols, beta-glucans, alpha-glucans, and melanin, it has been proven to possess anti-cancer, anti-inflammatory, anti-viral, antioxidant, hypoglycemic, and hypolipidemic activity(73).

Within this formulation, Chaga will be primarily used for their prebiotic properties, detox, and antioxidant activity.

Prebiotic for Gut Health

Melanin, one of the active components from chaga, has been shown to be an effective prebiotic for the positive bifidobacteria activation because of its ability to serve a variety of functions(74). Melanin contains proteins and polysaccharides; melanin can be used as an additional source of carbon and nitrogen for the bifidobacteria.

In addition, as an antioxidant, it can change the oxidation-reduction potential of the culture media and create conducive conditions for positive bacteria growth. Melanin can also associate with bifidobacteria, which helps their activity(74).

Chaga has also been successfully used to ameliorate the negative effects of DSS colitis model in mice, and the polysaccharides of this fungus have shown a positive regulatory effect on the microbiota(75).

In a model of mice with chronic pancreatitis, the gut microbiota profile, compromised by the disease, was partially restored by the administration of I. obliquus polysaccharides, which led to increased diversity and richness of gut microbiota and improved the clinical condition of the mouse(76).

The changes in glutathione peroxidase (GSH-PX), total antioxidant capacity (TAOC), tumor necrosis factor alpha (TNF-α), transforming growth factor beta (TGF-β), lipase and trypsin levels were measured, and gut microbiota composition and diversity were analyzed by high throughput sequencing. The chaga treatment increased GSH-PX and TAOC levels, and decreased TNF-α, TGF-β, lipase and trypsin levels in the pancreatitis mice(76). IOP increased the proportion of Bacteroidetes and decreased that of Firmicutes at phylum level. Bacteroidetes was found positively correlated with GSH-PX and TAOC, and Firmicutes correlated with TNF-α, TGF-β, and lipase. In conclusion, administration of IOP could regulate gut microbiota composition and diversity to a healthy profile in mice with pancreatitis, and some bacterial phylum significantly correlated with characteristic parameters(76).

Detox Benefits

Chaga has shown protective effects against the oxidative stress in liver induced by tert-butyl hydroperoxide in primary-cultured rat hepatocytes. This property maybe due to its ability to scavenge the free radicals and thereby it inhibits the leakage of liver marker enzymes because of liver damage(77). The high total phenolic contents maybe the reason for its strong antioxidant activity(77).

BioGenixUSA

Bacillus *coagulans* – Gut Health & Absorption

Bacillus coagulans are considered probiotics, essentially live bacteria that are beneficial for improving gut and digestive health. Probiotics work by encouraging the growth of 'good' or beneficial bacteria, this helps keep the harmful, 'bad' bacteria from over taking the gut. These good bacteria help encourage the production of immune cells and natural anti-bodies that can bolster the immune system, reduce inflammation and help improve nutrient absorption.

Supplementation with probiotics has been shown to have many healthful benefits and outcomes including modulation of the production of various bacterial species in the gut, bolster gut barrier function, and improvement in many properties of the human immune system(78).

Probiotics can also limit pathogen adhesion to host tissue and modulate the production of different metabolites such as vitamins, short-chain fatty acids, and molecules that act as neurotransmitters involved in gut-brain communication(79). Beyond benefiting physiological systems, probiotics have been shown to impact the absorption and production of key nutrients, including minerals, carbohydrates, protein, cholesterol, and various digestive enzymes(79,80,81).

Protein and Amino Acid Absorption

Bacillus coagulans GBI-30, 6086 also known as the trademarked ingredient, BC30 is a lactic acid producing, spore-forming bacterial species. Due to the formation of spores, BC30 can withstand the acidic environment of the stomach to reach the intestine where it germinates. Once active in the small intestine after germination, it has been shown to aid the digestion of carbohydrates and proteins.

Consumption of BC30 creates an intestinal environment that is not hospitable to various pathogens, creating a healthier and more efficient intestinal tract that is better able to utilize nutrients that have been consumed81. In addition to better utilization of consumed foods, BC30 increases the benefits of prebiotics by promoting populations of beneficial bacteria as well as the production of short-chain fatty acids essential for the health of cells lining the gut(82).

