

PRODUCT DESCRIPTION:

The goal when developing **FREE TEST™** was to create a formula that focuses on decreasing the homeostatic mechanisms that lower endogenous testosterone production, while at the same time increasing both the testosterone to cortisol (T:C) and testosterone to estrogen (T:E) ratios favorably. This in turn would allow for sustained increases in testosterone and subsequently lean body mass. An additional objective was to develop a product that could be safely and effectively taken for longer periods of time, and when combined with proper dietary and training factors, would be deemed equally as effective as the recently eradicated class of products commonly classified as prohormones.

Simply put, **FREE TEST** is the most effective DSHEA* compliant dietary supplement available for increasing free testosterone levels without harmful side effects. It has been shown to elevate free testosterone levels by 50-300% in human subjects with no evidence of Hypothalamic-Pituitary-Testicular-Axis (HPTA) disruption.

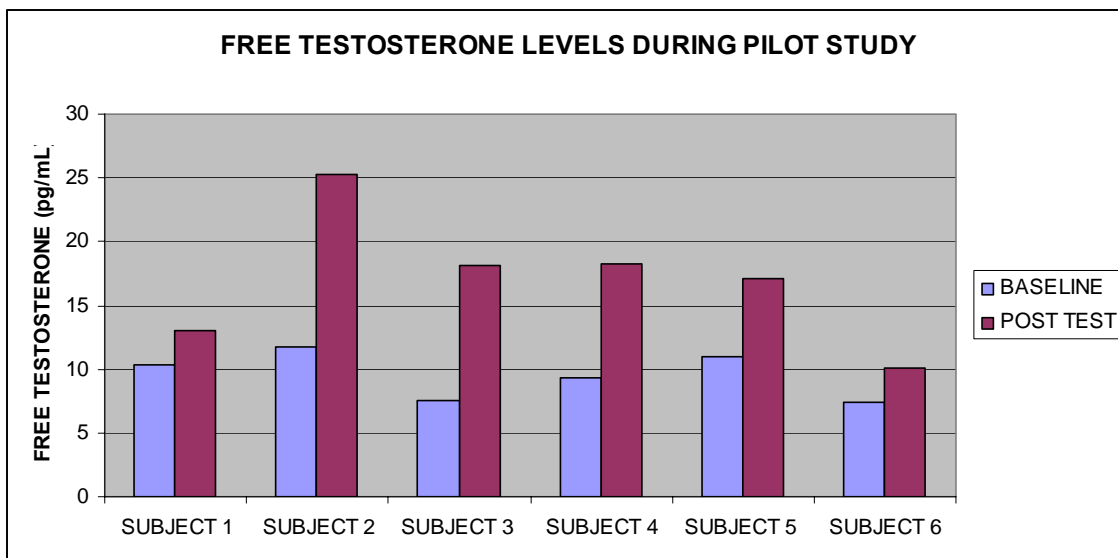
*NOTE: DSHEA (The Dietary Supplement Health and Education Act of 1994) defines a set of criteria for the ingredients, labeling and manufacturing along with other health and safety aspects for dietary supplement products. One key aspect of DSHEA that has particular relevance in this article is the requirement that all ingredients are “naturally occurring or found in nature”. For more information a complete copy of DSHEA can be found at www.FDA.gov.

How Free Test™ differs from other testosterone enhancement products:

- Over the past 10 years a variety of products that increase testosterone have been widely promoted in the sports supplement market. While some of these products do indeed increase testosterone, many of the most effective products (“designer” anabolic steroids and prohormones) consequently have the most undesirable and unhealthy side effects. Therefore, these products cannot be safely used for any appreciable period of time and are usually only effective for a maximum of 10-12 weeks; often less. (See Figure 2). Although users of these products often achieve rapid gains of 10-20 pounds or even more, said gains are often largely lost upon cessation of use.
- Prohormones are extremely effective in increasing androgen levels as they directly bind the androgen receptor, which is the most direct way of increasing testosterone levels. However, as mentioned above these products have some potentially deleterious side effects. Furthermore, the United States FDA has recently increased its level of scrutiny of these products as to whether they meet the criteria for DSHEA compliance – many of which do not. Consequently most supplement companies have discontinued the manufacture and distribution of these products - both voluntarily and involuntarily.
- There are numerous natural (non-prohormone and non-steroid-based) testosterone boosters currently offered in the sports nutrition market. While these products are typically DSHEA compliant, the lion’s share of them are completely ineffective and employ a hodgepodge of low-concentration herbal extracts, ineffective or unproven “anabolic” compounds and / or basic amino acids. Additionally, a common practice is to create “kitchen sink” formulas (inclusion of numerous popular ingredient compounds to attract the largest possible pool of customers) and claim that the combination is “scientifically proven” to work due to the long list of “proven” ingredients. While some of these ingredients may indeed be backed by clinical research and have been proven effective, upon closer inspection you’ll find that all too often these ingredients are included at dosages that are a small fraction of the amounts used in the clinical trials. Unfortunately the large ingredient deck disallows a sufficient amount (e.g. an effective dose) of any one of the ingredients to be included in a normal daily dose – rendering the entire product a diluted combination of trace ingredients that accomplish nothing.

- The only solution to this problem is to use fewer but more highly concentrated herbal extracts, which are often expensive and / or difficult to work with due to their physical properties. Many of these cost thousands of dollars per kilogram and have moist, sticky consistencies which wreak havoc on automated manufacturing equipment. While these ingredients provide the best way to deliver potent and effective products, many sports supplement companies and their contract manufacturers avoid them; opting instead for the more cost effective and efficiently manufactured (albeit inferior) products. This is the reason testosterone “boosters” have gotten a bad name; there simply aren’t many good ones out there.
- However, *there’s good news...* Applied Nutraceuticals has been working exclusively with these highly concentrated extracts since they were founded and has developed unique processes that allow them to employ even the most resinous of ingredients on their custom designed, cGMP-certified manufacturing line. This allows for the development and production of dramatically superior products that other companies simply are not capable of producing.
- Lastly, the Applied Nutraceuticals research and development team continuously pushes the limits of nutritional science with the application of never before used compounds. One such compound is a key component of **FREE TEST™**, which will be discussed in detail later in this article.

Figure 1: FREE TESTOSTERONE LEVELS DURING PILOT STUDY WHILE TAKING FREE TEST™



FREE TEST™ PRODUCT CHARACTERISTICS:

- Influences the heightened production of natural testosterone and free testosterone;
- Positively alters 7 homeostatic mechanisms of action that effect free and total testosterone output:
 - Contains a natural suicide inhibitor of aromatase, and will improve the Testosterone: Estrogen (T:E) profile and concurrently increase testosterone levels;
 - Increases NO/cGMP levels, which leads to increases in LH and greater endogenous testosterone production (1-4);
 - Increases testicular Acetyl L-Carnitine (ALCAR) levels; high testicular ALCAR levels are conducive to heightened spermatogenesis (28,30);
 - Lowers levels of cytokines IL-6, IL-1, and TNF-alpha- cytokines inhibit endogenous testosterone production by reducing levels of luteinizing hormone (33);
 - Lowers cortisol levels and heightens Testosterone: Cortisol (T:C) ratio via competitive inhibition;
 - Increases cAMP levels, which also increase LH levels (11,12,14);
 - Influences/Increases androgen receptor (AR) binding via altering receptor electronegativity (54).

