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# The genetic influence on the placebo effect specific to exercise performance

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**Abstract**

Placebo treatments can be used to elicit many different physiological responses; however, the underlying mechanisms responsible remain unclear. Recent research has shown the possibility of a genetic influence on the placebo response in patients with mood disorders. In this study, we attempted to establish a similar relationship in healthy college-aged students. Force production was measured by isometric knee extension of the quadriceps muscles using maximum voluntary contractions (MVC). Subjects were given placebo treatments disguised as an undisclosed sports supplement with the information that the supplement was previously shown to provide immediate strength improvements following ingestion. Subject DNA was genotyped for two genetic polymorphisms, tryptophan hydroxylase-2 (TPH2) and monoamine oxidase A (MAO-A). These particular polymorphisms were chosen for study based on previous research and their possible relationships to athletic performance. Results showed a 4.4% improvement in peak force with the ingestion of the placebo for both men and women ($p < 0.05$). We also found that the average placebo effect was similar for both genders (3.37% improvement in males, 7.47% in females). Neither polymorphism displayed a significant effect on the presence of the placebo response. We concluded that while a placebo response was evident with MVC isometric force production, TPH2 and MAO-A were not likely to be responsible for the effect.

**Chapter I: Introduction**

The most common protocol for proving the efficacy of a new drug or supplement is to design a randomized, double-blind study that allows the effects of the drug or supplement to be compared to those of a placebo. If the drug or supplement provides more positive or beneficial results than the placebo, the drug or supplement is said to be effective. Until recently, placebo effects have always been attributed to biological or psychological factors yet to be fully identified (24). However, recent research has evaluated specific genetic polymorphisms predisposing responders to placebo treatments. Furmark et al. monitored amygdala response in participants with social anxiety disorder (SAD) (6). The amygdala is the portion of the brain that monitors and processes emotions. Decreased activity in the amygdala is associated with low amounts of stress; SAD patients strive to achieve this through medications and therapy. Furmark et al. observed the greatest placebo response with subjects who carried the G allele in the tryptophan hydroxylase-2 (TPH2) gene (6). These same subjects experience a naturally lower amount of amygdala activity in the brain as a result of their genotype. Similarly, Leuchter et al. studied patients diagnosed with major depressive disorder (MDD) and found that subjects with a moderate or intermediate rate of monoamine oxidase A (MAO-A) enzymatic activity experienced the greatest degree of placebo response (11). MAO-A is an enzyme that facilitates the catabolism of norepinephrine, a process that can lead to symptom relief for MDD patients. The intermediate rate of enyzme activity was found in females with the heterozygous G/T expression of the gene. Because of the sex-linked nature of this polymorphism, males can only code for the high-rate expression (G allele) or low-rate activity (T allele).

Research has shown that placebos are capable of producing similar or greater effects than many sports supplements. Beedie et al. demonstrated that athletes given a hypothetical ergogenic aid coupled with positive information regarding the substance maintained sprint times in a repeat-sprint trial of 30 meters, whereas negative information led to trial times 2% slower than baseline values (1). Clark et al. evaluated differences in power output during 40-km cycling time trials when subjects were given a placebo versus a carbohydrate supplement dissolved into a drink (4). Subjects in each supplement group were further divided into three subgroups based on what each subgroup was instructed about their particular drink contents: told carbohydrate, told placebo, and not told. Subjects who were given the placebo but were told they received the carbohydrate beverage experienced an average of 7% improvement in power, the greatest change out of any subgroup. Pollo et al. showed that the perception of taking an ergogenic aid could increase quadriceps muscle performance and decrease muscle fatigue (17). Two groups were used: a supplement group and a control group. Subjects were not blinded to which group they were assigned to; however, neither group actually received any form of an ergogenic aid. Muscle performance was assessed over the course of four trials as the number of repetitions generated by the quadriceps at 60% of 1RM and the total work performed in each session. Without informing the subjects, trials 2 and 3 were actually performed at 45% of 1RM to further the deception of the efficacy of the ergogenic agent. Compared with baseline data, the supplement group showed an improvement of 22%. Countless other studies have shown significant placebo responses to ergogenic aids; however, the potential genetic effect on the placebo response to an ergogenic aid has not been evaluated (1, 2, 4, 5, 8, 12, 14, 17). The primary objective of this study, therefore, is

to determine if a genetic link exists between a specific genetic polymorphism and the ergogenic response to a placebo.

More specifically, we seek to determine if a genetic link exists between a person's susceptibility to a placebo purported to be an ergogenic aid and either the monoamine oxidase A or the tryptophan hydroxylase-2 gene polymorphisms. These polymorphisms have been chosen because of the promising data shown previously (6, 11). Furthermore, because of their relations to emotional processing and pain relief, both polymorphisms may play a role in athletic performance. The implications of establishing such a connection are considerable. Particularly, if certain people are genetically inclined to react to a placebo, the "gold standard" of drug testing with comparison to a placebo may no longer apply without prior genotype screening of participants. Also, if medical conditions can be treated and alleviated with a placebo, a moral issue arises of whether or not to lie to a patient about a treatment. The two major studies focusing on genetics and the placebo response that were previously mentioned focused on patients with mood disorders. To date, no study has yet examined the placebo effect in healthy subjects or in conditions where the placebo is supposed to elicit improvements in physical performance. Therefore, this study will compare the maximum voluntary contractions (MVC) of the quadriceps muscles in physically active subjects on two separate occasions – with and without ingestion of a supposed performance-enhancing supplement. Results will then be characterized by the genotypes of the subjects to determine if there is a genetic link.