BC30 produce digestive enzymes in the gut, including proteases – digestive enzymes that breakdown protein. Proteases have been shown to increase amino acid concentrations in the blood when administered with protein. In addition, BC30 enhances the health of the cells of the gut lining by decreasing inflammation, thereby improving nutrient absorption through optimum development of the absorptive area of the villi.

In a randomized, double-blind, placebo-controlled, crossover human clinical trial. Healthy subjects consumed either whey protein or whey protein plus 1 billion CFU BC30, for 2 weeks(81). BC30 increased absorption of amino acids including BCAAs (leucine, isoleucine, valine), as well as amino acids involved in blood flow regulation (citrulline), and recovery (glutamine)(81).

Another study examined the impact of adding BC30 to a 25 g dose of milk protein concentrate (MPC) on post-prandial changes in blood amino acids concentrations(82). Participants were instructed to track their dietary intake and ingest a daily 25 g dose of MPC with or without BC30. Essential Amino Acids (EAA) and Branched Chain Amino Acids (BCAA) all had significantly greater concentrations, and faster absorption with MPC + BC30 compared to just MPC(82).

Performance and Recovery Benefits

In a clinical trial, healthy resistance-trained individuals consumed either 20 g of casein protein or 20 g of casein protein plus 500 million BC30 twice daily in combination with a resistance training program, consisting of full body workouts 4 times per week for 8-weeks(83). The addition of BC30 showed a trend to increase vertical jump power in comparison to casein protein alone, it was also shown to have a beneficial effect on peak power and fat mass(83).

In a follow-up study, the effects of supplementation of both BC30 and protein, was evaluated on a group of trained males. Twenty-nine recreationally trained males were assigned to consume either 20 g of casein (PRO) or 20 g of casein plus probiotic (1 billion CFU) BC30 in a crossover, diet-controlled design(84). Participants performed a muscle damaging one legged exercise bout and perceived recovery, muscle soreness, strength and power, as well as markers of muscle damage and hypertrophy were measured post exercise(84).

PRO + BC30 significantly increased recovery at 24 and 72 h, and decreased soreness at 72 h post exercise in comparison to PRO. Perceptual measures were confirmed by increases in CK with PRO + BC30 showing a trend towards reduced muscle damage(84). The muscle-damaging exercise resulted in significantly increased muscle swelling and Blood Urea Nitrogen levels in both conditions with no difference between groups. The strenuous exercise significantly reduced athletic performance in PRO group whereas PRO + BC30 maintained performance(84).

BioGenixUSA

Gut Health

In a randomized double-blind placebo controlled dual site clinical trial compared a BC30 to placebo regarding effects on gastrointestinal symptoms in adults with post-prandial intestinal gas-related symptoms (abdominal pain, distention, flatulence) but no gastrointestinal (GI) diagnoses to explain the symptoms(85). Subjects were given either BC30 or placebo, daily and were evaluated every two-weeks over a four-week period.

The group receiving the BC30 achieved significant improvements in Gastrointestinal Symptom Rating Scale (GSRS) including abdominal pain, distention, bloating and gas. BC30 was effective in improving the quality of life and reducing gastrointestinal symptoms in adults with post prandial intestinal gas related symptoms and no GI diagnoses(85).

In another randomized, double-blind, placebo-controlled clinical trial, subjects either received placebo or BC30 once daily for 8-weeks. Self-assessments of the severity of IBS symptoms (abdominal pain and bloating) were recorded every day for 8 weeks(86). No treatment-related adverse events or serious adverse events were reported during the 8-week study period. BC30 can be considered a safe and effective option for the relief of abdominal pain and bloating for patients with IBS.