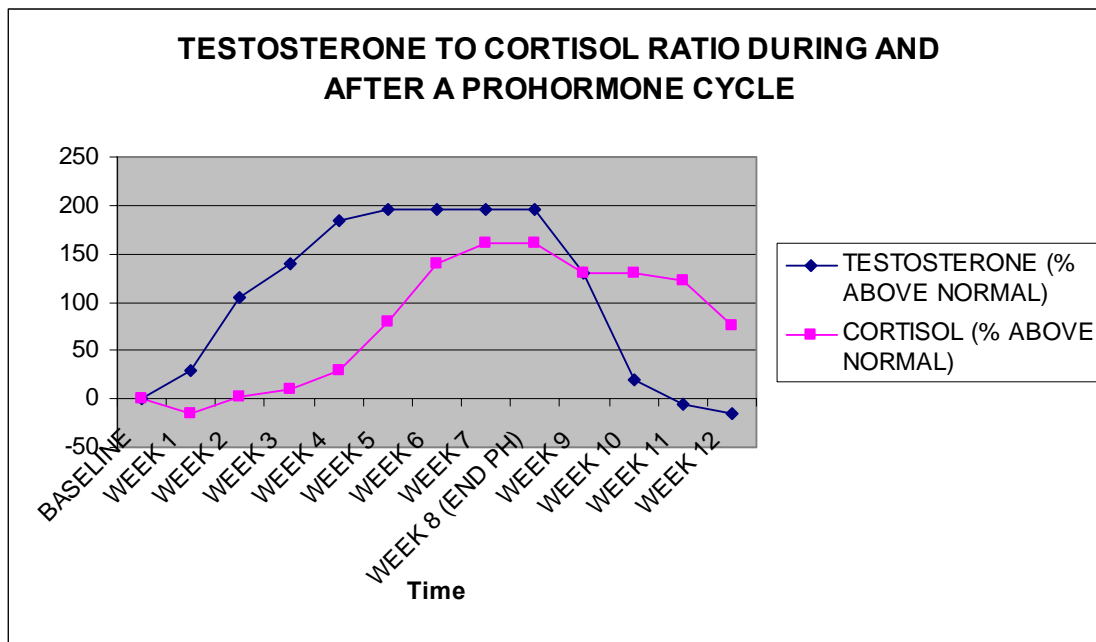
EFFECTS RELATED TO PHYSIQUE AND LIFE ENHANCEMENT:

- Greater lean muscle mass
- Lowered body fat
- Better/Faster recovery from training
- Increased muscular strength
- Heightened libido and sexual drive

The basics of how FREE TEST works:

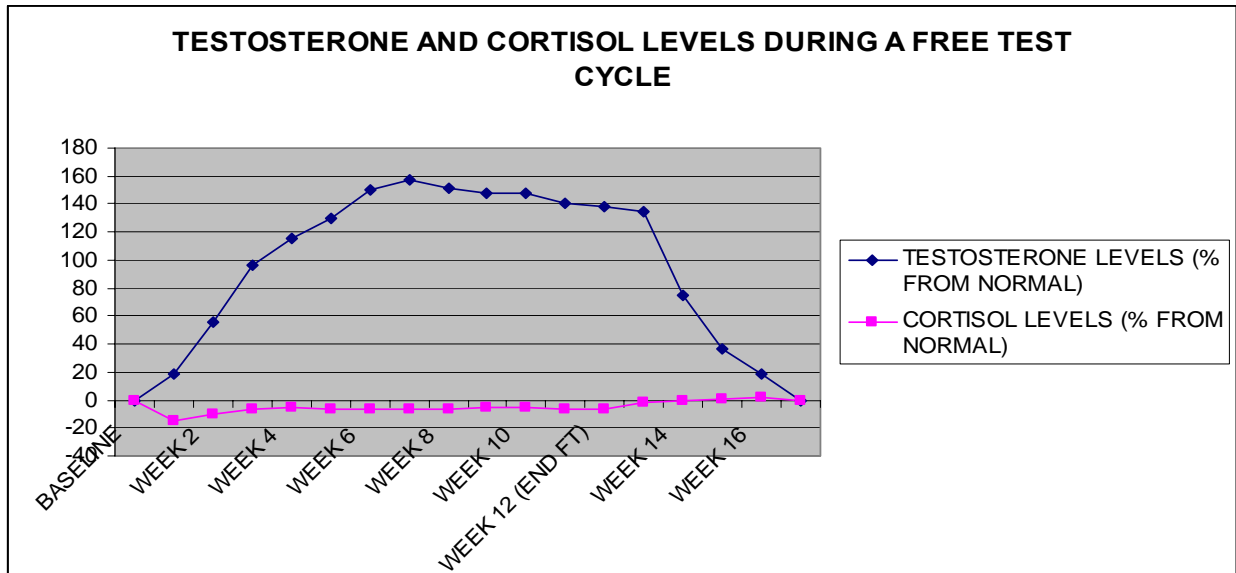
- The main mechanism of action in **FREE TEST** has to do with the anabolic/catabolic ratio in skeletal muscle, catabolic principles, and how they relate to homeostasis. During an anabolic steroid or prohormone cycle, testosterone levels rise, creating an anabolic state; as the testosterone to cortisol ratio (T:C) will become very positive. (See Figure 2: Notice the separation between the blue and pink lines at the beginning of the cycle). This allows for increased protein synthesis, and increases in lean body mass. However, due to homeostatic mechanisms in the body, cortisol levels begin to rise along with the increase in testosterone after the first 1-2 weeks (Note how the blue and pink lines come closer and closer together). The body seeks to gain balance (homeostasis) over the increased anabolism via modulating cortisol levels. The body recognizes that it cannot be in a state in which it requires such large amounts of nutrients for protein synthesis and lean tissue accretion for very long (a very high positive nitrogen balance), so it essentially tries to put the brakes on, via increasing cortisol levels to meet heightened testosterone levels (53).

Figure 2: TESTOSTERONE AND CORTISOL LEVELS DURING A PROHORMONE OR ANABOLIC / ANDROGENIC STEROID CYCLE



- This phenomenon occurs during every cycle and it is why, after week 6 of a cycle, gains slow dramatically, or stop altogether due to the homeostatic rise in cortisol. When this occurs, the user has one of two options: (1) Cessation of the cycle, followed by post-cycle therapy ((PCT) - note the pink line above the blue in wks. 10-12, indicative of a catabolic state), or: (2) Increase the dosage, and take the level of testosterone up even higher (not recommended for safety reasons).
- Neither option is very good for keeping gains. The mechanism of action of Free Test seeks to circumvent this via manipulating the T:C ratio via non-androgen receptor mediated means. One key ingredient, 3, 7 keto DHEA plays a key role in accomplishing this. Here's how it works: 7-Keto derivatives competitively interact with the 11beta-HSD1 enzyme, which is the enzyme that allows the transfer of inactive glucocorticoids to active ones. This is important as it therefore helps keep cortisol levels lower than normal by slowing (but not stopping) the conversion of inactive cortisol to active cortisol - allowing for mitigated levels of this catabolic hormone. (46-48).
- Non-androgen receptor (AR) mediated methods of increasing testosterone are extremely crucial to the inner workings of Free Test. Another mechanism of action is that it exerts its effects through decreased estrogen levels, increased testicular function, and increased levels of luteinizing hormone (LH) (53). Since these controls are non-AR mediated (at least not directly), the rise in testosterone is more gradual and not as traumatic, consequently the body will not try to maintain homeostasis as stringently as with the dramatic increases seen with an AR-mediated cycle (see Figure 3 below).

Figure 3: THEORETICAL TESTOSTERONE AND CORTISOL LEVELS DURING A FREE TEST™ CYCLE



- These more gradual increases in testosterone levels allow for cortisol levels to remain relatively low during the cycle. Additional factors that directly suppress cortisol further improve the anabolic ratio (T:C), with anabolism far outweighing catabolism throughout the cycle, with minimal disruption of hormone levels post-cycle (53).

