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# The effects of androstenedione supplementation on testosterone levels, exercise performance, and mood in older males

Samantha J. Pannier  
*Ithaca College*

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**THE EFFECTS OF ANDROSTENEDIONE SUPPLEMENTATION ON  
TESTOSTERONE LEVELS, EXERCISE PERFORMANCE, AND MOOD  
IN OLDER MALES**

**A Thesis Presented to the Faculty of the  
Graduate Program in Exercise and Sport Sciences  
Ithaca College**

**In Partial Fulfillment of the  
Requirements for the Degree  
Master of Science**

**by**

**Samantha J. Pannier**

**September 2004**

Ithaca College  
School of Health Sciences and Human Performance  
Ithaca, New York

CERTIFICATE OF APPROVAL

MASTER OF SCIENCE THESIS

This is to certify that the Thesis of  
Samantha J. Pannier  
submitted in partial fulfillment of the requirements for the  
the degree of Master of Science in the School of  
Health Sciences and Human Performance  
at Ithaca College has been approved.

Thesis Advisor:

Committee Member:

Candidate:

Chair,  
Graduate Program :

Dean of Graduate Studies:

Date:

August 25, 2004

## ABSTRACT

Previous research has shown that androstenedione supplementation does not increase testosterone levels in young to middle age men. It has been speculated that the supplement does not alter testosterone in these men because their testosterone levels were normal prior to participating in the respective studies. Androstenedione may only increase testosterone in individuals who have a low testosterone level. The primary purposes of this study, therefore, was to determine if androstenedione supplementation alters testosterone in older men, who have lower natural testosterone levels than younger counterparts. A secondary purpose was to determine if the supplement altered mood, perceived exertion, or testosterone levels with exercise. Twenty-five males, 55 to 85 years of age, served as subjects. Each had been exercising consistently for a minimum of 3 months and had a relatively stable health background (no cardiac events within the past 5 years). Subjects were randomly assigned to either the treatment or control group, which consumed 200 mg of androstenedione or placebo, respectively. Immediately after treatment, subjects completed a short form of the Profile of Mood Questionnaire (POMS) and provided a saliva sample, which was subsequently analyzed for testosterone concentration. Subjects then completed a prescribed 30 min workout that included resistance and cardiovascular exercise. Immediately after completing each exercise, subjects rated their perceived exertion (RPE) with the Borg scale. Additional saliva samples were taken at 60 and 120 min post-treatment, whereas mood was reassessed immediately following exercise and 180 min post treatment. A 2 x 3 (group x time) analysis of variance (ANOVA) with repeated measures on the second factor was used to

examine differences in testosterone levels and mood over time and between groups. A composite RPE score for all exercises performed was calculated. A two-sample t-test was used to assess between group differences in perceived exertion. Androstenedione supplementation did not alter testosterone levels across time or between groups. There were also no differences between the groups in the variables measured by the POMS, which included anger, vigor, and fatigue. Relative to baseline, however, fatigue increased during exercise and decreased after exercise in both groups, whereas anger decreased both during exercise and after in both groups regardless of treatment. There was also no difference in RPE between the groups. Single dose androstenedione supplementation did not affect testosterone levels in older males even though they had below normal initial testosterone levels. These results suggest that a ceiling effect of naturally occurring testosterone does not appear to explain the lack of conversion of orally consumed androstenedione to testosterone. In addition, since there were no differences between the groups in RPE or POMS, the supplement also does not alter perception or mood during exercise. Collectively, these data show that older males will not benefit from androstenedione supplementation.

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## Chapter 1

### INTRODUCTION

Androgens are hormones secreted by the testes and adrenal glands that stimulate the formation of secondary male sexual characteristics such as: auxiliary hair growth (both facial and body), deepening of the voice, bone and muscle development, acne, and maturing of sexual organs (Flieger, 1995). The most predominant androgen is testosterone. Testosterone levels are elevated prior to birth, then drop substantially afterwards. A sharp rise is seen at puberty, with peak levels reached in the twenties (Greenspan & Baxter, 1994). There is a gradual decline in testosterone production throughout the remainder of the lifespan. This progressive testosterone decline is linked to andropause, which is characterized by a decline in intellectual acumen and sexual functioning, along with an increase in abdominal obesity and muscle atrophy (Smith, 1994). These symptoms are similar to those of hypogonadism, which affects an estimated four to five million men in America (Rodgers, 1995).