Nano ^{2} Delivery Technology

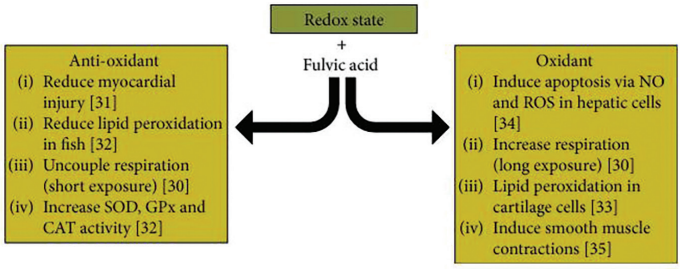
Fulvic Acid – Absorption & Chelation

Fulvic acid is a subclass of diverse compounds known as humic substances, which are by-products of organic degradation from microorganisms. Fulvic acid consist of small molecular weight, hydrophilic, carboxylic-containing molecules including a mixture of covalently linked phenolic, quinoid and benzene carboxylic acid compounds(87). Depending on where the fulvic acid is derived can change its composition of oxygen, nitrogen, aromatic ring, and carbon content. Fulvic acid is soluble at all pHs, has a small molecular weight and a high oxygen content8(88)

Anti-Inflammatory & Pro-Inflammatory

There is some evidence to suggest that fulvic acid can work as both an anti-inflammatory and a pro-inflammatory. In human monocyte models, fulvic acid was shown to reduce TNF-α expression after exposure to the endotoxin Lipopolysaccharide (LPS)(88). Fulvic acid was also shown to reduce COX2 and prostaglandin E2 (PGE2) secretion after homocysteine stimulation in primary human monocytes. Additionally, it can blunt interleukin release.

Figure 13: Redox Balance of Fulvic Acid



Fulvic acid may help with redox balance, allowing it to induce oxidation pathways – inducing apoptosis via NO and ROS, increase respiration, lipid peroxidation and inducing smooth muscle contractions; as well as antioxidant pathways by reducing lipid peroxidation, uncoupling respiration, and increasing SOD, glutathione and CAT activity(98).

Gut Health & Nutrient Absorption

Fulvic acid has been shown to influence the soil microbe composition and is able to conjugate or bind itself to various minerals increasing uptake in plants(89). Fulvic acid is therefore suggested to improve the gut flora, nutrient and mineral absorption and may help heal the gut.

Fulvic acid was shown to influence the bioavailability of heavy metals in animal models. Fulvic acid can increase the absorption of copper in porcine oviductal epithelial cells and reduce its toxicity via chelation(89). Fulvic acid has been shown to mediate drug delivery in rats as well. A low bioavailable drug, when conjugated to fulvic acid, increased absorption across the rat intestinal sac along with concentrations of the drug in blood plasma.

Another study showed the interaction between fulvic acid and transferrin (TF), a potential delivery agent for anti-cancer drugs(90). Results showed that fulvic acid binds to TF and forms a new complex. This study indicated a mechanism of the interaction between fulvic acid and TF, which may provide information for possible design of methods to deliver drug molecules via transferrin to target tissues and cells effectively(90).

The Complete Edge System – CE6

CE6 delivers a complete edge, when it comes to regenerative support for slowing or reversing the male aging process. As discussed, this formulation will support male endocrinology system using multiple complexes including:

Muscle Gene Regulation Complex

- DAA
- Shilajit Extract (50% Fulvic Acid)
- Phosphatidyl Serine
- DHEA
- Pregnenolone - The Steroid Hormone Precursor

BioGenixUSA

Heart Health Support Complex

- Policosanol – Potential Cholesterol Lowering Agent

Endocrine & Antioxidant Support System

- Vitamin D – Testosterone Support & Muscle Function
- Zinc – Role in Testosterone Synthesis
- Boron – Regulation of Sex Hormones
- Vitamin E – Antioxidant Support

Gut Microbiome Absorption Complex

- Chaga Mushroom Extract
- Bacillus Coagulans – Gut Microbiome & Absorption

Gut Microbiome Absorption Complex

- Improvements in Energy & Focus
- Improvements in Metabolism
- Improvements in Muscle Mass
- Maintenance of Bone Density
- Improvements in Free Testosterone Levels
- Improvements in Heart Health

BioGenixUSA

Scientific References

1. Maddalena Di Fiore M, et al. D-Aspartic Acid in Vertebrate Reproduction: Animal Models and Experimental Designs. Biomolecules. 2019. 9(9): 445.

2. Usiello A, et al. New Evidence on the Role of D-Aspartate Metabolism in Regulating Brain and Endocrine System Physiology: Preclinical Observations to Clinical Applications. Int J Mol Sci. 2020. 21(22): 8718.