Figure 4: OVERVIEW OF THE HYPOTHALAMIC PITUITARY TESTICULAR AXIS

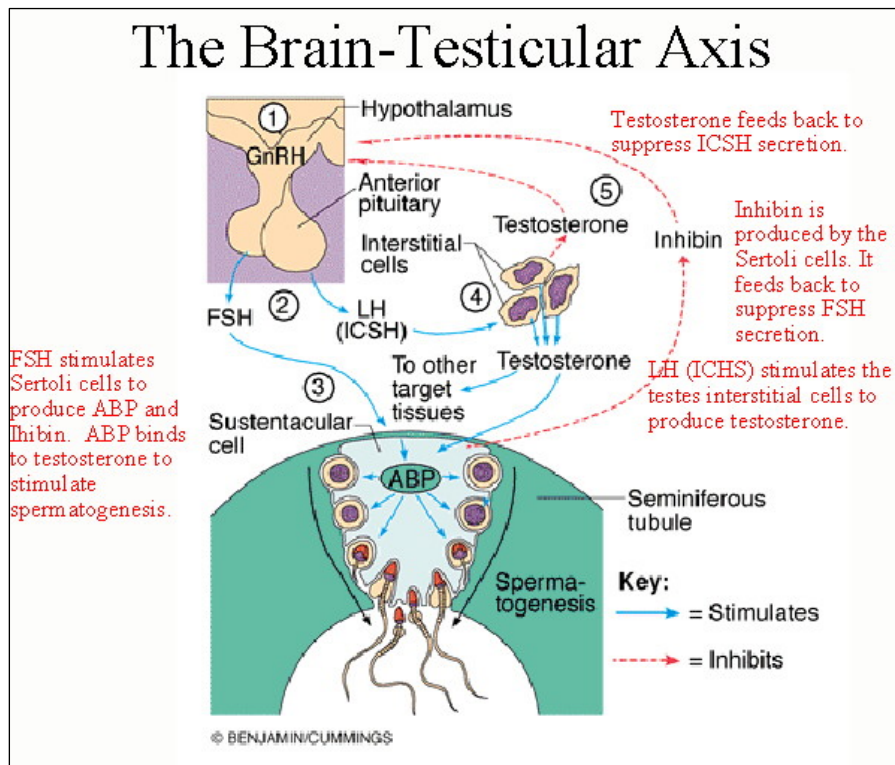
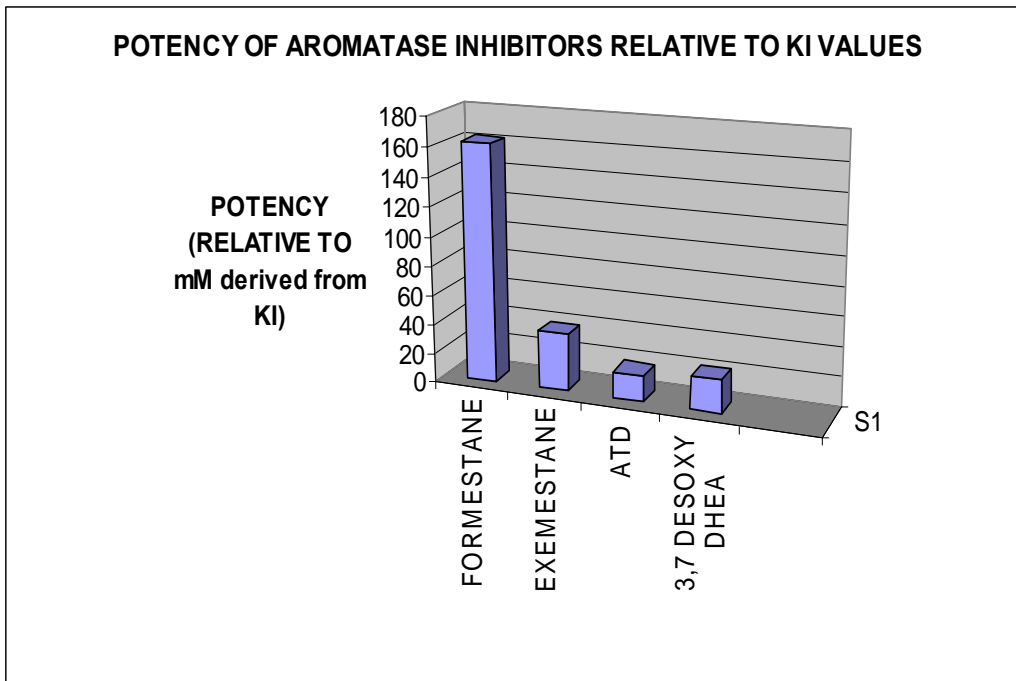


Figure 5: POTENCY OF AROMATASE INHIBITORS, INCLUDING 3-DESOXY 7-KETO DHEA (KI VALUES)



FREE TEST™ MAIN MECHANISMS OF ACTION:

Regulating Estrogen and Increasing Testosterone via Suicide Aromatase Inhibition: The Role of 3, 7-Keto DHEA:

- 3, 7-Keto DHEA is a naturally-occurring metabolite of dehydroepiandrosterone (DHEA), and is a potent aromatase inhibitor with some very unique qualities. Aromatase is an enzyme that transforms testosterone into estrogen, and the more active aromatase is, the more estrogen will ultimately be present. Therefore, aromatase inhibitors significantly decrease the level of estrogen in the body. This is important as increased estrogen in men can signal the hypothalamic pituitary testicular axis (HPTA) to shut down the release of gonadotropin-releasing hormone (GnRH). GnRH signals the production of luteinizing hormone (LH), which signals the production of testosterone. Therefore, increased estrogen levels can lower endogenous testosterone production (21,29,31).
- 3, 7-Keto DHEA has demonstrated strong ability to lower estrogen, thus mitigating this effect. It has a high binding affinity (Ki value = 0.22 mM) to the aromatase enzyme, and binds in an irreversible manner, making it a suicide inhibitor of aromatase. Ki Values measure how efficiently a compound binds to its associated receptor. The lower the Ki value; the higher the binding affinity. This inhibition allows for the production of less estradiol (E2) and estrone (E1) and allows the user of the compound to maintain a higher level of testosterone; hence improving the Testosterone: Estrogen (T:E) ratio. The mechanism through which aromatase inhibitors raise testosterone is fairly simple; the HPTA senses low levels of estrogen, and because the body seeks to maintain homeostasis (it likes to maintain at least some estrogen, even in men), there is a concurrent increase in the amount of testosterone that is being produced, as a way to compensate for the low estrogen levels. The increased testosterone levels normally will result in increased estrogen since there is no estrogen being produced. Essentially, the brain is tricked into trying to produce more estrogen, so it releases more luteinizing

hormone releasing hormone (LHRH) and subsequently more LH, leading to even higher testosterone levels (20,21-23).

- All aromatase inhibitors share this characteristic of positively altering the T:E ratio, and all will raise serum testosterone levels in men, which has been referenced in numerous studies. 3,7-Keto DHEA is comparable in potency to several other commonly available aromatase inhibitors. As explained above, a lower K_i value means higher potency, making it more potent than both Formestane and Exemestane, and very similar to androstentrione (ATD) (31,59). (See Figure 5)
- 3,7-Keto DHEA is unique from other commonly used aromatase inhibitors in sports supplements in that it is a natural metabolite of 7-Keto DHEA and it cannot directly bind to the androgen receptor. 3,7-Keto DHEA (like 7-Keto DHEA) also cannot convert to testosterone, estrogen, or progesterone via any type of enzymatic reaction, so by strict definition it cannot in any way be considered a prohormone. This clearly differentiates it from other recently banned products that allow for the direct conversion to a controlled substance in the body (in either in trace amounts or full-scale conversion). This can not occur with 3,7-Keto DHEA, as it is formed naturally in humans from 7-Keto DHEA and can be readily found in humans in the amount of 5-7 ug/day (23-24).

