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The Effects of a Dietary Supplement, Free Test™, on Serum Free and Total Testosterone Levels in Weight-Trained Male Subjects

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Abstract

The objective of many weight-trained men is to raise circulating testosterone levels, both through training, and supplementation. In light of these facts, a dietary supplement formulation, Free Test™, was created, combining 3,7-Keto DHEA, Acetyl-L-Carnitine, N-Acetyl-Cysteine, Coleus Forskolii (Forslean®), Resveratrol, Quercetin Dihydrate, B-Vitamins, Magnesium, Zinc, Bioperine®, and Selenium. The product focuses on decreasing the homeostatic mechanisms that lower endogenous testosterone production, while at the same time allowing for sustained, safe increases in free and serum testosterone levels. The purpose of this study was to determine the effects of Free Test™ on serum hormone levels in resistance trained males. **Methods:** 6 men (mean age, 32.5 yr. ± 10.01, range 18–49 yr) with at least 4 years of weight-training experience were studied. Total (TT) and free (FT) testosterone levels were measured via blood work (LabCorp®) on two separate occasions before (Pre) and after (Post) having taken the dietary supplement, with serum and free testosterone being measured on days (d)= Day 0 and Days 21-61 (mean time period 31.6 d ± 14.87, range 21-61 d). One (1) blood draw per analysis period was taken; Two (2) total draws per subject over two separate days, and during the trial period, subjects were given a 4 capsule daily dosage of the supplement. **Results:** Each of the 6 weight-trained men (100%) had significant increases in free testosterone levels after 21-61 days of ingesting a 4 capsule daily dosage of Free Test™ when compared to baseline values. Average baseline FT was 9.55 pg/mL ± 1.80 (SD). Average FT following administration of the supplement was 16.98 pg/mL± 5.2, a 78% increase in FT ($p<0.10$) values for the group.

Introduction

Numerous studies have documented the anabolic effects of testosterone on skeletal muscle, and this has led to a huge increase in dietary supplement products on the current market that claim to raise testosterone levels and improve body composition. Many testosterone-enhancing supplements have been shown to be either ineffective or dangerous, due to various factors. Some of these products do indeed increase testosterone, but many of the most effective products (Anabolic Steroids and Prohormones) in turn have the most unsafe side effects. Prohormones (PH) and AAS are extremely effective in increasing androgen levels, as they directly bind the androgen receptor (AR), which is the most direct way of increasing testosterone levels. However, as aforementioned, these products can also cause some deleterious side effects, and recently the United States FDA has cracked down on the manufacture of this type of products, and has openly and actively pursued product recalls against companies that distribute prohormones. Similarly, many non-PH and AAS-based testosterone boosters also exist on the current market. Unfortunately, many of these products are completely ineffective, and use a hodgepodge of low extraction-percentage herbals, ineffective or unproven anabolic compounds and/or amino acids. Most of these products use a “kitchen sink” formula, and claim that their combination is “scientifically proven” to work. Some herbal components boost testosterone levels, but most of the time, it is only when taken in completely unattainable dosages that would make a normal person extremely sick or make the product outrageously expensive to produce.

Therefore, an opportunity exists to create a product that can provide a safe way to increase testosterone levels, while being compliant with the Dietary Supplement and Health Act (DSHEA) and other current United States regulatory guidelines. The goal of Free Test™ was to create a formula that focuses on decreasing the homeostatic mechanisms that lower endogenous testosterone production, while at the same time allowing for sustained increases in testosterone, and its positive subsequent effects on lean body mass. The formulation is intended to work not by stimulating the androgen receptor (AR) and having direct androgenic activity, but by manipulating factors that can increase or decrease TT and FT in the body. TT is nearly 100% bound by several different factors- 40% is bound by Steroid Hormone Binding Globulin

(SHBG, a β-Globulin that binds hormones such as testosterone and estrogen), 40% is bound by albumin, and 17% is bound by other proteins. The remaining testosterone is considered to be FT, or the unbound, biologically active component of testosterone (TST). Once in the blood, FT can be bound by the AR, and converted to dihydrotestosterone (DHT), or converted to estradiol, via the aromatase enzyme (29).