3. D'Aniello, et al. D-Aspartate, a Key Element for the Improvement of Sperm Quality. Adv Sex Med. 2012. 45–53

4. Topo E, et al. The role and molecular mechanism of D-aspartic acid in the release and synthesis of LH and testosterone in humans and rats. Reprod Biol Endocrinol. 2009. 7: 120.

5. Willoughby DS, et al. 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. Nutrition Res. 2013. 33(10): 803-10.

6. Melville GW, et al. The effects of D-Aspartic Acid supplementation in resistance trained men over training period: A randomized controlled trial. PLoS One. 2017. 12(8): e0182630.

7. Das A, et al. The human skeletal muscle tanscriptome in response to oral shilajit supplementation. J Med Food. 2016. 19(7): 701-9.

8. Bhattacharyya S, et al. Beneficial effect of processed shilajit on swimming exercise induced impaired energy status of mice. Pharmacologyonline. 2019. 1: 817-25.

9. Das A, et al. The human skeletal muscle transcriptome in response to oral shilajit supplementation. J Med Food. 2016. 19(7): 701-9.

10. Keller JL, et al. The effects of Shilajit supplementation on fatigue-induced decreases in muscular strength and serum hydroxyproline levels. J Int Soc Sports Nut. 2019. 16:3.

11. Mishra RK, Jain A, Singh Sk. Profertility effects of Shilajit on cadmium-induced infertility in male mice. Andrologia. 2018. 50(8).

12. Pandit S, et al. Clinical evaluation of purified shilajit on testosterone levels in healthy volunteers. Andrologia. 2016. 48(5): 570-5.

13. Biswas TK, et al. Clinical evaluation of spermatogenic activity of processed shilajit in oligospermia. Andrologia. 2010. 42(1): 48-56.

14. Starks MA, et al. The effects of phosphatidylserine on endocrine response to moderate intensity exercise. J Int Sports Nutr. 2008. 5:11.

15. Monteleone P, et al. Effects of phosphatidylserine on the neuroendocrine response to physical stress in humans. Neuroendocrinology. 1990. 52(3): 243-8.1992. 42(4): 385-8.

16. Monteleone P, et al. Blunting by chronic phosphatidylserine administration of the stress-induced activation of the hypothalamo-pituitary-adrenal axis in healthy men. Eur J Clin Pharmacol. 1992. 42(4): 385-8.

17. Fahey T, Pearl MS. The hormonal and perceptive effects of phosphatidylserine administration during two weeks of resistive exercise-induced overtraining. Bio Sport. 1998. 15(3):135-144.

18. Kingsley MI, et al. Effects of phosphatidylserine on exercise capacity during cycling in active males. Med Sci Sports Exerc. 2006. 38(1): 64-71.

19. Fernholz KM, Seifert JG, Bacharach DW, Burke ER, Gazal O. The Effects of Phosphatidyl Serine on Markers of Muscular Stress in Endurance Runners [abstract]. Med Sci Sports Exerc. 2000. 32: S321.

20. Benton D, et al. The influence of phosphatidylserine supplementation on mood and heart rate with an acute stressor. Nutr Neurosci. 2001. 4(3): 169-78.

21. Hellhammer J, et al. Effects of soy lecithin phosphatidic acid and phosphatidylserine complex (PAS) on the endocrine and psychological response to mental stress. Stress. 2004. 7(2): 119-26.

22. Hellhammer J, et al. A soy-based phosphatidylserine/phosphatidic acid complex (PAS) normalizes the stress reactivity of the hypothalamus-pituitary-adrenal-axis in chronically stressed male subjects: a randomized, placebo-controlled study. Lipids Health Dis. 2014. 13: 121.

23. Crook TH, et al. Effects of phosphatidylserine in age-associated memory impairment. Neurology. 1991. 41(5): 644-9.

24. More M, et al. Positive effects of soy lecithin-derived phosphatidylserine plus phosphatidic acid on mood, memory, cognition, daily functioning and mood in elderly patients with Alzheimer's disease and dementia. Adv Ther. 2014. 31(12): 1247-62.