Increasing luteinizing hormone via elevating nitric oxide and cyclic Guanosine Monophosphate (cGMP): The role of Acetyl-L-Carnitine, Resveratrol, and their interaction with the male reproductive system and StAR:

- **FREE TEST™** strongly influences testosterone production through the nitric oxide pathway and cGMP by increasing luteinizing hormone levels. Luteinizing Hormone (via receptors found on the surface of Leydig cells) is important as it controls the production and secretion of testosterone (1-4).
- The inclusion of Acetyl L-Carnitine (ALCAR) (a derivative of L-Carnitine which is an amino acid that is crucial for fatty acid metabolism and male reproductive function) allows for this manipulation of cGMP, as does Resveratrol. cGMP is classified as a second messenger, meaning that it exerts its effects by acting in a manner **secondary and in response to** a first messenger signaling molecule. When a first messenger signaling molecule binds to a cell surface, another secondary pathway is activated that increases cGMP production (1,15,19,27).
- Increasing cGMP is extremely important to the action of **FREE TEST**, in that increased cGMP levels equate to increased levels of luteinizing hormone (LH). LH has a stimulatory action on endogenous testosterone production; so when cGMP levels increase, so do LH levels, and when LH levels increase, so does endogenous testosterone production. This effect has been well documented in recent research on PDE5 inhibitors (compounds that increase cGMP levels such as sildenafil (Viagra®). Researchers found that high cyclic GMP levels ultimately equate to higher testosterone levels.
- ALCAR has also been shown to be an acetate donor to coenzyme A, allowing it to aid in the synthesis and function of acetylcholine, both peripherally and centrally. 7-Keto derivatives of DHEA (like 3,7-Keto DHEA) antagonize GABA-A receptors and functionally increase cholinergic expression. Through this mechanism of action, ALCAR and 3,7-Keto DHEA can increase cGMP levels, through increasing the amount of available acetylcholine- as mentioned above, more cGMP, more LH, more testosterone. (18,20,32).
- ALCAR levels are very important for testicular function, in that low testicular ALCAR and L-Carnitine levels are strongly associated with testicular dysfunction and infertility. (28,30,33)

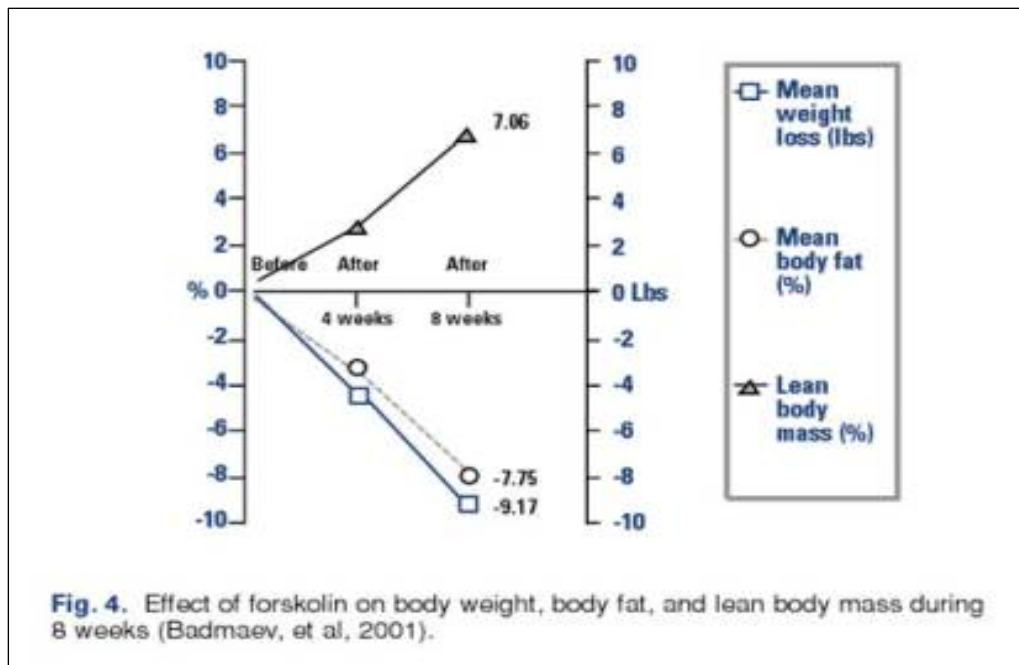
- ALCAR has also been shown to have a significant effect on increasing Leptin levels, and has also been shown to be a potent factor in the male reproductive system. Leptin releases LHRH (luteinizing releasing hormone) by activating nitric oxide synthase (NOS), an enzyme that speeds the formation of nitric oxide (NO) from the amino acid L-Arginine. LHRH controls the release of luteinizing hormone (LH), and greater amounts of LHRH release equal greater amounts of luteinizing hormone (LH) release, which can allow for higher amounts of endogenous testosterone. Therefore, by increasing leptin levels and boosting LHRH release, ALCAR can have some additional positive effects on endogenous testosterone production (1-4,18).
- L-Carnitine, ALCAR, and several derivations thereof have been shown to elevate Nitric Oxide (NO) levels. NO is important in that it regulates vascular tone, central nervous system stimulation, induces the release of LHRH and regulates cGMP levels. ALCAR and 7-Keto derivatives of DHEA have also been shown to elevate acetylcholine levels, which allow for a concurrent increase in cGMP production. NO also activates the release of LHRH which reaches the pituitary and activates the release of gonadotropins via the activation of neural NO synthase (NOS) in the pituitary gland (1-4, 15, 33-34).
- The fact that cGMP has a stimulatory action on steroidogenesis via increased LH production is an important one. In numerous pathway studies, increases in cGMP increased phosphorylation of the **steroidogenic acute regulatory protein** (StAR). Increased phosphorylation of StAR is important, in that (StAR) is a Leydig cell cholesterol transfer protein that provides the building blocks for testosterone synthesis. Increased phosphorylation allows for increased StAR activation, and increased StAR activation is necessary for the stimulation of steroidogenic enzymes involved in the transfer of cholesterol to testosterone. This increase in StAR, and the greater transfer of cholesterol to testosterone can result in greater endogenous testosterone synthesis (15,33-34).
- By increasing the intermediary between these two processes, a resulting positive shift in anabolism can occur. Therefore, high cGMP levels also equate to high levels of luteinizing hormone (the hormone responsible for mediating endogenous spermatogenesis), and when cGMP levels are elevated it serves as an intermediate in the signaling cascade that ranges from LH binding to testosterone production (15,33-34).
- These results suggest that cGMP contributes to the control of basal steroidogenesis (endogenous testosterone production) in Leydig cells through the protein kinase G (PKG, and enzyme responsible for the activation of second messengers) -dependent modification of the StAR protein and interaction with LH. LH controls the production and secretion of testosterone, and the subsequent binding of LH with its receptor allows signaling through the cyclic AMP pathway through GTP binding proteins. Signal transduction occurs through the protein kinase A pathway as its principal signal transduction mechanism, and this ultimately allows for the release of testosterone after 30-60 minutes of LH stimulation (15-17,33-34).
- To summarize, **FREE TEST™** contains several potent direct mechanisms of action that strongly influence the production of increased testosterone via the NO / cGMP pathway, through a cascade of regulatory proteins and second messengers.

Forskolin and Resveratrol: Increasing endogenous testosterone production via increasing Cyclic AMP (cAMP) levels:

- Forskolin has been the subject of research in the supplement industry since the early 1980s. Scientific studies have discovered that along with increasing thyroid activity and thermogenesis, forskolin is also a potent anabolic. This occurs primarily through elevation of **3,5 cyclic adenosine monophosphate** (cAMP) which is another second messenger that is important in hormone signaling (14,16).

- cAMP elevation is a crucial piece of the puzzle in creating both an anabolic and thermogenic state, and forskolin is one of the best compounds available for triggering dramatic increases in cAMP levels. One study showed that forskolin was able to increase cAMP levels 4.82 times more than a placebo. Another study demonstrates that forskolin can raise cAMP levels in fat cells. This is important because it demonstrates the ability of the compound to enhance lipolysis; meaning that forskolin exerts powerful fat-burning effects as well as being a potent anabolic agent; plus forskolin can also enhance endurance capacity as well (5-10,14,16).
- Another added bonus of forskolin and resveratrol are that they both increase cAMP independently of epinephrine, thus providing increased energy without the need to take any type of traditional stimulant. Increased cAMP also is a signal for steroidogenesis (testosterone production) in the Leydig cells of the testes by increasing levels of **steroidogenic acute regulatory protein** (StAR- as mentioned above) (14,16,36-38).