# Chapter II: Background

## I. Eliciting placebo response through deception in athletic performance

| Author/Year | Sample Size | Training Status | Intervention | | Findings |
|---|---|---|---|---|---|
| | | | **Informed** | **Received** | |
| Maganaris et al. 2000 (12) | 11 | Elite weightlifters | Trial 1 – both groups given anabolic steroid; Trial 2 – Group 1: anabolic steroid, Group 2 - placebo | Placebo for all groups and trials | 3.8% improvement in strength performance when subjects believed they were given steroids |
| Clark et al. 2000 (4) | 43 | Sub-elite endurance cyclists | Group 1 – carbohydrate beverage; Group 2 – non-caloric sweetener beverage; Group 3 – 50:50 chance of receiving carbohydrate beverage | Each group randomized – half received carbohydrate, half received placebo | 4.3% increased mean power when subjects believed they were ingesting carbohydrate and were given placebo |
| Beedie et al. 2006 (2) | 7 | Competitive cyclists | 0 mg/kg, 4.5 mg/kg or 9.0 mg/kg of caffeine | Placebo | 2.2% increased power output when subjects believed they had ingested caffeine |
| Beedie et al. 2007 (1) | 42 | team-sport athletes | Group 1 – repeat-sprint and endurance performance enhancer Group 2 – endurance performance enhancer, negative impact on repeat-sprint performance | 200mg of cornstarch | Group 1(with positive information regarding treatment) – no difference in times between baseline and experimental data; Group 2 (with negative information regarding treatment) – ran 1.7% slower than baseline data |
| McClung and Collins 2007 | 17 | Sub-elite endurance | Treatment 1 – sodium | Treatment 1 – sodium | 1.5% improvement in 5,000m time |

| | | | | | |
|---|---|---|---|---|---|
| (14) | | athletes | bicarbonate; Treatment 2 – sodium bicarbonate; Treatment 3 – no treatment; Treatment 4 – no treatment | bicarbonate; Treatment 2 – placebo; Treatment 3 – sodium bicarbonate; Treatment 4 – no treatment | trials with placebo |
| Kalasountas et al. 2007 (8) | 42 | Untrained students | Trial 1 – amino acids; Trial 2 – Group 1: amino acids, Group 2: no treatment (negative information regarding supplement) | Placebo for all groups and trials | 19.6% improvement in strength performance with positive information regarding supplement |
| Pollo et al. 2008 (17) | 44 | Sub-elite athletes | Caffeine | Placebo | Two experiments: Leg extension strength increased by 11.8% without conditioning, 22.1% with conditioning |
| Foad et al. 2008 (5) | 14 | Sub-elite cyclists | Treatment 1 – caffeine; Treatment 2 – caffeine; Treatment 3 – no treatment; Treatment 4 – no treatment | Treatment 1 – caffeine; Treatment 2 – placebo; Treatment 3 – caffeine; Treatment 4 – no treatment | 0.7% improvement in mean power with placebo |

Placebos have been shown to enhance performance for a variety of athletic performances. Placebo treatments can improve endurance performance time trials with both running and cycling (1, 14). Strength performance can also improve when subjects believe that a supplement will benefit their power output (2, 4, 5, 8, 12, 17). Maganaris et al. found that giving placebo steroid treatments to elite weightlifters collectively improved 1RM of bench press, dead lift, and squat by 3.8% (12). Beedie et al. found that

mean power in a 10km cycling time trial increased 1.3% when subjects believed they had ingested 4.5 mg/kg of caffeine and 3.1% when subjects believed they had ingested 9.0 mg/kg of caffeine (2). Clark et al. found an improvement of 4.3% in mean cycling power during a 40km time trial in response to a placebo carbohydrate beverage (4).

Interventions in these studies usually inform the subjects that one of several widely accepted ergogenic aids – such as steroids, caffeine, sodium bicarbonate, carbohydrate, etc. – will be given to improve athletic performance. It seems that the greatest effects are also seen when subjects receive positive information or reinforcement regarding the supplement (1, 8). Conversely, negative information can lead to a negative impact on performance (1). Very little data has been seen on the ability to elicit a placebo effect using a supplement that is unfamiliar to subjects. Using aids that are familiar to subjects may allow for preconceived notions regarding such supplements to dominate the subjects' response to the placebo treatment and thus contribute to the high degree of variability seen in placebo studies. By using an unknown sport supplement, every subject will begin at baseline knowledge and will hopefully negate the effects of prior beliefs.

Several other contributors may influence the magnitude of placebo response, including sample size, subject training status, and method of performance testing. In most cases, a larger sample size seems to relatively magnify the percent of change for placebo treatments (4, 8, 17). Perhaps a larger sample size is needed to clearly identify if a placebo response exists. Also, the differences in training status may greatly affect the outcome of each study. In several studies, sub-elite athletes were used. When compared to elite and competitive athletes, these subjects have a greater room for improvement,

allowing for a greater chance of variability and change in performance measures. Finally, the procedures utilized in these studies are not standardized. It is very difficult to compare performance measures for strength improvements in 1RM to 40km cycling time trials. Furthermore, our protocol will utilize untrained, recreationally active students and will use force production strength measures to quantify the degree of placebo response. By doing so, it will be much easier to compare any performance improvements seen to existing data and will help with interpreting the results of testing.