Numerous treatments have been developed to rectify andropause and hypogonadism. One such purported treatment is androstenedione, a natural precursor to testosterone, which is converted to testosterone within the human testes by the enzyme 17 $\beta$ -hydroxysteroid dehydrogenase (King et al., 1999). Sold over-the-counter as a dietary supplement, androstenedione is administered through a nasal spray, subcutaneous patch, or as a pill, which is the most commonly used method due to its low cost and convenience. If androstenedione supplementation can elevate testosterone levels, then the effects of male menopause would be reduced, as sexual functioning, energy levels, psychological well-being, bone density, and lean body mass should improve (Sadovsky,

1997). In short, androstenedione supplementation may be a form of androgen replacement therapy (ART) for men similar to the estrogen therapy that women have been prescribed since the 1940's (Coney, 1994).

To be an effective therapeutic modality, androstenedione supplementation must increase testosterone levels. Recent research has shown, however, that oral consumption of androstenedione by young and middle age men has little to no effect on testosterone levels (King et al., 1999; Rasmussen, Volpi, Gore, & Wolfe, 2000; Wallace, Lim, Cutler, & Bucci, 1999). Instead of elevating testosterone in this subject cohort, androstenedione supplementation elevated estrogen levels. Apparently androstenedione bypassed the more common testosterone pathway and was instead converted into estrone, a strong estrogen.

Since the young subjects in the aforementioned studies had normal testosterone levels, which are typically greater than in males from 55 to 80 years, it is possible that the ingested supplement was not be converted to testosterone (Simon, Nahoul, & Chades, 1996; Vermeulen, 1996). The authors offered that a ceiling had been reached. Such an event explains the increase in estrogen. Since the androstenedione could not be converted to testosterone, the only way to eliminate it was via the less common estrogen pathway. Mahesh & Greenblatt (1962) provided additional indirect evidence for the ceiling effect hypothesis, as they showed that androstenedione increased testosterone levels in women. Since women have lower testosterone levels than men, the androstenedione supplement elevated testosterone in the females rather than being converted to estrogen.

The primary purpose of this study was to test the ceiling hypothesis by determining if androstenedione supplementation increases testosterone levels in older

males, who typically have lower testosterone levels than those tested in previous studies. The testosterone levels of the subjects used in this study typically range from 376-617 ng·dl<sup>-1</sup>. Subjects in the aforementioned studies, who were 35 and younger, had an average testosterone level between 617-668 ng·dl<sup>-1</sup>. Since inadequate testosterone levels lower energy and depress mood states, they can theoretically decrease exercise performance (FDA Consumer, 1996). A secondary purpose of this study was to determine if the supplement altered mood, perceived exertion, or testosterone levels with exercise.

#### Statement of the Problem

To study effects of androstenedione supplementation on testosterone levels and mood state in older men before and after exercise.

#### Major and Minor Hypotheses

The major null hypothesis for this study was:

1. Androstenedione will have no effect on testosterone levels before or after exercise.

The minor hypothesis for this investigation was:

1. Androstenedione will have no effect on mood state before or after exercise.
2. Androstenedione will have no effect on perception of exertion during exercise performance.

#### Assumptions of the Study

For the purpose of this study, the following assumptions were made when planning the investigation:

1. Saliva testing is an accurate tool used to measure testosterone.

2. The subjects were representative of typical older males.
3. Androstenedione is a completely safe dietary supplement.
4. Alterations in testosterone levels that may be seen are due only to androstenedione supplementation.
5. A ceiling effect for testosterone conversion is operating, which necessitates this study.

#### Delimitations of the Study

The delimitations of this investigation are as follows:

1. Older males were the subjects.
2. Only the effects of Ultimate Nutrition Androstenedione were investigated in a single 200 mg dose.
3. Only saliva testing was used to measure testosterone levels.
4. Only bikes, treadmills, and rowers were used in a specific exercise plan.
5. Only RPE was used to measure the effort level of exercise workloads.
6. Only anger, vigor, and fatigue were measured to assess mood.