FIGURE 6: Forskolin-Induced Lean Body Mass Gains (Badmaev et al. 2001)



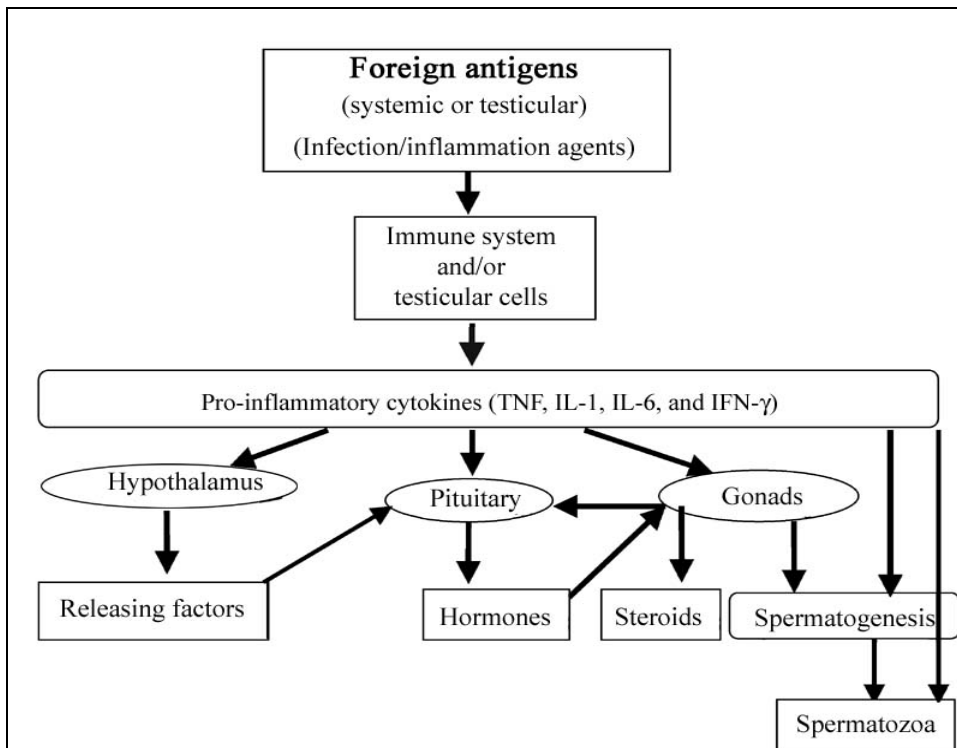
- As mentioned earlier, StAR activation is necessary for the stimulation of the transfer of cholesterol to testosterone. By this process, and the fact that high cAMP levels also equate to high levels of luteinizing hormone, significant increases in endogenous testosterone production can occur along with a resulting increase in anabolism and protein synthesis (14-15).
- Numerous other studies have shown parallels between increased cAMP levels and increased anabolism, and a ground-breaking 2005 study in *The Journal of Obesity Research* found that obese men taking 250 mg of 10% forskolin a day for 12 weeks (roughly the dosage included in the daily dosage of Free Test™) experienced an averaged 33% increase in free testosterone levels, averaged a 10 lbs. fat loss per person and increased lean mass an average of 8 lbs! A 2001 study by Badmaev (see Figure 6) also yielded similar results (6,8,54).
- **Quercetin** is a citrus bioflavanoid that has been shown to boost the effects of resveratrol (this is the primary rationale for its inclusion in the formula) via prevention of precocious *glucorindiation* (deactivation of the compound) by the liver. Similarly,

quercetin has also been shown to have some estrogen-antagonizing effects. Quercetin has also been shown to have some additional anti-oxidant effects, and has been shown to lower cortisol levels in several animal studies. Lastly, the combination of resveratrol and quercetin also has been shown in some cellular studies to actually destroy fat cells. (39,40,44)

Mitigating the factors that decrease testosterone, and how FREE TEST™ combats them via N-Acetyl-Cysteine and Selenium:

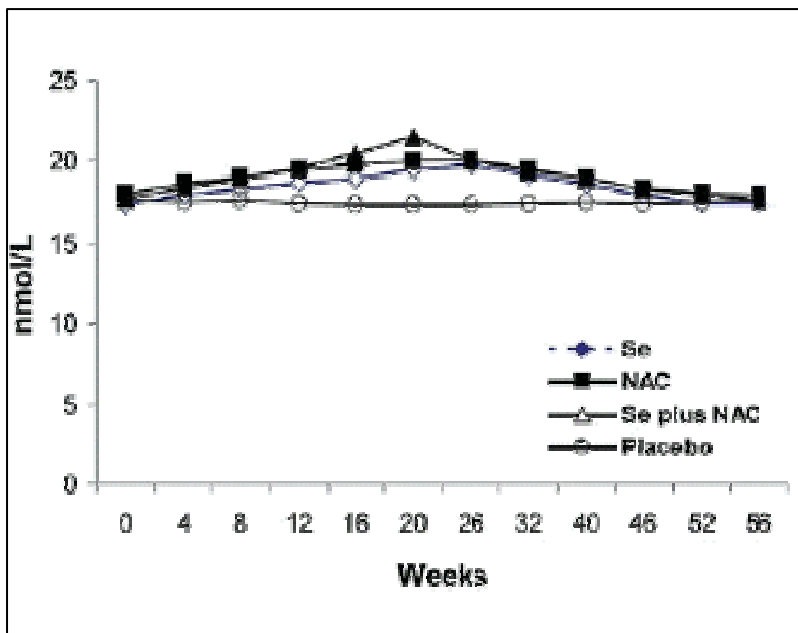
- **FREE TEST** offers yet another benefit in the production and elevation of free testosterone, as it serves as a strong anti-oxidant in the reproductive system.
- Cytokines are substances secreted by the immune system that carry signals between cells, and have effects on the function of many different bodily systems, including the function of endogenous testosterone modulation (25-26,35). Cytokines can be disruptive to the endocrine system, in that they can be involved in feedback inhibition during the production of testosterone, which can result in a downgrade of natural testosterone production. Under conditions such as systemic or testicular stress, the systemic and local (within the testis) amounts of pro-inflammatory cytokines (TNF, IL-1, and IL-6) will be increased significantly. Elevated amounts of cytokines can therefore be associated with decreased testicular function. Elevated levels of these cytokines will effect the endocrine system and alter the amount of hormones released by the system (mainly GnRH, LH and FSH), which regulate the spermatogenic process. Cytokines (mainly IL-6) can also significantly raise cortisol levels, which can also lead to a reduced anabolic stimulus (13,25,35).
- In addition, Pro-inflammatory cytokines can suppress the cells of the testis, and lead to a decrease in spermatogenesis and thus to affect the function and/or viability of the testes in the production of testosterone (33-34).

Figure 7: ALTERATION OF HORMONAL PRODUCTION VIA CYTOKINES



- A recent study involving men taking a combination of **N-Acetyl-Cysteine** (or NAC, a precursor to glutathione), and **selenium**, (a trace mineral that has been shown to have direct effects on the biosynthesis pathways of testosterone), found that the combination of the two compounds has a mild synergistic effect on increasing testosterone levels in men. Selenium is rate-limiting in testosterone production in men, and if not enough selenium (selenium deficiency is very common) is available, testosterone will not be produced in optimal levels (25-26,49). The researchers also believe that the combination has an antioxidant effect in the testes via increasing glutathione levels. Glutathione is a tripeptide thiol found in all cells of the body, and is responsible for regulating protein synthesis and detoxifying cell structures. Selenium is needed for the detoxifying enzyme glutathione peroxidase, and NAC significantly increases glutathione levels. Increases in glutathione and glutathione peroxidase seem to be negatively correlated with cytokine release; as levels of these anti-oxidants increase, cytokine levels decrease. This effect is readily apparent in the male reproductive system, as several studies have shown that NAC can reverse testicular damage cross-indicated with cytokine release and cytokine-related testicular suppression. This characteristic alone promotes conditions that are extremely favorable for optimal testosterone production (25.49).

FIGURE 8: INCREASED TESTOSTERONE LEVELS RELATED TO N-ACETYL CYSTEINE AND SELENIUM SUPPLEMENTATION: STUDY DATA



Modulating glucocorticoid and cortisol: Another way FREE TEST™ preserves the anabolic environment:

- Glucocorticoids are a class of hormones that bind to the glucocorticoid receptor, and serve to prevent inflammation in the body. Cortisol is the most prevalent of the glucocorticoid hormones. It is a corticosteroid produced in the adrenal cortex of the kidney, and is used by the body as a response to stress. The release of cortisol is controlled by adreno-corticotropin-releasing hormone (45-47, 53).
- Cortisol does have some benefits; for example cortisol is necessary for obtaining an optimal response to stimulant products, along with many body processes. The main function of cortisol is to increase blood sugar, but it can also counteract several different anabolic hormones in the body, including insulin and testosterone, thus acting in a

catabolic function by tearing down muscle tissue when it is present in excessive amounts (53).