## II. Genetic determinants of placebo response

| Author/Year | Disorder | Treatment | Effect of Treatment | Genetic Polymorphism Identified |
|---|---|---|---|---|
| Furmark et al. 2008 (6) | Social Anxiety Disorder (SAD) | study drug provided by GlaxoSmithKline or placebo | reduced anxiety associated with public speaking | TPH2 gene promoter: homozygous GG - greatest response |
| Leuchter et al. 2009 (11) | Major Depressive Disorder (MDD) | placebo, or 1 of 3 commonly prescribed antidepressants (fluoxetine, venlafaxine, or sertraline) | alleviated symptoms of depression | monoamine oxidase A (MAO-A): GG or G - lowest response, GT – greatest response; Val-Met catechol-O-methyltransferase (COMT): Met-Met - lowest response, Val-Met or Val-Val – higher response |

A genetic polymorphism exists when a particular gene can be clearly expressed by two or more phenotypes or morphological traits. Each polymorphism can be identified according to the coding sequence found on DNA in the region specific to each particular gene. In two separate studies, patients with mood disorders that possessed specific alleles in two known genetic polymorphisms experienced a greater degree of placebo response than patients who were lacking those same alleles (6, 11). The specific

polymorphisms in question were the G-703T polymorphism of the tryptophan hydroxylase-2 (TPH2) gene promoter in patients with social anxiety disorder and GT polymorphism of the monoamine oxidase A (MAO-A) enzyme. Furmark et al. studied subjects that were diagnosed with social anxiety disorder (SAD), which is characterized by anxiety and fear from the judgment of others (6). The symptoms of SAD can be treated with medication to alleviate anxiety by reducing stress-related activity in the amygdala of the brain. Placebo treatments, disguised as SAD medications, have also been shown successful in treating SAD. Patients who carry the T allele in the TPH2 gene tend to naturally display a higher amount of amygdala activity then patients who are homozygous for the G allele. It was observed that patients homozygous for the G allele demonstrated the greatest response to placebo treatments with regard to SAD symptom alleviation. Leuchter et al. evaluated subjects diagnosed with major depressive disorder (MDD), a mood disorder characterized by chronic depression associated with symptoms of sadness, loss, anger, or frustration (11). Monoamine oxidase A (MAO-A) is an enzyme that facilitates the catabolism of norepinephrine. The sex-linked gene that codes for MAO-A determines the rate of enzymatic activity: males with a single T allele or females with T/T demonstrate the lowest-activity rate; G/T females have a moderate or intermediate rate; G males or G/G females show the highest-activity rate. In patients with MDD, the highest activity of MAO-A (G or G/G alleles) showed the lowest response to placebo treatments. The intermediate rate of MAO-A activity, coded by the G/T alleles, showed the most promising response to placebo treatments.

Current research findings cannot be generalized to a greater population because the only research performed thus far has focused on patients with mood disorders.

Specifically, it is unknown if genetic polymorphism can influence individuals without mood disorders. Furthermore, it is unknown if these polymorphisms would influence the placebo response in other situations, such as athletic performance. Thus the present study will look at healthy subjects who are recreationally active to see if a similar genetic link can be established for placebo effect.

### III. Reliability and validity of knee extension MVC through isometric testing

| Author/Year | Sample Size | Tool Utilized | Intersession Correlation |
|---|---|---|---|
| Bohannon 1986 (3) | 30 | Handheld dynamometer | 0.98 |
| Stuberg and Metcalf 1988 (20) | 14 | Handheld dynamometer | 0.98 |
| Rainoldi et al. 2001 (18) | 9 | Specially designed bed with force transducer | 0.70 |
| Larsson et al. 2003 (10) | 20 | Isokinetic dynamometer | 0.93 |
| Symons et al. 2005 (21) | 19 | Biodex | 0.91 |
| Kelin et al. 2008 (9) | 20 | Handheld dynamometer | 0.79 |

A maximum voluntary contraction (MVC) is the peak force produced by one single contraction. Knee extension isometric MVCs have been accepted as a reliable measure of muscular strength. Most research on the reliability of isometric MVC testing has shown a high degree of correlation between trial sessions on different days (3, 10, 20, 21). This shows a great amount of repeatability with minimal day-to-day variability in maximal force production.