#### Limitations of the Study

The limitations of this study are as follows:

1. The results may only apply to older males with low testosterone levels.
2. The results may only apply to the effects of Ultimate Nutrition Androstenedione supplementation in the specified dose.
3. Results may only apply when the tool of measurement used is saliva testing.
4. Results for RPE may only apply when a similar exercise program is employed.

5. Aspects of mood other than anger, vigor, and fatigue may not be affected as the results described by this study.

### Definition of Terms

The following terms were operationally defined for the purpose of this investigation:

1. Treatment group- those subjects who received androstenedione.
2. Control group – those subjects who received a placebo.
3. Older males – men who are over the age of 55.
4. Supplementation – an artificial substance added to the human body to gain normal levels, or exceed normal limits to profit from the effect.
5. Over-the-counter – a medicine that can be purchased at a drug store without a prescription, such as aspirin and cough syrup.
6. Andropause – a male form of menopause, characterized by low testosterone levels, intellectual slowing, abdominal obesity, and muscle atrophy.



## Chapter 2

### REVIEW OF LITERATURE

Menopause, the cessation of reproductive life in females, is a well-known condition that begins in a woman's early forties. Menopause typically builds slowly over a period of 10 years known as the climacteric, during which the levels of female sex hormones, such as estrogen and progesterone, decline by up to 75%, marking the end of ovarian functioning (Johnson & Everitt, 1984). Menopause symptoms include: irregular menstrual periods, hot flashes, night sweats, disturbed sleep patterns, vaginal dryness and shrinkage of genital tissues, dry skin, more frequent urination or leakage of urine, more frequent minor vaginal and urinary tract infections, fatigue, loss of sexual desire, anxiety, depression, tearfulness, irritability, sleeplessness, lack of concentration, and memory trouble (University of Michigan Health System). These symptoms are often reduced by hormone replacement therapy (HRT). Recent research suggests that long-term HRT is contraindicated, but short-term use is still seen favorably (Biscup, 2003; Chew & Ng, 2002; Shah & Alexander, 2003).

Men, like women, also experience a decrease in sex hormone production as they age. The process is more gradual and is called andropause, which does not have a noticeable start, but its symptoms usually become noticeable around 50 years of age. These symptoms include: weakness, fatigue, reduced muscle and bone mass, impaired hematopoiesis, decrease in spermatozoa, sexual dysfunction, depression, anxiety, irritability, insomnia, memory impairment, and reduced cognitive function (Lund, Bever-Stille, & Perry, 1999). Similar to menopause, treatment for andropause is androgen replacement therapy (ART). The most common type of ART is testosterone supplementation in the form of pills, patches, or injections (Flieger, 1995).

Some over-the-counter products are purported to be ART substitutes, such as androstenedione, an androgen produced within the body, which when taken as a supplement theoretically boosts testosterone levels. If testosterone levels in older males

are increased, than their psychological well-being, bone density, and lean body mass should improve (Sadoesky, 1997). Such benefits would be appreciated, but the claimed actions of androstenedione are largely untested. This literature review focuses on the following: (a) what is androstenedione, (b) the biochemical relationship among androstenedione, testosterone, and estrogen, (c) effects of testosterone and its relationship to andropause, (d) effects of testosterone on exercise performance and mood, (e) research on androstenedione, and (f) summary.

### What is Androstenedione

Androstenedione is a C<sup>19</sup> steroid produced primarily in the testes and secondarily in the adrenal glands from testosterone or dehydroepiandrosterone (DHEA) as shown in Figure 1 (Schneider, 1978). The biochemical relationship among the sex hormones will be explored more fully subsequently. After production, androstenedione is converted back into testosterone, converted into estrogen, or secreted into the circulatory system. From the blood it enters other tissues where it directly or indirectly exerts its physiological effects (King et al., 1999). The normal secretion rate for  $\Delta^4$  androstenedione-3,17 is 5 mg·d<sup>-1</sup> (Sawin, 1969). Secreted androstenedione represents about 8% of the circulating androgens in the body; relative to testosterone and 5 $\alpha$ -dihydrotestosterone androstenedione is mildly androgenic (Johnson & Everitt, 1984). The most critical aspect of androstenedione for this study, however, is its potential for conversion into either testosterone or estrogen.