The product formulation seeks to increase FT via several different indirect means:

- Via Improving the Testosterone:Estrogen (T:E) profile and concurrently increasing testosterone levels through using a naturally-occurring aromatase inhibitor (21-22,27)
- Through Increasing Nitric Oxide (NO) and cyclic guanosine monophsophate (cGMP) levels, which lead to increases in luteinizing hormone (LH), which is a trigger for greater endogenous testosterone production (1-4,15, 27-28).
- Lowering levels of certain cytokines- many cytokines are inflammatory agents that can inhibit endogenous testosterone production by reducing levels of lutenizing hormone (LH) and decreasing testicular function (25-26,33-34)
- Increasing cyclic adenosine monophosphate (cAMP) levels, which also increase LH levels (14, 16,17)

The purpose of this pilot work is twofold, in that it allows the researchers to establish that the product does indeed increase TT and FT in a directional manner, and can be used safely for varying periods of time (measured via questionnaire).

Methods

Subjects

Six men between the ages of 18 and 49 yr (mean age, 32.5 ± 10.01) were studied. Eight men were initially chosen; however, one patient had to be excluded because of a pre-existing history of thyroid dysfunction, and another had to be excluded due to the use of an anabolic substance 4 weeks prior to the test. No patients had a history of glucocorticoid or anticonvulsant use, diabetes mellitus, gastrointestinal disease, gastrointestinal surgery, acromegaly, malignancy, or any other known metabolic disease. No patient had a history of alcoholism. All subjects were required to have at least 4 years of weight training experience. There was no history of pubertal or adult testosterone deficiency or growth disturbance, no history of delayed puberty, and no history of pituitary disease or deficiency. As aforementioned, subjects who had used various prohormone derivatives or pharmacologic agents such as anabolic steroids within 6 weeks prior to the study were not allowed to participate. All subjects gave written informed consent, and a questionnaire was given to all subjects regarding how well they tolerated the supplement, protocol for the trial, and also a section where any adverse events or symptoms could be reported (29,31,37).

List of Exclusionary Substances

Refer to WADA 2009 Guidelines for entire list (35)

Testing Sessions

There were two separate days of testing: Baseline Testing Day (Pre), where each subject had TT and FT measured via blood draw, and Testing Day 2 (Post), where subjects had FT and TT measured via blood draw after having received a 4 capsule daily dosage of the product over a varying time interval (Range 21-61 days). The subject questionnaire was returned after the second blood draw for each subject (29,31,37).

Blood Collection and Assay Methods

Blood samples were taken from an antecubital vein into a 10 ml collection tube, with a 2 mL sample. The samples were handled via Lab Corp® protocol 140103, being allowed to stand at room temperature for 10 min and then centrifuged. The serum was removed and frozen for later analysis. Measured via Lab Corp® protocol 140103 Testosterone,Free (Direct), Serum with Total Testosterone. Routine serum biochemical measurements were made using standard techniques. FT was measured by direct analog/ radioimunnoassay (RIA), and TT was measured by Electrochemiluminescence Immunoassay (ECLIA). FT values were measured in pg/mL; TT values were measured in ng/dL (36).

Supplementation Protocol

Subjects were randomly assigned differing lengths of trial supplementation for the product. Two subjects were assigned a 20-22 day trial, three subjects were assigned a 28-30 day trial, and one subject was assigned a 60-62 day trial. The reasoning behind the variable length trials between subject groups was to see if the formulation was sound for elevations in TST over longer, medium, and shorter duration, and to get an idea of whether or not the supplement could be used safely and effectively over varying periods of time. Subjects were instructed to orally ingest 4 capsules upon waking in the AM, preferably with food. During the supplementation period, the physical activity and diet of the subjects was not monitored. Instead, each participant was instructed not to change any of their dietary habits and physical training regime during the trial period (18-19,29,31,37).