Many factors can contribute to variations and limit correlation. Maintaining the same position for each test repetition is crucial. Bohannon manually braced each limb during testing, which could contribute to intersession variance (3). Less controllable factors such as subject moods or degree of motivation can also add day-to-day differences (18). Kelin et al. evaluated intratester and intersession reliability by utilizing three separate testers of varying degrees of experience in using a handheld dynamometer (9). Both intratester and intersession results showed high correlation values and low standard error of measurements. However, it was observed that handheld dynamometers were likely to be contraindicated in any instance where the strength of the subject may overpower that of the tester. For example, it was discovered during pilot testing that the subjects' strength in plantar flexion was greater than the testers' ability to resist the motion (9). This could not be remedied in any way by changing the angle at which the test was being administered, so plantar flexion strength could not be evaluated by this method. Therefore, the most ideal method for using these dynamometers may be to place the subjects in a position that offers a greater mechanical advantage to the tester. Symons et al. used a Biodex to test single-session repeatability in older men (21). The coefficient of variation ranged from 8 to 17% for peak torque (21). The most notable aspect about this protocol was the system developed by the tester. Every subject was given verbal instructions regarding the testing procedure. His or her leg was then moved passively through the range of motion. Once completed, each subject had the opportunity to practice the test before the actual recorded test was administered. Throughout the entire protocol, the subject was given consistent encouragement. In order to standardize this procedure, it is important that the tester provide consistent and similar encouragement to

every subject.  Verbal feedback regarding specific force data should not be given as it could change the subject's motivation for the next test.  The use of the Biodex also limits the variability created by using the handheld dynamometers by facilitating reproducible setups.

### IV. Proposed mechanisms for the placebo effect

| Author/Year | Treatment type | Proposed mechanism |
|---|---|---|
| Voudouris et al. 1989 (22) | Analgesic cream | Classical conditioning |
| Voudouris et al. 1990 (23) | Analgesic cream | Classical conditioning |
| Montgomery and Kirsch 1996 (16) | Topical anesthetic | Classical conditioning, Expectancy theory |
| Mayberg et al. 2002 (13) | Antidepressant medication | Expectancy theory |
| McRae et al. 2004 (15) | Transplantation of human embryonic dopamine neurons in patients with advanced Parkinson's disease | Expectancy theory |
| Wager et al. 2007 (25) | Analgesic cream | Expectancy theory |

Two main theories have been proposed to explain the psychological mechanism for the placebo effect: classical conditioning and expectancy theory.  Classical conditioning, as introduced by Ivan Pavlov in 1927, states that a response can be learned when the same stimulus is applied repeatedly (19).  When the subject is properly conditioned, a conditioned response will occur when the same stimulus is applied.  The famous example of classical conditioning, Pavlov's dogs, demonstrated the dogs' conditioned response (salivating) in response to the stimulus (bell) that had previously been associated with the serving of food.  Expectancy theory states that a placebo will elicit a particular response simply because the recipient believes that it will (19).  Such

expectations can result from advertisements, support from credible people (such as doctors, scientists, etc.), or referrals made by trusted family or friends.

In two separate studies, Voudouris et al. placed subjects into groups to determine which theory was responsible for the placebo effect (22, 23).  In the 1989 study, two groups of subjects were instructed that an analgesic cream would reduce skin sensitivity and block pain (22).  Three trials were established where the pain stimulus was incrementally increased until the subject could no longer tolerate the pain.  They were then treated with the cream and the stimulus was again applied until it was no longer tolerable.  During the second trial, the rate of increment was decreased for group I and increased for group II without the subjects' knowledge to simulate the conditioning phase.  Despite the expectancy that the cream would diminish the pain, group II subjects showed a decrease in mean pain tolerance as a result of the learning that occurred in trial 2.  These results were confirmed by the second study published in 1990.  Using a different methodology that both separated expectancy and conditioning and combined the two, it was found that conditioning alone elicited a greater placebo response than expectancy alone (23).

Mayberg et al. focused only on the expectancy theory (13).  In treating clinically depressed men, half of the men who experienced symptom remission had been given placebos (13).  No conditioning was performed on these subjects, but they were clearly instructed on how the treatment was supposed to improve mood.  Similarly, McRae et al. found that out of the 30 subjects, the 18 that received a sham surgery versus a neuron transplantation surgery experienced greater improvements in quality of life than the 12

who actually received the transplant (15).  Again, only the expectation that the treatment should work was responsible for the outcome.

It seem that the literature is unclear whether one theory holds greater responsibility than the other.  Stewart-Williams and Podd suggest that perhaps both play a key role in facilitating a placebo response (19).  One can also function independently of the other, as seen in the studies by Mayberg et al. and McRae et al. (13, 15).

Very little research has been conducted on the physiological mechanisms surrounding the placebo response.  Wager et al. has shown that despite the lack of a pharmacological treatment in placebo pain therapy, a physiological effect can still take place (25).  Similar to the body's response to pain medication, the expectancy of a treatment can elicit opioid release that relieves pain.  However, little is known about why or how this occurs.

**Chapter III: Methods**

**Subjects.**

Informed consent was obtained from 54 subjects (34 male, 20 female), aged 18-22. Subjects were recreationally active (minimum: 1 bout of exercise per week for 30 minutes) college students from James Madison University. Upper level Kinesiology/Exercise Science students with prior knowledge of typical supplement testing procedures were excluded from participation to protect the deception necessary in this study.

**Study Design.**

Subjects participated in three trials on separate occasions. The first was a familiarization trial. Subjects were asked to perform a number of maximum voluntary contractions (MVC) of the quadriceps muscle using a custom-built muscle function device designed at James Madison University. The test was performed with the subject seated upright with the self-reported dominant leg positioned at approximately 70° of knee flexion. Each MVC was held for 3 seconds against the stationary bar, and force production was measured from a force transducer for each contraction. A minimum of three repetitions with a maximum of eight were used to determine maximal force output for the trial until two numbers were generated within 20N of each other, and the results were averaged.