- A negative Testosterone:Cortisol (T:C) ratio essentially starves muscle cells of nutrients by reducing muscle glycogen and potassium and weakening the immune system which can cause a general loss of muscle mass. A positive T:C ratio is necessary to produce an anabolic environment in the human body (53).
- 7-Keto DHEA, DHEA, and most derivations of this molecule have been found in many research studies to have the ability to significantly reduce glucocorticoid levels by altering cytokine levels, as discussed earlier in this article. They also modulate the negative immune effects of glucocorticoids on skeletal muscle that occur via competition for the 11beta-hydroxysteroid dehydrogenase 1 enzyme. The finding from initial pilot studies strongly suggest that 3, 7-Keto DHEA will produce potent immuno-modulating and cortisol-lowering effects similar to or possibly even greater than those associated with 7-Keto DHEA (22-23,45-47).
- One of the aforementioned means through which 7-Keto DHEA and its metabolites serve this function is via acting on 11beta hydroxysteroid dehydrogenase 1 (11beta-HSD1), which is an enzyme that converts inactive glucocorticoids to active substrate. 7-Keto DHEA and its analogs have been shown to compete with inactive glucocorticoids for the 11beta-HSD1 enzyme, thus reducing the conversion of inactive glucocorticoids to their active, muscle-catabolic form via receptor site antagonism. A good example would be a reduction in the conversion of cortisone to cortisol, which is a process dependant on the 11beta-HSD1 enzyme. 7-Keto DHEA has been shown to reduce this conversion; thereby lowering cortisol levels (45-47).

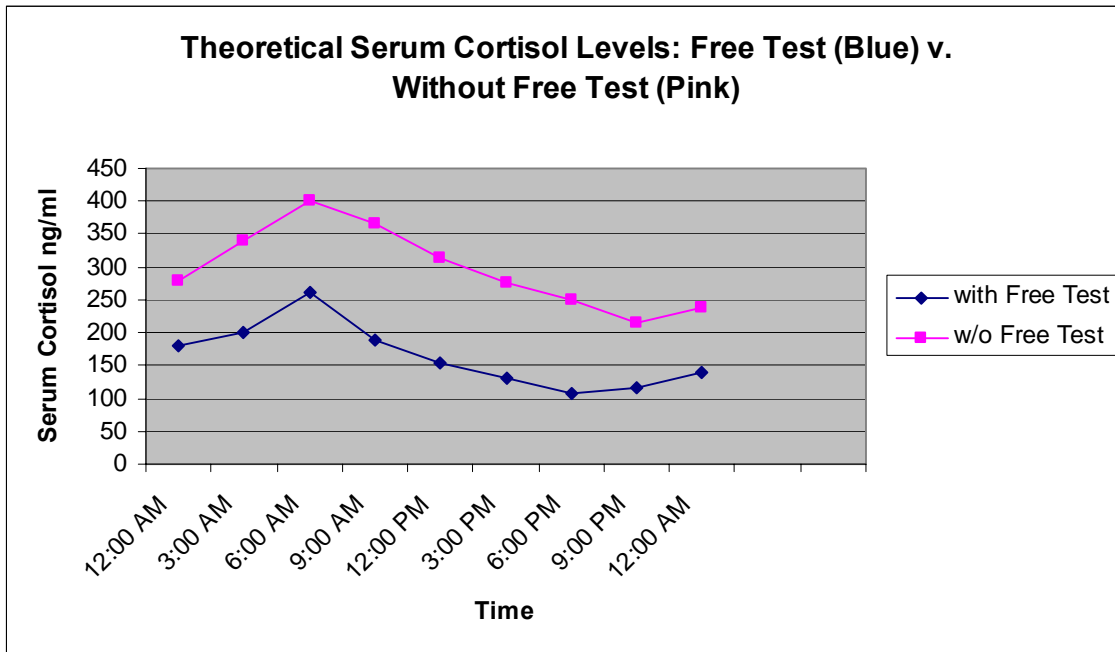
FREE TEST™ creates a superior binding environment for existing androgens: The role of Zinc and Magnesium in promoting a better binding environment for Free Testosterone:

- Magnesium has been shown in more recent studies to inhibit the binding of steroid hormone binding globulin (SHBG) and free testosterone. SHBG binds free testosterone and allows it to be excreted from the body without binding the androgen receptor. Magnesium keeps this from happening by altering the binding affinity of testosterone to SHBG, and thereby allowing for increased amounts of free testosterone to remain active in the bloodstream (41-43).
- Other human research has shown that supplemental magnesium, when taken along with other ingredients like DHEA and Zinc, can significantly increase free testosterone. Low magnesium levels have been found to be correlated with high cortisol levels, and this can lead to a negative skewing of the T:C ratio (20,41-43) .
- Zinc is involved in over 200 enzymatic reactions, allowing for the action of several different hormones: GH, testosterone, and insulin all require zinc for synthesis (42-43,53).
- The inclusion of these elements in **FREE TEST** allows for greater numbers of androgen receptors and a better binding environment for testosterone in skeletal muscle, hence creating a better target for circulating free testosterone and allowing for greater binding of testosterone to the additional receptors. All of these factors contribute to the multiple, synergistic mechanisms that lead to the dramatically increased protein synthesis, better recovery, and increased muscle mass users experience while taking **FREE TEST**.

Administration, Timing, and Dosing

- Under normal conditions, diurnal cortisol levels tend to be highest shortly after waking, and these levels tend to decline during the day, reaching their lowest point in the evening. Although cortisol levels tend to decline during the day, stressful events punctuate spikes into said decline (see Figure 5). Similarly, diurnal testosterone levels tend to be highest in the early morning, and tend to wane as the day goes on, also spiking in a periodic fashion. Taking this into account, the timing of the Free Test™ dosage is crucial in contributing to the effectiveness of the product (53).
- Dosing the product within 1-2 hours of waking will allow the product to be most effective, in regards to both diurnal hormone regulation, and also the relative half-lives of the compounds included in **FREE TEST™**. This will allow for a reduction in heightened cortisol levels associated with diurnal fluctuations, and will allow for a continued time period of increased testosterone levels, for up to 12-18 hours after the AM dosage (See Figure 7).

Figure 8: THEORETICAL DIURNAL CORTISOL LEVELS WITH AND WITHOUT FREE TEST™



- A second dose of the product most likely will not be required later in the day due to the inclusion of 3, 7-Keto DHEA, as suicide aromatase inhibitors normally only require dosing every 18-24 hours (21-22).
- A dosage of 4-6 Capsules per day of Free Test™ is recommended, with food, within 1-2 hours of waking, depending on body weight.
- Research involving blood testing of free and serum testosterone levels indicates heightened levels of testosterone within 1-2 weeks of the first dosage, with increases occurring up to weeks 3-4, and possibly longer (55).

- **FREE TEST** can be run for much longer periods of time than conventional prohormones due to its effects on endogenous (naturally produced) testosterone, and the fact that it does not directly antagonize the androgen receptor. Instead, the product minimizes the homeostatic mechanisms in your own body that lower testosterone levels, and increases testosterone via secondary mechanisms of anabolism (manipulation of T:E and T:C). This allows the user to be able to run the compound for longer periods of time, up to 4-5 months while remaining safe and effective.

Stacks and Tips to Maximize the Product

- **Stack product with HGH-Up♂**- While each is extremely potent and effect on their own, there are distinct synergies with taking these product simultaneously. Their combined effects with rival that of even the most powerful prohormones without any of the risk associated with them.
- Take Bio-Mend Anti-Oxidant formula
 - High ORAC Value
 - Protects cellular membrane
 - Protects transcriptional factors (mRNA and DNA)
- In general, maintain a healthy diet and lifestyle:
 - Drink Plenty of water; at least 64 oz. per day
 - Ingest at least 1 gram of protein per lb. of body weight daily
 - Sleep at least 7 hours per night
 - Eat lots of fruits and vegetables
 - Eat lots of complex carbs
 - Eat 5-6 smaller protein and carb-rich meals throughout the day
 - Increase calories to at least 500 Kcal/day over your normal intake
 - BCAAs and Creatine will be helpful
 - Avoid alcohol and tobacco

In conclusion, FREE TEST™ is the most effective DSHEA compliant Testosterone enhancement product yet to be offered in the history of sports nutrition. For more information, you can contact Applied Nutraceuticals at info@appliednutraceuticals.com.