Reported Side Effects from Trial

After completing their final blood draw, subjects reported by questionnaire any issues they may have had with the supplementation or the protocol. Subjects were also asked to list any kind of medical side effects that might have occurred during the research.

Statistical Analysis
Due to the nature of pilot research (low number of subjects and only two variables measured)- the design of the study is a quasi-experimental One Group Pretest-Posttest Design. Therefore, a t-test paired for pretest-posttest scores for both TT and FT was utilized. All statistical procedures were performed using GraphPad software and a probability level of $p < 0.10$ was adopted throughout the study (37).

Results

Posttest Subject Questionnaire

Subjective analysis of the posttest subject questionnaire found that all 6 participants appeared to have exhibited full adherence to the supplement protocol, and were able to finish the required dosing regimen and testing procedures with no side effects. Evaluation of the questionnaires also revealed no noticeable changes in dietary habits or physical activity levels during the trial period.

Serum FT and TT Levels

Compared to baseline, posttests showed FT levels to be significantly higher, indicating a significant main effect for FT ($p = 0.092$). TT levels increased, but the increases were not enough to be deemed significant ($p = 0.1484$) (37).

Table 1: FT Levels Baseline v. Post

Group	Baseline FT	FT After Free Test™ Supplementation
Mean	9.550 pg/mL	16.983 pg/mL
SD	1.807	5.204
SEM	0.738	2.125
N	6	6

Table 2: TT Levels Baseline v. Post

Group	Baseline TT	TT After Free Test™ Supplementation
Mean	436.67 ng/dL	649.83 ng/dL
SD	200.60	266.31
SEM	81.90	108.72
N	6	6

Table 3: Individual Values By Subject- TT

Subject	Age	Duration of Usage	Baseline TT	TT Post
1	29 yr	21 d	450 ng/dL	998 ng/dL
2	34 yr	21 d	306 ng/dL	425 ng/dL
3	18 yr	61 d	689 ng/dL	983 ng/dL
4	34 yr	29 d	321 ng/dL	516 ng/dL
5	31 yr	28 d	196 ng/dL	458 ng/dL
6	49 yr	30 d	658 ng/dL	519 ng/dL

Table 4: Individual Values By Subject- FT

Subject	Age	Duration of Usage	Baseline TT	TT Post
1	29 yr	21 d	11.7 pg/mL	25.3 pg/mL
2	34 yr	21 d	10.4 pg/mL	13.0 pg/mL
3	18 yr	61 d	11.0 pg/mL	17.1 pg/mL
4	34 yr	29 d	9.3 pg/mL	18.3 pg/mL
5	31 yr	28 d	7.5 pg/mL	18.1 pg/mL
6	49 yr	30 d	7.4 pg/mL	10.1 pg/mL

Discussion

In this research, we sought to find the effects of Free Test™ supplementation at 4 capsules per day over varying time periods on TT and FT. No adverse side effects were reported by the subjects, and subjective data gathered from posttest questionnaires found that Free Test™ was well-tolerated over time periods ranging from three to nine weeks.

In reference to changes in TT, while subjects did show a directional increase, but this increase was not significant (20). The significant change over the course of the pilot was in FT, which underwent an increase of 78%. The increases in FT were most likely related to several different mechanisms of action in the product. Free Test™ contains Acetly-L-Carnitine, which has been shown to increase nitric oxide (NO) and cyclic GMP (cGMP) levels via elevating acetylcholine levels. NO is important in that it regulates vascular tone, CNS stimulation, and, most importantly, induces the release of luteinizing hormone releasing hormone (LHRH) and regulates cyclic guanosine monophosphate (cGMP) levels. NO also activates the release of LHRH which reaches the pituitary and activates the release of luteininzing hormone (LH) via the activation of neural NO synthase (NOS) in the pituitary gland (17,18,30,32).