25. Prough RA, et al. Novel Mechanism for DHEA Action. J Mol Endocrinol. 2016. 56(3): R139-55.

26. Miller WL, Auchus RJ. The molecular biology, biochemistry and physiology of human steroidogenesis and its disorders. Endocr Rev. 2011. 32(1): 81-151.

27. Traish A, et al. Dehydroepiandrosterone (DHEA) – a precursor steroid or an active hormone in human physiology. J Sex Med. 2011. 8(11) 2960-82.

28. Liu TC, et al. Effect of acute DHEA administration on free testosterone in middle-aged and young men following high-intensity interval training. Eur J App Physiol. 2013. 113(7): 1783-92.

29. Morales AJ, et al. The effect of six months treatment with a 100 mg daily dose of dehydroepiandrosterone (DHEA) on circulating sex steroids, body composition and muscle strength in age-advanced men and women. Clin Endocrinol. 1998. 49(4):421-32.

30. Morales AJ, et al. Effects of replacement dose of DHEA in men and women of advancing age. J Clin Endocrinol Metab. 1994. 78(6): 1360-7.

31. Murugnan S, et al. The neurosteroid pregnenolone promotes degradation of key proteins in the innate immune signaling to suppress inflammation. J Biol Chem. 2019. 294(12): 4596-607.
32. Miller WL. Steroid hormone synthesis in mitochondria. Mol Cell Endocrinol. 2013. 379(1-2): 62-73.
33. Ferri C, Fioranelli M. The Role of Pregnenolone in Inflammatory Degenerative Brain Disease. Interdiscip J Microinflammation. 2014. 1:121.
34. Kilts JD, et al. Neurosteroids and self-reported pain in veterans who served in the US Military after Sept 11, 2001. Pain Med. 2010. 11(10): 1469-76.
35. Naylor JC, et al. Effect of Pregnenolone vs Placebo on Self-reported chronic low back pain among US Military Vets. JAMA Netw Open. 2020. 3(3): e200287.
36. Serra M, et al. Social isolation-induced decreases in both the abundance of neuroactive steroids and GABA(A) receptor function in rat brain. J Neurochem. 2000. 75(2):732-40.
37. Vallée M, et al. Neurosteroids: Deficient cognitive performance in aged rats depends on low pregnenolone sulfate levels in the hippocampus. PNAS. 1997. 94(26): 14865-70.
38. Mayo W, et al. Pregnenolone sulfate and aging of cognitive functions: behavioral, neurochemical and morphological investigations. Horm Behav. 2001. 40(2): 215-7.
39. George MS, et al. CSF neuroactive steroids in affective disorders: pregnenolone, progesterone and DBI. Bio Psychiatry. 1994. 35(10): 775-80.
40. Osuji J, et al. Pregnenolone for cognition and mood in dual diagnosis patients. Psychiatry Res. 2010. 178(2): 309-12.
41. Sherwood Brown E, et al. A Randomized, double-blind, placebo-controlled trial of pregnenolone for bipolar depression. Neuropsychoparmacology. 2014. 39(12): 2867-2873.
42. Policosanol – An Overview. Complementary and Alternative Therapies and the Aging Population. 2009.
43. Burg JS, Espenshade PJ. Regulation of HMG-CoA reductase in mammals and yeast. Prog Lipid Res. 2011. 50(4): 403-10.
44. Bananerjee S, et al. Activation of AMP-Kinase by policosanol requires peroxisomal metabolism. Lipids. 2011. 46(4): 311-21.
45. Da-Eun N, et al. Policosanol Attenuates Cholesterol Synthesis via AMPK Activation in Hypercholesterolemic Rats. J Med Food. 2019. 22(11).
46. Cho K, et al. Cuban sugar cane wax acid and policosanol showed similar atheroprotective effects with inhibition of LDL oxidation and cholesteryl ester transfer via enhancement on high-density lipoproteins functionality. Cardiovasc Ther. 2019.