Studies and Clinical Info

1. Young ME, Leighton B.(1998) Evidence for altered sensitivity of the nitric oxide/cGMP signaling cascade in insulin-resistant skeletal muscle. *Biochem J.* Jan 1;329 (Pt 1):73-9
2. Aghdasi B, Reid MB, and Hamilton SL. (1997) Nitric oxide protects the skeletal muscle Ca²⁺ release channel from oxidation induced activation. *J Biol Chem* 272:25462-25467
3. Balon TW, Nadler JL. (1997)Evidence that nitric oxide increases glucose transport in skeletal muscle. *J Appl Physiol.* 1997 Jan;82(1):359-63.
4. Etgen GJ Jr, Fryburg DA, Gibbs EM. (1997) Nitric oxide stimulates skeletal muscle glucose transport through a calcium/contraction- and phosphatidylinositol-3-kinase-independent pathway. *Diabetes Nitric oxide*; 46(11):1915-9.
5. Asano Tsuguyoshi (2001). Clinical report on root extract of perilla plant (*Coleus forskohlii*) ForsLean® in reducing body fat. Asano Institute. Tokyo , Japan.
6. Kreider RB, et al. Effects of *Coleus forskohlii* supplementation on body composition and markers of health in sedentary overweight females. *Experimental Biology 2002 Late Breaking Abstracts.* LB305: 2002.

7. LEAMON, KB; PADGETT, W; DALY, JW. "Forskolin: Unique diterpene activator of adenylate cyclase in membrane and intact cells" Proc. Natl. Acad. Sci. USA 1981,78,3363-67
8. DE SOUZA, NJ; DOHADWALLA, AN; REDEN, J; Forskolin, A. "Labdane Diterpenoid with Antihypertensive, Positive Inotropic, Platelet Aggregation Inhibitory, and Adenylate Cyclase Activating Properties" Medicinal Research Reviews 1983, 3(2), 201-219
9. Badmaev, V. Majeed, M. Conte, A, Parker, J. "Diterpene Forskolin (Coleus forskohlii, Benth.): A possible new compound for reduction of body weight by increasing lean body mass" Sabinsa Corporation <http://www.b5srl.com/articles/nu/2002/marapr/badmaev.htm> 1/21/2004
10. Ding X, Staudinger J. Induction of Drug Metabolism by Forskolin, the Role of The Pregnane X Receptor and the PKA Signal Transduction Pathway. J Pharmacol Exp Ther. 2004 Sep 30
11. Forskolin, a diterpene compound isolated from the roots of Coleus forskohlii, activates adenylate cyclase in membranes from a variety of mammalian tissues. adenylate cyclase in human basophils and mast cells. Biochem Pharmacol. 1987 Jan 1;36(1):13-20.
12. Asif AR, Ljubojevic M, Sabolic I, Shnitsar V, Metten M, Anzai N, Müller GA, Burckhardt G, Hagos Y. Regulation of steroid hormone biosynthesis enzymes and organic anion transporters by forskolin and DHEA-S treatment in adrenocortical cells. Am J Physiol Endocrinol Metab. 2006 Dec;291(6):E1351-9. Epub 2006 Jul 11.
13. Tsai SC, Lu CC, Lin CS, Wang PS. Antisteroidogenic actions of hydrogen peroxide on rat Leydig cells. J Cell Biochem. 2003 Dec 15;90(6):1276-86.
14. Cronin MJ, Evans WS, Hewlett EL, Thorner MO. LH release is facilitated by agents that alter cyclic AMP generating system. Am J Physiol. 1984 Jan;246(1 Pt 1):E44-51.
15. Andric SA, Janjic MM, Stojkov NJ, Kostic TS. Protein kinase G-mediated stimulation of basal Leydig cell steroidogenesis. Am J Physiol Endocrinol Metab. 2007 Nov;293(5):E1399-408. Epub 2007 Sep 11.
16. Manna PR, Dyson MT, Stocco DM. Regulation of the steroidogenic acute regulatory protein gene expression: present and future perspectives. Mol Hum Reprod. 2009 Jun;15(6):321-33. Epub 2009 Mar 25. Review.
17. Kostic TS, Stojkov NJ, Janjic MM, Maric D, Andric SA. The adaptive response of adult rat Leydig cells to repeated immobilization stress: the role of protein kinase A and steroidogenic acute regulatory protein. Stress. 2008;11(5):370-80.
18. Kraemer WJ, Spiering BA, Volek JS, Ratamess NA, Sharman MJ, Rubin MR, French DN, Silvestre R, Hatfield DL, Van Heest JL, Vingren JL, Judelson DA, Deschenes MR, Maresh CM. *Androgenic responses to resistance exercise: effects of feeding and L-carnitine*. Med Sci Sports Exer., 2006 Jul; 38(7): 1288-96.
19. Kraemer WJ, Volek JS, French DN, Rubin MR, Sharman MJ, Gómez AL, Ratamess NA, Newton RU, Jemiolo B, Craig BW, Häkkinen K. *The effects of L-carnitine L-tartrate supplementation on hormonal responses to resistance exercise and recovery*. J Strength Cond. Res. 2003 Aug; 17(3): 455-462.
20. André C, Berthelot A, Robert JF, Thomassin M, Guillaume YC. *Testimony of the correlation between DHEA and bioavailable testosterone using a biochromatographic concept: effect of two salts*. J Pharm Biomed An., 2003 Dec 4; 33(5): 911-21.
21. J. Raman, P. Schlegel AROMATASE INHIBITORS FOR MALE INFERTILITY *The Journal of Urology*, Volume 167, Issue 2, Pages 624-629.
22. Burnett-Bowie SA, Roupenian KC, Dere ME, Lee H, Leder BZ. Effects of aromatase inhibition in hypogonadal older men: a randomized, double-blind, placebo-controlled trial. Clin Endocrinol (Oxf). 2008 Jun 25. [Epub ahead of print]
23. Schubert K, Wehrberger K, Hobe G; Androsta-3,5-diene-7,17-dione: isolation from urine and formation from 7-keto-dehydro-epiandrosterone sulphate under various conditions of hydrolysis; Endocrinol Exp. 1971 Dec;5(4):205-10
24. Numazawa M, Mutsumi A, Tachibana M, Hoshi K; Synthesis of androst-5-en-7-ones and androsta-3,5-dien-7-ones and their related 7-deoxy analogs as conformational and catalytic probes for the active site of aromatase; J Med Chem. 1994 Jul 8;37(14):2198-205