Similarly, the fact that the product increases the action of NO and cGMP are important one, as both entities have a stimulatory action on steroidogenesis via increased LH production. Therefore, high cGMP levels also equate to high levels of LH, and when cGMP levels are elevated, it serves as an intermediate in the signaling cascade that ranges from luteinizing hormone (LH) binding to testosterone production. In numerous pathway studies, increases in cGMP increased phosphorylation of the steroidogenic acute regulatory protein (StAR). Steroidogenic acute regulatory protein (StAR) is a Leydig cell cholesterol transfer protein that provides the building blocks for testosterone synthesis. StAR activation is necessary

for the stimulation of steroidogenic enzymes involved in the transfer of cholesterol to testosterone. These results suggest that cGMP contributes to the control of basal steroidogenesis (endogenous testosterone production) in Leydig cells through the PKG-dependent modification of the StAR protein and interaction.with LH. Even more important is the fact that LH, via receptors found on the surface of Leydig cells, controls the production and secretion of testosterone. The subsequent binding of LH with its receptor allows signalling through the cyclic AMP pathway via 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. This is most likely one of the mechanisms that contributed to increased FT (1-3,15-16,32).

Another contributing factor to increased FT has to do with This occurs primarily through elevation of 3,5 cyclic adenosine monophosphate (cAMP), a second messenger important in hormone signaling, via forskolin. One study (Bristow et al, 1984) showed that forskolin was able to increase cAMP levels 4.82 times more than a placebo. Increased cAMP production is responsible for the activation of protein kinase A, which is an enzyme that has positive effects androgen receptor binding *even in the absence of other androgens*. 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). By this process, and the fact that high cAMP levels also equate to high levels of luteinizing hormone (the hormone responsible for mediating endogenous spermatogenesis), significant increases in endogenous testosterone production may occur, along with a resulting increase in anabolism and protein synthesis. A 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 (5-12).

The product also seems to have a strong anti-oxidative effect on the testes, which can allow the testes an optimal environment for testosterone production. The combination of N-Acetyl-Cysteine and selenium has been shown in recent studies to cause elevations in FT. 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, and several types of cytokines have been linked to testicular impairment via decreasing LH levels. 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 alone can allow for conditions that are extremely favorable for optimal testosterone production (13,25-26,33).

The increase in FT levels may also be linked to the inclusion of a natural aromatase inhibitor in the product. 3-Desoxy 7-Keto DHEA has demonstrated strong ability to lower estrogen, as It has shown a high binding affinity (Ki value = 0.22 mM) to the aromatase enzyme, and binds in an irreversible manner. 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- a balance referred to as the Testosterone:Estrogen (T:E) ratio. 3,7-Keto DHEA is unique from other commonly available aromatase inhibitors on the sports supplement market, in that it is a natural metabolite of 7-Keto DHEA, and cannot directly bind to the androgen receptor (AR). 3,7-Keto DHEA (like 7-Keto DHEA) also cannot convert to testosterone, estrogen, or progesterone via any type of enzymatic reaction, so it cannot be considered a prohormone.

Another recent problem with commonly available aromatase inhibitors on the supplement market is the direct conversion of the compound to a controlled substance in the body or during synthesis, either in trace amounts, or full-scale conversion. This does not occur with 3,7-Keto DHEA, as it is formed naturally under various conditions in humans from 7-Keto DHEA and can be readily found in humans in the amount of 5-7 ug/day. 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 but the brain is essentially tricked into trying to produce more estrogen, so it releases more LHRH and subsequently LH, leading to even higher testosterone levels (23-24).

Contributions

DT designed the study, drafted the manuscript, supervised coordination and data acquisition, and performed the statistical analysis. DO and DL participated in the data acquisition.

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Can you have them forward our department with any (preferably full text) studies illustrating toxicology, efficacy, and sourcing (DSHEA compliance) data for the active ingredient 3,7 KETO DHEA?

Also, it'd be great to know what methods were used to demonstrate a 200% increase in free test levels. I notice the D. Orrell name from this email string is the same as referenced on the bottle—is the email from someone who co-authored a study or conducted an in-house test? I cannot find it in any of the databases used to conduct these types of inquiries.