The same MVC protocol was applied during the two subsequent treatment trials. Prior to each trial, subjects ingested 50 mL of a commercial sports beverage. On one occasion, the subjects were told that the drink contained an ergogenic aid already

dissolved into the drink. On the other occasion (control trial), the subject was told that the drink did not contain the ergogenic aid. Nothing was actually dissolved into the drink on either day. MVC tests were conducted immediately following ingestion and a 5-min warm-up (3mph on treadmill). The order of the treatment trials was randomly counter-balanced.

To prevent subjects from attempting to research the ergogenic aid thought to be used in this study, subjects were informed that the supplement was still in its testing phase and was not readily available on the market to consumers.

**Genotyping.**

Blood samples were obtained from each subject during the Familiarization Trial. DNA was extracted from whole blood using a Qiagen kit following the manufacturer's protocol (Qiagen, Valencia, California). DNA samples were sent to the Center for Genetic Medicine Research in Washington, DC for genotyping. Specifically, the genotyping was conducted for MAO-A and TPH-2 gene promotor. Genotyping was blinded to subject, treatment, and treatment response.

*MAOA* **T941G Polymorphism**

To determine the presence of T- or G- allele located at mRNA position 1072 in the coding sequence of the *MAOA* gene (Gene ID: 4128), a PCR product was amplified using forward primer 5′-GAC CTT GAC TGC CAA GAT-3′ and reverse primer: 5′-CTTCTTCTTCCAGAAGGCC-3′ with methods developed by Hotamisligil and Breakefield (7). The polymerase chain reaction (PCR) was performed according to the manufacturer's instructions (Accuprime PCR kit, Invitrogen, US). PCR reactions (total

volume: 20 µl) contained 50g DNA, Buffer II (40 mM Tris-HCl [pH 8.4], 100 mM KCl,

3 mM MgCl$_2$, 400 µM dGTP, 400 µM dATP, 400 µM dTTP, 400 µM dCTP, 1U

AccuPrime *Taq* DNA Polymerase, thermostable AccuPrime protein, and stabilizers)

(Invitrogen), 0.1 mM/L of each primer. After the initial denaturation step at 94°C for 2

min, DNA was amplified in 35 PCR cycles (94°C for 30 sec; 60°C for 30 sec; 68°C for 1

min). Ten microliters of the PCR product was digested with 3U *Fnu IV* (New England

Biolabs, US), analyzed by gel electrophoresis in a 2% agarose gel containing ethidium

bromide, and visualized under UV light. When a G allele is present, digestion results in

two fragments of 65 bp whereas the absence of the *Fnu IV* recognition site (GCNGC)

leaves the 130-bp PCR product intact.

### *TPH2* (-G703T) Polymorphism

The *TPH2* -G703T (rs4570625) is located in the putative transcriptional control

region of *TPH2* (Gene ID: 121278). PCR was performed with the forward primer 5′-

TTTTATGAAAGCCATTACACAT-3′ and the reverse primer 5′-

TTCCACTCTTCCAGTTATTTTA-3′ developed by Furmark et al. (6). The PCR

amplification mixture (total volume = 20 µl) contained 50g gDNA, Buffer II (40 mM

Tris-HCl [pH 8.4], 100 mM KCl, 3 mM MgCl$_2$, 400 µM dGTP, 400 µM dATP, 400 µM

dTTP, 400 µM dCTP, 1U AccuPrime *Taq* DNA Polymerase, thermostable AccuPrime

protein, and stabilizers) (Invitrogen), 0.1 mM/L of each primer. Samples were amplified

using a Thermocycler (GeneAmp PCR system 2720, Applied Biosystems) for 35 cycles.

After an initial 2 min at 94°C, each subsequent cycle consisted of 30 s at 94°C, 30 s at

60°C, and 1 min at 68°C. The amplified DNA (10 µl) was digested with the 5U of the

restriction enzyme *PsiI* (New England Biolabs, US), which cuts at the -703T site. The

product was electrophoresed in 2% agarose gels and stained with ethidium bromide. The undigested PCR product carries the G variant, whereas the digested product with two fragments of 55 and 149 bp contains the T allele. Homozygous genotypes were identified by the presence of a single 204 bp band (G/G), or bands of 55 and 149 bp (T/T). The heterozygous genotype had three bands: 204, 55, and 149 bp (G/T).

**Statistical Analysis.**

Statistical analyses were performed using the Statistical Package for the Social Sciences version 16 (SPSS Inc, Chicago, Illinois). The placebo effect was quantified as the percent difference between the trial in which the subject believed they were ingesting the ergogenic aid and the control trial. Potential differences in the placebo effect were compared across genotypes using repeated measures analysis of variance (ANOVA) with one within-subjects effect (treatment) and two between-subjects effects (genotype and gender). Post-hoc differences were determined using a t-test with a Bonferroni correction.

**Chapter IV: Results**

　　　Subjects displayed a significant increase in MVC when told they were receiving a supplement (P < 0.05) (Figure 4.1). Mean force production for the treatment without the supplement was 487 ± 137N while the mean force production for the treatment with the perceived supplement was 508 ± 145N.