25. Safarinejad MR, Safarinejad S. Efficacy of selenium and/or N-acetyl-cysteine for improving semen parameters in infertile men: a double-blind, placebo controlled, randomized study. *J Urol*. 2009 Feb;181(2):741-51. Epub 2008 Dec 16.
26. Farombi EO, Ugwuezunmba MC, Ezenwadu TT, Oyeyemi MO, Ekor M. Tetracycline-induced reproductive toxicity in male rats: effects of vitamin C and N-acetylcysteine. *Exp Toxicol Pathol*. 2008 Jun;60(1):77-85. Epub 2008 Apr 11.
27. Fernanda BM Priviero, Julio A Rojas-Moscoso, Alexandre S Silva, Edson Antunes and Angelina Zanesco Effect of L-carnitine supplementation on the sGC/cGMP pathway in vascular relaxing responses from exercised rats *BMC Pharmacology* 2009, **9**(Suppl 1):P54doi:10.1186/1471-2210-9-S1-P54
28. Cheng HJ, Chen T. [Clinical efficacy of combined L-carnitine and acetyl-L-carnitine on idiopathic asthenospermia] *Zhonghua Nan Ke Xue*. 2008 Feb;14(2):149-51.
29. Rohle D, Wilborn C, Taylor L, Mulligan C, Kreider R, Willoughby D. Effects of eight weeks of an alleged aromatase inhibiting nutritional supplement 6-OXO (androst-4-ene-3,6,17-trione) on serum hormone profiles and clinical safety markers in resistance-trained, eugonadal males. *J Int Soc Sports Nutr*. 2007 Oct 19;4:13.
30. Zhou X, Liu F, Zhai S. Effect of L-carnitine and/or L-acetyl-carnitine in nutrition treatment for male infertility: a systematic review. *Asia Pac J Clin Nutr*. 2007;16 Suppl 1:383-90.
31. Willoughby DS, Wilborn C, Taylor L, Campbell W Eight weeks of aromatase inhibition using the nutritional supplement Novedex XT: effects in young, eugonadal men. *Int J Sport Nutr Exerc Metab*. 2007 Feb;17(1):92-108.
32. Lee NP, Cheng CY. Nitric oxide and cyclic nucleotides: their roles in junction dynamics and spermatogenesis. *Adv Exp Med Biol*. 2008;636:172-85. Review.
33. Ulcova-Gallova Z, Gruberova J, Vrzalova J, Bibkova K, Peknicova J, Micanova Z, Topolcan O. Sperm antibodies, intra-acrosomal sperm proteins, and cytokines in semen in men from infertile couples. *Am J Reprod Immunol*. 2009 Mar;61(3):236-45.
34. Tang LF, Jiang H, Shang XJ, Zhao LM, Bai Q, Hong K, Liu DF, Liu JM, Yuan RP, Chen Q, Ma LL. [Seminal plasma levocarnitine significantly correlated with semen quality] *Zhonghua Nan Ke Xue*. 2008 Aug;14(8):704-8.
35. Cheol Yi Hong,¹ Jin Hee Park,¹ Ryun Seop Ahn, Suh Young Im, Hueng-Sik Choi, Jaemog Soh, Synthia H. Mellon, and Keesook Lee^{*} Mechanism of Suppression of Testicular Steroidogenesis by Proinflammatory Cytokine Tumor Necrosis Factor Alpha Hormone Research Center, School of Biological Sciences and Technology, Department of Biology, Chonnam National University, Gwangju 500-757, Republic of Korea, Department of Obstetrics and Gynecology, University of California, San Francisco, California 94143 Corresponding author. Hormone Research Center, School of Biological Sciences and Technology, Chonnam National University, Gwangju 500-757, Republic of Korea. Phone: 82-62-530-0509. Received August 7, 2003; Revised September 23, 2003; Accepted December 29, 2003.
36. Dell'Agli M, Galli GV, Vrhovsek U, Mattivi F, Bosisio E. In vitro inhibition of human cGMP-specific phosphodiesterase-5 by polyphenols from red grapes. *J Agric Food Chem*. 2005 Mar 23;53(6):1960-5.
37. Wang Y, Lee KW, Chan FL, Chen S, Leung LK. The red wine polyphenol resveratrol displays bilevel inhibition on aromatase in breast cancer cells. *Toxicol Sci*. 2006 Jul;92(1):71-7. Epub 2006 Apr 11.
38. Wang Y, Ye L, Leung LK. A positive feedback pathway of estrogen biosynthesis in breast cancer cells is contained by resveratrol. *Toxicology*. 2008 Jun 27;248(2-3):130-5. Epub 2008 Mar 29.
39. Kawabata K, Kawai Y, Terao J. Suppressive effect of quercetin on acute stress-induced hypothalamic-pituitary-adrenal axis response in Wistar rats. *J Nutr Biochem*. 2009 May 6. [Epub ahead of print]
40. Kuppusamy UR, Das NP. Potentiation of beta-adrenoceptor agonist-mediated lipolysis by quercetin and fisetin in isolated rat adipocytes. *Biochem Pharmacol*. 1994 Feb 9;47(3):521-9.
41. Excoffon L, Guillaume YC, Woronoff-Lemsi MC, André C. *Magnesium effect on testosterone-SHBG association studied by a novel molecular chromatography approach*. *J Pharm Biomed An*. 2009 Feb. 20: 49(2). 175-180.

42. Age-Related Eye Disease Study Research Group (2001). "A Randomized, Placebo-Controlled, Clinical Trial of High-Dose Supplementation With Vitamins C and E, Beta Carotene, and Zinc for Age-Related Macular Degeneration and Vision Loss. *Arch Ophthalmology*. 119(10): 1417.
43. Yamaguchi S, Miura C, Kikuchi K, Celino FT, Agusa T, Tanabe S, Miura T. Zinc is an essential trace element for spermatogenesis. *Proc Natl Acad Sci U S A*. 2009 Jun 30;106(26):10859-64. Epub 2009 Jun 18.
44. Yang JY, Della-Fera MA, Rayalam S, Ambati S, Hartzell DL, Park HJ, Baile CA. *Life Sci*. Enhanced inhibition of adipogenesis and induction of apoptosis in 3T3-L1 adipocytes with combinations of resveratrol and quercetin. 2008 May 7;82(19-20):1032-9. Epub 2008 Mar 21.
45. Van den Berghe G, de Zegher F, Wouters P, Schetz M, Verwaest C, Ferdinande P, Lauwers P. Dehydroepiandrosterone sulphate in critical illness: effect of dopamine. *Clin Endocrinol (Oxf)*. 1995 Oct;43(4):457-63.
46. Hazeldine J, Arlt W, Lord JM. Dehydroepiandrosterone as a regulator of immune cell function. *J Steroid Biochem Mol Biol*. 2010 Jan 12. [Epub ahead of print]
47. Balázs Z, Nashev LG, Chandsawangbhuwana C, Baker ME, Odermatt A. Hexose-6-phosphate dehydrogenase modulates the effect of inhibitors and alternative substrates of 11beta-hydroxysteroid dehydrogenase 1. *Mol Cell Endocrinol*. 2009 Mar 25;301(1-2):117-22. Epub 2008 Oct 25.
48. Swali A, Walker EA, Lavery GG, Tomlinson JW, Stewart PM. 11beta-Hydroxysteroid dehydrogenase type 1 regulates insulin and glucagon secretion in pancreatic islets. *Diabetologia*. 2008 Nov;51(11):2003-11. Epub 2008 Sept 9
49. Jana K, Samanta PK, Manna I, Ghosh P, Singh N, Khetan RP, Ray BR. Protective effect of sodium selenite and zinc sulfate on intensive swimming-induced testicular gamatogenic and steroidogenic disorders in mature male rats. *Appl Physiol Nutr Metab*. 2008 Oct;33(5):903-14.
50. G. Shoba, D. Joy, T. Joseph, M. Majeed, R. Rajendran and P.S. Srinivas Influence Of Piperine On The Pharmacokinetics Of Curcumin In Animals And Human Volunteers. *Planta Med*. (1998) 64(4):353-356.
51. Vladimir Badmaev, M.D., Ph.D., Muhammed Majeed, Ph.D. and Edward P. Norkus Ph.D. Piperine, An Alkaloid Derived From Black Pepper, Increases Serum Response Of Beta-Carotene During 14-Days Of Oral Beta-Carotene Supplementation. *Nutrition Research* (1999) 19(3) 381-388.
52. Vladimir Badmaev, M.D., Ph.D., Muhammed Majeed, Ph.D., and Lakshmi Prakash, Ph.D. Piperine Derived From Black Pepper Increases The Plasma Levels Of Coenzyme Q10 Following Oral Supplementation. *J. Nutr. Biochem*. (2000) 11: 109-113.
53. MARIEB, *Essentials of Human Anatomy and Physiology*, 4th Edition.
54. Godard MP, Johnson BA, Richmond SR. Body composition and hormonal adaptations associated with forskolin consumption in overweight and obese men. *Obes Res*. 2005 Aug;13(8):1335-43.
55. Tanis, D., Orrell, D. and Long, Dr. W. *The Effects of a Dietary Supplement, Free Test™, on Serum Free and Total Testosterone Levels in Weight-Trained Male Subjects*. Applied Nutriceuticals® In-House Pilot Work, April 2010, Copyright © 2010 by Applied Nutriceuticals® Lab Corp® and Applied Nutriceuticals® Research, Charlotte, North Carolina 28269