### The Biochemical Relationship among Androstenedione, Testosterone, and Estrogen

Approximately 95% of the testosterone that is synthesized in the male body is manufactured in the testes, primarily in the leydig cells and secondarily in the sertoli cells (Jones, 1997). The other 5% of testosterone is produced in the adrenal glands. Irrespective of where it is made, most testosterone is manufactured from acetate and

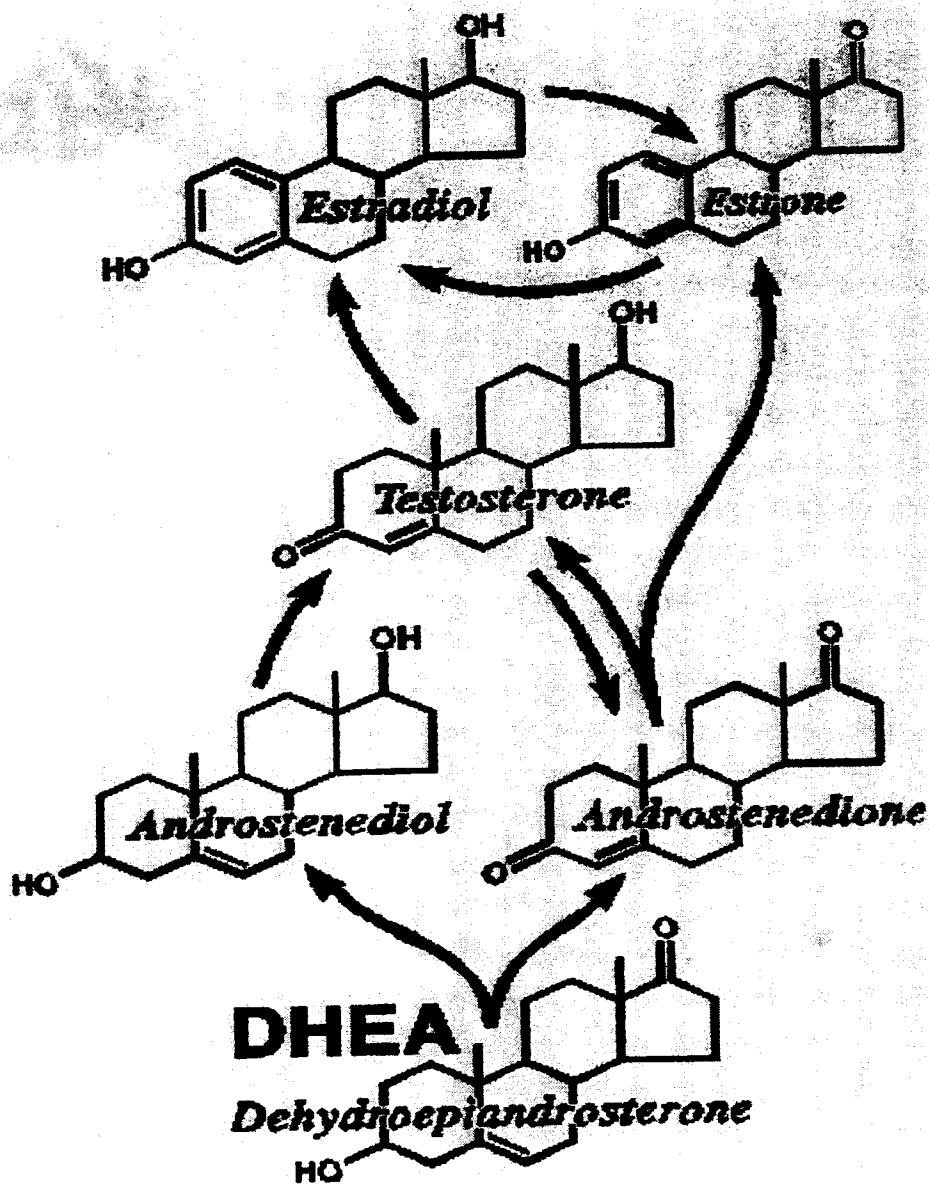
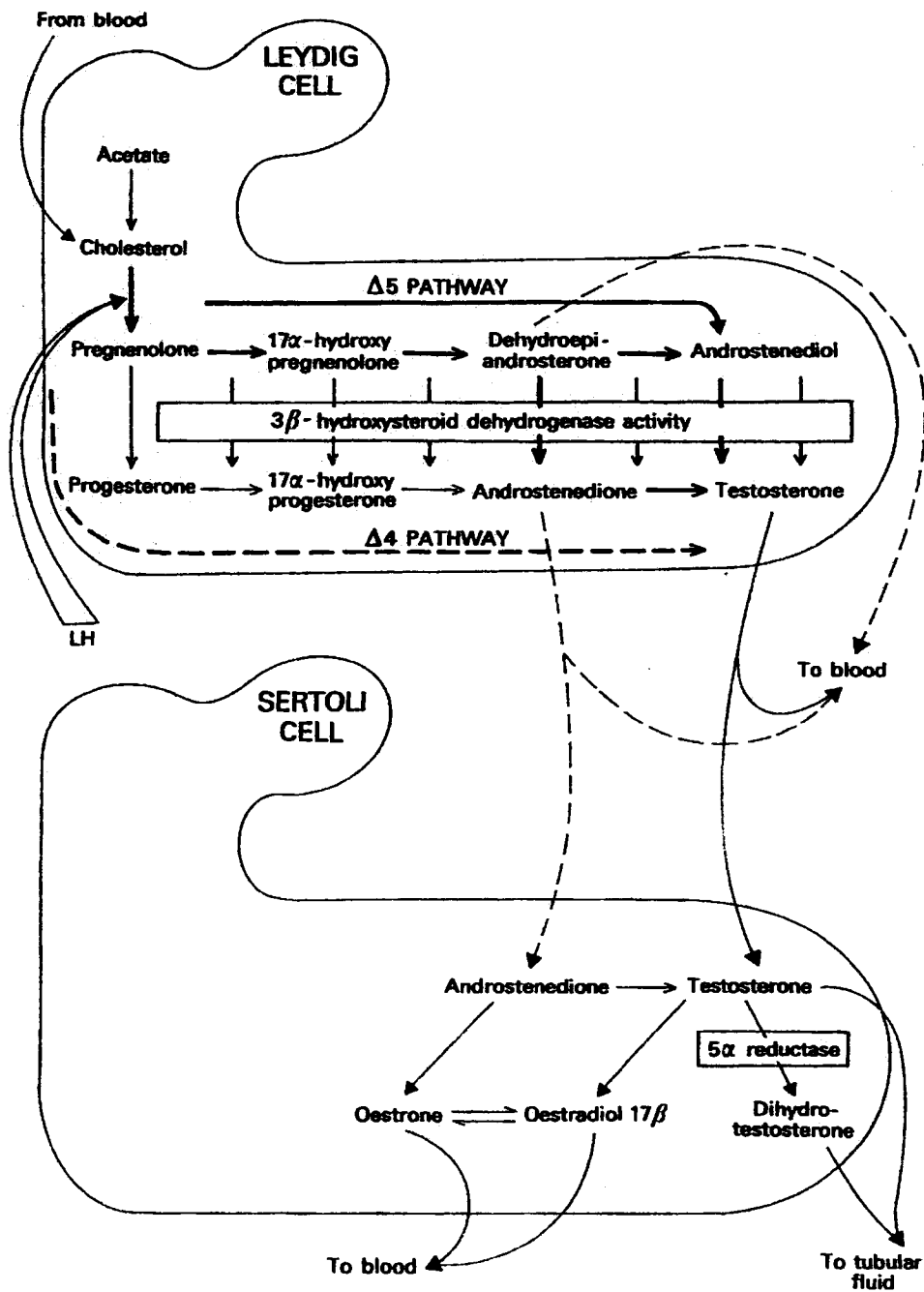