　　　Tables 4.1 and 4.2 summarize the percent improvement for the placebo response for each genotype by gender. There was no main effect for gender on the placebo response (P > 0.05). There was a 3.37% increase in force production for males and 7.47% for females with the perceived supplement. Furthermore, there was no main effect for genotype nor a genotype x treatment interaction for either the MAO-A or TPH2 polymorphisms (P > 0.05), suggesting that neither polymorphism impacted the magnitude of the placebo response in this sample of subjects.

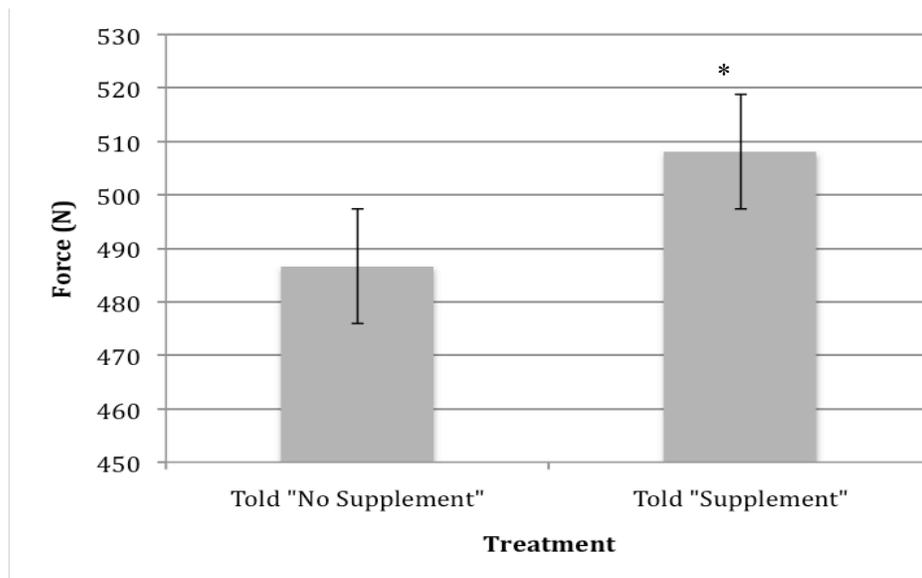Figure 4.1: Force production (* denotes significance at p < 0.05)



Table 4.1: Percent Improvement for the placebo for MAO-A

| Gender | Genotype | Mean | Std. Deviation | N |
|--------|----------|------|----------------|---|
| Male | G | 4.6536 | 10.93301 | 8 |
| | T | 2.9713 | 12.87901 | 26 |
| | Total | 3.3672 | 12.31007 | 34 |
| Female | G | 4.4591 | 13.07367 | 4 |
| | T | 8.2193 | 10.22442 | 16 |
| | Total | 7.4672 | 10.57825 | 20 |
| Total | G | 4.5888 | 11.07650 | 12 |
| | T | 4.9706 | 12.08465 | 42 |
| | Total | 4.8857 | 11.76702 | 54 |

Table 4.2: Percent Improvement for the placebo for TPH2

| Gender | Genotype | Mean | Std. Deviation | N |
|--------|----------|------|----------------|---|
| Male | GG | 3.5772 | 12.84906 | 31 |
| | GT | 1.1968 | 4.01843 | 3 |
| | Total | 3.3672 | 12.31007 | 34 |
| Female | GG | 7.3231 | 10.99320 | 17 |
| | GT | 8.2839 | 9.75005 | 3 |
| | Total | 7.4672 | 10.57825 | 20 |
| Total | GG | 4.9039 | 12.23930 | 48 |
| | GT | 4.9706 | 12.08465 | 6 |
| | Total | 4.8857 | 11.76702 | 54 |

**Chapter V: Discussion**

The primary finding of the present study is that MVC force production was elevated by 4.4% when subjects believed they had ingested an ergogenic aid. This placebo effect reaffirms that supplement and drug efficacy studies should utilize double-blind protocols. If subjects are not blinded to which intervention they are receiving, it would be hard to prove that any given substance is more effective than not giving any substance at all. Also, by limiting the subjects' prior knowledge about the sport supplement, the tester was able to provide the same information regarding the supplement and its supposed effects to every subject, likely increasing the impact of the placebo response.

The increase in force production also confirms that a placebo response can be demonstrated with maximal strength testing. The change of +4.4% in maximal force production is consistent with both the 3.8% improvement in 1RM demonstrated by Maganaris et al. and 4.3% increase in mean power during a 40km cycling time trial by Clark et al. (4, 12). The study conducted by Kalasountas et al. was most similar in subjects and methods to our study (8). Specifically, the authors also examined untrained, recreationally active college-aged students as subjects, and performance measures were strength measurements. Kalasountas et al. also included a treatment/no-treatment group similar to the intervention protocol we used (8). However, our findings showed a much smaller improvement in change in force (4.4% versus 19.6%). This could be due to the use of 1RM bench press and leg press to evaluate strength changes in the study by Kalsountas et al. while we looked at isometric strength of the quadriceps muscles only (8).

Gender did not impact the magnitude of the placebo effect; both males and females demonstrated a similar degree of placebo response. Males experienced a 3.39% increase while females experienced a 6.70% increase in force production. These changes were not significantly different from one another. Therefore, future placebo studies need not discriminate potential subjects based on gender.