47. Chen JT, et al. Meta-analysis of natural therapies for hyperlipidemia: plant sterols and stanols versus policosanol. Pharmacotherapy. 2005. 25(2): 171-83.
48. Gouni-Berthold I, Berthold H. Policosanol: clinical pharmacology and therapeutic significance of a new lipid-lowering agent. Am Heart J. 2002. 143(2): 356-65.
49. Kim S, et al. Long term consumption of Cuban policosanol lowers central and brachial blood pressure and improves lipid profile with enhancement of lipoprotein properties in healthy Korean participants. Front Physiol. 2018. 9: 412.
50. Park H, et al. Short-term consumption of Cuban policosanol lowers aortic and peripheral blood pressure and ameliorates serum lipid parameters in healthy Korean participants: randomized, double-blind, and placebo-controlled study. Int J Environ Res Pub Health. 2019. 16(5): 809.
51. Jae-Yong K, et al. Consumption of policosanol enhances HDL functionality via CETP inhibition and reduces blood pressure and visceral fat in young and middle-aged subjects. Int J Mol Med. 2017. 39(4):889-99.
52. Sharma R, et al. Octacosanol and policosanol prevent high-fat diet-induced obesity and metabolic disorders by activating brown adipose tissue and improving liver metabolism. Sci Rep. 2019. 9: 5169.
53. Cegla L, et al. Vitamin D and it's role in skeletal muscle. Curr Opin Clin Nutr Metab Care. 1296): 628-33.
54. Sorenson OH, et al. Myopathy in bone loss of ageing: improvement by treatment with 1alpha-hydroxycholecalciferol and calcium. Clin Sci. 1979. 56(2): 157-61.
55. Sato Y, et al. Low-dose vitamin D prevents muscular atrophy and reduces falls and hip fractures in women after stroke: a randomized controlled trial. Cerebrovasc Dis. 2005. 20(3): 187-92.
56. Wyon AM, et al. Acute Effects of Vitamin D3 Supplementation on Muscle Strength in Judoka Athletes: A Randomized Placebo-Controlled, Double-Blind Trial. Clin J Sport Med. 2016. 26(4): 279-84.
57. Tomlinson PB, et al. Effects of vitamin D supplementation on upper and lower body muscle strength levels in healthy individuals. A systematic review with meta-analysis. J Sci Med Sport. 2015. 18(5): 575-80.
58. Zhang L, et al. Effect of vitamin D supplementation on upper and lower limb muscle strength and muscle power in athletes: A meta-analysis. PLoS One. 2019. 1494): e0215826.
59. Wang N, et al. Vitamin D is associated with testosterone and hypogonadism in Chinese men: results from a cross sectional SPECT-China study. Reprod Biol Endocrinol. 2015. 13: 74.

60. Carrillo AE, et al. Impact of vitamin D supplementation during a resistance training intervention on body composition, muscle function and glucose tolerance in overweight and obese adults. Clin Nutr. 2013. 32(3): 375-81.
61. Pilz S, et al. Effect of vitamin D supplementation on testosterone levels in men. Horm Metab Res. 2011. 43(3): 223-5.
62. Zamir A, et al. Manipulation of dietary intake on changes in circulating testosterone concentrations. Nutrients. 2021. 13(10): 3375.
63. Santos HO, et al. Use of medicinal doses of zinc as a safe and efficient coadjutant in the treatment of male hypogonadism. Aging Male. 2020. 23(5): 669-78.
64. Prasad AS, et al. Zinc status and serum testosterone levels of healthy adults. Nutrition. 1996. 12(5): 344-8.
65. Kilic M. Effect of fatiguing bicycle exercise on thyroid hormone and testosterone levels in sedentary males supplemented with oral zinc. Neuro Endocrinol Lett. 2007. 28(5): 681-5.
66. Kilic M, et al. The effect of exhaustion exercise on thyroid hormones and testosterone levels of elite athletes receiving oral zinc. Neuro Endocrinol Lett. 2006. 27(1-2): 247-52.
67. Naghii MR, et al. Comparative effects of daily and weekly boron supplementation on plasma steroid hormones and proinflammatory cytokines. J Trace Elem Med Biol. 2011. 25(1): 54-8.
68. Bello M, et al. Several effects of boron are induced by uncoupling steroid hormones from their transporters in blood. Med Hypotheses. 2018. 118: 78-83.
69. Nielsen FH, et al. Effect of boron depletion and repletion on blood indicators of calcium status in humans fed a magnesium-low diet. J Trace Elements in Exp Med. 1990. 45-54.
70. Miyazawa T, et al. Vitamin E: Regulatory Redox Interactions. IUBMB Life.
71. Fischer CP, et al. Supplementation with Vitamins C and E inhibit release of IL-6 from Contracting Human Skeletal Muscle. J Physiol. 2004. 558(Pt 2): 633-45.
72. Khatami PG, et al. The effects of high-dose vitamin E supplementation on biomarkers of kidney injury, inflammation, and oxidative stress in patients with diabetic nephropathy: A randomized, double-blind, placebo-controlled trial. J Clin Lipidol. 2016. 10(4): 922-929.
73. Lu Y, et al. Recent developments in Inonotus obliquus (Chaga mushroom) polysaccharides: isolation, structural characteristics, biological activities and application. Polymers. 2021. 13(9): 1441.
74. Burmasova MA, et al. Melanins of Inonotus Obliquus: Bifidogenic and Antioxidant Properties. Biomolecules. 2019. 9(6): 248.