Figure 1. Pathways of Androstenedione

cholesterol, a process influenced by luteinizing hormone (LH), a gonadotropin released from the anterior pituitary gland. LH catalyzes the combination of acetate and cholesterol into pregnenolone, the precursor of all sex hormones, including testosterone, progesterone, and estrogen. In males, the enzyme  $17\alpha$  hydroxylase converts the pregnenolone into  $17\alpha$ -hydroxypregnenolone, which is then converted into DHEA by  $17, 20$  desmolase. DHEA has several fates; it is either converted into androstenediol by the enzyme  $17\alpha$ -hydroxysteroid dehydrogenase or androstenedione by the enzyme  $3\beta$ -hydroxysteroid dehydrogenase. Androstenediol, in turn, is converted into testosterone by the enzyme  $3\beta$ -hydroxysteroid dehydrogenase (Johnson & Everitt, 1984). In contrast, androstenedione takes two pathways with the predominant one being conversion to testosterone by the enzyme  $17\alpha$ -hydroxysteroid dehydrogenase. Less commonly, the leydig cells secrete androstenedione into the general circulation. Some of the secreted androstenedione enters nearby sertoli cells, which convert it into either testosterone or estrogen with the enzyme aromatase (Johnson & Everitt, 1984). Approximately  $3 \text{ mg}\cdot\text{d}^{-1}$  of androstenedione is converted into estrogen at a transfer rate of 1.5%, yielding  $125\text{-}150 \text{ }\mu\text{g}\cdot\text{d}^{-1}$  of estrogen (Longcope, Kato, & Horton, 1969). Indeed, androstenedione is the precursor for 20-30% of estrogen in the body (Horton & Tait, 1966). The biochemical interrelationship among androstenedione, testosterone, and estrogen in the testes is illustrated in Figure 2.

After its production in the sertoli cells, leydig cells, or cells of the adrenal gland, testosterone has two fates: it is converted into other products by these cells or secreted into the general circulation. Once in the general circulation, testosterone enters target tissues, where it exerts physiological effects or is converted into other products, which then exert physiological effects. Irrespective of location, testosterone is ultimately converted into one of three products: androstenedione via  $17\alpha$ -hydroxysteroid dehydrogenase, estrogen by aromatase, or  $5\alpha$  dihydrotestosterone (DHT) by  $5\alpha$



**Figure 2.** The Biochemical Interrelationship among Androstenedione, Testosterone, and Estrogen in the Testes.

reductase. DHT is the most prevalent and potent of the male androgens (Johnson & Everitt, 1984).

### Effects of Testosterone and its Relationship to Andropause

Testosterone is the primary sex hormone in males (The Testosterone Source, 1999). It is responsible for producing the male reproductive system and influencing the development of the brain, kidney, liver, muscle tissue, as well as other parts of the body. Testosterone production begins in the testes around the eighth week of fetal development (Flieger, 1995). The testosterone concentration increases slowly until birth, when it drops substantially to a minute level, which is maintained until puberty, whereupon production dramatically increases. The large surge in testosterone in males at puberty induces many changes within the body, such as: voice deepening, facial hair growth, genital enlargement, increased libido, and bone and muscle maturation. The testosterone concentration continues to rise until it peaks in the twenties. After peaking, testosterone levels drop by about 1% each year until death (Saunders, Montague, Levine, & Guay, 1998). Table 1 shows that the testosterone levels typically decrease from 617 to 376  $\text{ng}\cdot\text{dl}^{-1}$ , a 35% reduction, in males between the ages of 25-85.