Neither of the genetic polymorphisms selected for this study (TPH2 and MAO-A) influenced the placebo response to a perceived sports supplement. These two polymorphisms selected were chosen based on the promising results seen in previous studies and the possibility of their impact on athletic performance (6, 11). Furmark et al. observed the greatest placebo response with SAD patients who carried the G allele in the TPH2 gene (6). As a result of their genotype, these same subjects experience a naturally lower amount of amygdala activity in the brain, which leads to lower stress levels. In the study by Leuchter et al., MDD patients with a moderate or intermediate rate of MAO-A enzymatic activity experienced the greatest degree of placebo response (11). This trait was found in females with the heterozygous G/T expression of the gene. Because of the sex-linked nature of this polymorphism, males can only code for the high-rate expression (G allele) or low-rate activity (T allele). In this study, subjects who carried either the G allele in the TPH2 gene or the G/T expression of the MAO-A gene or both did not experience a significantly higher magnitude of placebo response than the subjects who possessed other gene expressions. However, these genetic links may only be evident in patients with mood disorders. The underlying conditions of social anxiety disorder or major depressive disorder may have influenced the findings of the previous studies, and the lack of such conditions in this study could account for the different results. Also,

quantifying a psychological response to a treatment such as "alleviating anxiety" may be more subjective than measuring force production. It is difficult to maintain consistency in evaluation when subjects self-report the degree of their symptom resolution, and the data results may not be comparable to the results of our study.

There were several limitations to this study that should be noted. First, the sample size may not have been large enough to observe significant differences between genetic groups. With genetic research, larger sample sizes are needed because the testing pool quickly diminishes when subjects are divided into sub-groups based on their genetic characteristics. However, there was no trend present to suggest that a larger sample size may have led to any significant findings (MAO-A $p = 0.77$; TPH2 $p = 0.74$). The second limitation was that the polymorphisms selected for this study might have limited the scope of possibility. TPH2 and MAO-A have been shown to be influential in patients with mood disorders; however, they may not exhibit the same influence in healthy subjects. A third limitation was the use of isometric force as the measure of force production. Isometric force does not translate to other methods of force production because of the lack of movement through the joint's range of motion. Also, strength tests may not fatigue the subject enough to elicit a genetically-influenced placebo response. Endurance training protocols that are longer in duration may allow more time for the deception to affect the subject psychologically. Isometric testing was used in this study because of its simplicity and low daily variability. Data on the reliability of MVC force production on a day-to-day basis indicates a high degree of reproducibility (3, 10, 20, 21). However, our findings strongly suggest that while a placebo response can be generated

with such a simple protocol, there is a lack of genetic effect associated with this particular protocol.

In summary, a placebo response can be elicited with MVC force production of the quadriceps. This can be seen in both males and females. However, our findings suggest that the TPH2 and MAO-A gene polymorphisms do not influence the likelihood of a placebo response. Future studies should consider other polymorphisms that are not influenced by the presence of mood disorders or other underlying conditions. Furthermore, larger sample sizes and a different exercise protocol might influence future results.

**Appendix I**

**Consent to Participate in Research**

**Identification of Investigators & Purpose of Study**

You are being asked to participate in a research study conducted by Jennifer Wu from James Madison University. The purpose of this study is to determine if genetics affect the training response obtained by ingestion of a specific supplement.

**Potential Risks & Benefits**

If you choose to participate in this study, you will perform three separate trials of a maximum voluntary contraction of the thigh muscle. The investigator perceives the following are possible risks arising from your participation in the study: nausea, discomfort, dizziness, and in rare occurrences, heart attack, stroke or death. However, you were chosen for this study because of your low risk for these occurrences. In healthy individuals, the risk of death during vigorous exercise has been estimated at 1 death per year for every 18,000 individuals.

**Potential benefits from participation in this study include:**

1) Helping with research that may improve the effectiveness of supplementation by targeting people for whom it will be most effective.

2) Knowledge of your maximal voluntary contraction

3) Knowledge of whether you are a responder to this particular supplement.

**Research Procedures**

Should you decide to participate in this research study, you will be asked to sign this consent form once all of your questions have been answered to your satisfaction. This study consists of three separate trials of a maximum voluntary contraction of the thigh muscle. All testing will occur in Godwin Hall, room 209, on the campus of James Madison University. Furthermore, you will be asked to regulate your diet intake according to specific guidelines prior to every testing session. All tests will be separated by at least 48 hours, so that you will be tested three times over a two to three week period, for a total of approximately three hours of testing. A blood draw will also be taken at the first testing session for the purpose of genotyping.

Maximum voluntary contraction: These three test sessions will be performed at the same time of day each time. You will be asked to refrain from food and beverages (except water) for two hours prior to these tests. In addition, you will need to refrain from consumption of caffeine-containing beverages (coffee, tea, cola drinks, cocoa) for 24 hours prior to the test. During these three test sessions, you will maximally contract your thigh muscle against an unmoving knee extension bar. This test will be performed three times during each session, and the average score will be calculated for each session. Ten minutes prior to each test, you will ingest an 8 oz. portion of a popular sports beverage either with the supplement dissolved into the beverage or without the supplement. Between test preparation and completion of these exercise tests, each test should take approximately 20 minutes.