75. Chen YF, et al. Inonotus obliquus polysaccharide ameliorates dextran sulphate sodium induced colitis involving modulation of Th1/Th2 and Th17/Treg balance. Artif Cells Nanomed Biotech. 2019. 47(1): 757-66.
76. Hu Y, et al. Inonotus obliquus polysaccharide regulates gut microbiota of chronic pancreatitis in mice. AMB Express. 2017. 7:39.
77. Hong KB, et al. Hepatoprotective Activity of Water Extracts from Chaga Medicinal Mushroom, Inonotus obliquus (Higher Basidiomycetes) Against Tert-Butyl Hydroperoxide-Induced Oxidative Liver Injury in Primary Cultured Rat Hepatocytes. Int J Med Mushrooms. 2015. 17(11): 1069-76.
78. Pyne DB, et al. Probiotics supplementation for athletes – clinical and physiological effects. Eur J Sport Sci. 2015. 15(1): 63-72.
79. Duranti S, et al. Obesity and microbiota: an example of an intricate relationship. Genes Nutr. 2017. 12: 18.
80. Jager R, et al. International Society of Sports Nutrition Position Stand: Probiotics. JISSN. 2019.
81. Jager R, et al. Probiotic Bacillus coagulans GBI-30 6086 Improves Protein Absorption and Utilization. Probiotics Antimicrob Proteins. 2018. 10(4): 611-15.
82. Stecker RA, et al. Bacillus coagulans GBI-30 6086 improves amino acid absorption from milk protein. Nutrition & Metabolism. 2020. 17:93.
83. Georges J, et al. The effects of probiotic supplementation on lean body mass, strength and power and health indicators in resistance trained males: a pilot study. JISSN. 2014. P38.
84. Jäger R, Shields KA, Lowery RP, et al. Probiotic Bacillus coagulans GBI-30, 6086 reduces exercise-induced muscle damage and increases recovery. PeerJ 2016.
85. Kalman DS, et al. A prospective, randomized, double-blind, placebo controlled parallel-group dual site trial to evaluate the effects of a Bacillus coagulans-based product on functional intestinal gas symptoms. BMC Gastroenterology. 2009. 9: 85.
86. Hun L. Original research: Bacillus coagulans Significantly Improved Abdominal Pain and Bloating in Patients with IBS. Postgraduate Med. 2009. 121(2): 119-24.
87. Winkler J, Ghosh S. Therapeutic potential of fulvic acid in chronic inflammatory diseases and diabetes. J Diabetes Res. 2018.
88. Junek R, et al. Bimodal effect of humic acids on the LPS-induced TNF-alpha release from differentiated U937 cells. Phytomed. 2009. 16(5): 470-6.
89. Sanmanee N, Areekijseree M. The effects of fulvic acid on copper bioavailability to porcine oviductal epithelial cells. Biol Trace Elem Res. 2010. 135(1-3): 162-73.
90. Mirza MA, et al. Comparative evaluation of humic substances in oral drug delivery. Results Pharma Sci. 2011. 1(1): 16-26.