Blood Sampling: We will obtain about 5 ml of blood (about 1 teaspoon) prior to the first test session in order to extract DNA and determine specific genotypes.  These blood samples will be obtained from an arm vein.

DNA Sampling: We will extract a sample of your DNA from your blood sample.  The DNA will be stored in our laboratory, but the sample will be coded so that no one except the investigators can detect which sample is yours. The DNA testing will involve determining your sequence of DNA for a specific gene that may be related to the effectiveness of the supplement. The results of this genetic testing will only be available to the primary investigator and you. These results will not be made public and will be stored in a locked file cabinet.  Your samples and data will be discarded after a five year period; or earlier if requested by you.

**Confidentiality**

The results of this research will be presented at conferences and published in exercise science journals.  The results of this project will be coded in such a way that your identity will not be attached to the final form of this study.  The researcher retains the right to use and publish non-identifiable data.  However, you can ask that your data be removed from the study at any point prior to presentation and publication.  While individual responses are confidential, aggregate data will be presented representing averages or generalizations about the responses as a whole.  All data will be stored in a secure location accessible only to the researcher.  Final aggregate results will be made available to you upon request.

**Participation & Withdrawal**

Your participation is entirely voluntary.  You are free to choose not to participate.

Should you choose to participate, you can withdraw at any time without consequences of

any kind.  Your right to withdraw includes the right to request that your DNA and blood

samples be discarded at any time.

**Questions**

If you have questions or concerns during the time of your participation in this study, or

after its completion or you would like to receive a copy of the final aggregate results of

this study, please contact: Christopher J. Womack, Ph.D. at womackcx@jmu.edu or by

phone at 540-568-6515.

**Questions about Your Rights as a Research Subject**

Dr. David Cockley

Chair, Institutional Review Board

James Madison University

(540) 568-2834

cocklede@jmu.edu

**Giving of Consent**

I have read this consent form and I understand what is being requested of me as a

participant in this study.  I freely consent to participate.  I have been given satisfactory

answers to my questions.  The investigator provided me with a copy of this form.  I

certify that I am at least 18 years of age.

_____          _____
Name of Participant  (Printed)          Name of Researcher(s)  (Printed)


_____          _____
Name of Participant  (Signed)           Name of Researcher(s)  (Signed)


_____          _____
Date                                     Date

**Appendix II**

*AHA/ACSM Health/Fitness Facility Pre-participation Screening Questionnaire*

*Assess your health status by marking all true statements*

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*History*

You have had:

_____ a heart attack

_____ heart surgery

_____ cardiac catheterization

_____ coronary angioplasty (PTCA)

_____ pacemaker/implantable cardiac

_____ defibrillator/rhythm disturbance

_____ heart valve disease

_____ heart failure

_____ heart transplantation

_____ congenital heart disease

> If you marked any of these statements in this section, consult your physician or other appropriate health care provider before engaging in exercise. You may need to use a facility with a *medically qualified staff*.

*Symptoms*

_____ You experience chest discomfort with exertion

_____ You experience unreasonable breathlessness

_____ You experience dizziness, fainting, or blackouts

_____ You take heart medications

*Other Health Issues*

_____ You have diabetes

_____ You have asthma or other lung disease

_____ You have burning or cramping sensation in your lower

legs when walking short distances

_____ You have musculoskeletal problems that limit your

physical activity

_____ You have concerns about the safety of exercise

_____ You take prescription medication(s)

---

*Cardiovascular risk factors*

_____ You are a man older than 45 years

_____ You are a woman older than 55 years, have had a

hysterectomy, or are postmenopausal

_____ You smoke, or quit smoking within the previous 6 months

_____ Your blood pressure is > 140/90 mmHg

_____ You do not know your blood pressure

_____ You take blood pressure medication

_____ Your blood cholesterol level is > 200 mg/dl

_____ You do not know your cholesterol level

> If you marked two or more of the statements in this section, you should consult your physician or other appropriate health care provider before engaging in exercise. You might benefit from using a facility with a *professionally qualified exercise staff* to guide your exercise program.

_____ You have a close blood relative who had a heart attack or

heart surgery before age 55 (father or brother) or age 65

(mother or sister)

_____ You are physically inactive (i.e. you get < 30 minutes of

physical activity on at least 3 days of the week)

_____ You have a BMI > 30 kg•m$^2$ or waist circumference

> 102 cm (men) or > 88 cm (women)

_____

_____ None of the above

> You should be able to exercise safely without consulting your physician or other appropriate health care provider in a self-guided program or almost any facility that meets your exercise program needs.

**Appendix III**

## Subject Prescreening Information

**Please Complete the Following:**

Gender:        Male    Female    (circle one)

Age (yrs):

Height (inches):

Weight (lbs):

**Average Exercise Habits over the Past 2 Months:**

Avg. # days of exercise per week:

Avg. amount of time per bout of exercise:

**Do you have a muscle or joint injury that precludes the completion of the exercise protocol?  Explain.**



**Do you currently use medications for relief of pain and/or soreness?  Explain.**



**Do you have a blood clotting disorder (haemophilia, thrombocytopenia, etc)?**



**Do you currently use blood-thinning medications (Coumadin, etc)?**



**Do you currently use cardiac medications (Digoxin, Digitalis, etc)?**

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