As mentioned in the introduction of this chapter, somewhere around the age of 50 men may experience andropause, which is clinically diagnosed when testosterone levels fall below 300  $\text{ng}\cdot\text{dl}^{-1}$  (Jones, 1997). Affecting four to five million Americans, andropause is caused by a decreased testicular response to gonadotropins, resulting from reduced vascularization of the testes as well as a lowered number and availability of leydig cells within them (Timiras, Quay, Vernadakis, 1995). Collectively, these changes decrease the amount of testosterone produced by the body, which causes unwanted side effects, such as: abdominal obesity, intellectual slowing, elevated voice pitch, decreased facial hair growth, osteoporosis, depression, decreased muscle mass, and irritability, while also reducing sexual functioning (FDA Consumer, 1996; Jones, 1997; Smith,

**Table 1.****Mean Plasma Testosterone Levels for the Average Male by Age (ng·dl<sup>-1</sup>)**

Age	No. of Subjects	Total Testosterone	Standard Deviation	Free Testosterone	Standard Deviation
25-34	45	617	170	12.3	2.8
35-44	22	610	212	10.3	1.2
45-54	23	606	213	9.1	2.2
55-64	43	562	195	8.3	2.1
66-74	47	524	197	6.9	2.3
75-84	48	471	169	6.0	2.3
85-100	21	376	134	5.4	2.3

Note. source of chart: <http://www.weymouthclinic.co.uk/wellman/paper.html>

1994). Changes in sexual function include slower erections, and a decrease in the size of the scrotum, penis, and seminal vesicles (Jones, 1997).

Andropause is a form of hypogonadism, a hormonal deficiency that is estimated to afflict approximately 5 million American males (FDA Consumer, 1996).

Hypogonadism results from a multitude of reasons, but the effects are the same as andropause (Bagatell & Bremner, 1996). To combat either condition, many men use some form of ART, which slows the drop in testosterone, frequently returning it to normal levels, thereby abating or reversing the changes associated with hypogonadism or andropause.

Androstenedione is one purported type of ART. Theoretically it elevates testosterone levels and consequently prevents or delays the symptoms associated with andropause. It may also protect the body against heart disease, stroke, prostate cancer, and osteoporosis by maintaining a healthy range of testosterone within the body (Jones, 1997; Rudman et al., 1994). Since ART, including possibly androstenedione, increases psychological mood states in addition to improving physical capacities, it may also enhance ability to exercise, another well-known modality that attenuates the effects of aging (Craig, Brown & Everhart, 1989; Hurel et al., 1999; Izquierdo et al., 2001; Suominen & Rahkila, 1991; Young & Ismail, 1979). If ART were effective, then men may be more likely to initiate exercise, possibly sustaining it at a greater intensity for a longer duration, thereby improving the benefits derived from exercise (Suominen & Rahkila, 1991). In addition, if the exercise was of a greater intensity and duration, it may further improve natural androgen levels, as exercise is a known stimulus for androgen secretion (Craig, 1989; Hurel et al., 1999). Exercise and ART could very well act synergistically to improve vitality in older men.

#### Effects of Exercise Performance on Testosterone and Mood

It has been shown that abnormally low levels of testosterone can decrease lean body mass, muscular strength, and exercise tolerance (Perry, 1999). These changes can



reduce exercise duration, intensity, and possibly frequency. If testosterone is elevated in such individuals, mood, strength, and quality of life significantly improve (Ferrando et al., 2002; Lund, Bever-Stille, & Perry, 1999). These benefits occur independent of exercise (Wang et al., 2000). Exercise alone, however, can elevate testosterone levels over time (Hurel, 1999; Hakkinen, 2002). In older males, for instance, 18 months of exercise elevated testosterone levels, suggesting that exercise can partially counteract the normal decline in hormones seen with aging (Hurel et al., 1999). In contrast to chronic exercise, acute bouts of exercise have relatively no effect on testosterone levels (Borst, Vincent, Lowenthal, & Braith, 2002; Craig, Brown, & Everhart, 1989; Kraemer et al., 2003; Smilios, Pilianidis, Karamouzis, & Tokmakidis, 2003; Tremblay, Copeland, & Van Helder, 2004; Zhou, et al., 2000). Thus, if one only performs acute bouts of exercise, some form of ART may be needed to return testosterone to normal levels.

Even though acute bouts of exercise do not immediately raise testosterone, they can positively affect mood states regardless of the person's age and sex, as well as the duration, mode, intensity of the exercise bout (Annesi, 2002; Fox, 1999; Hansen, Stevens, & Coast, 2001; Lane & Lovejoy, 2001; McGowan, Pierce, & Jordan, 1991; Pierce & Pate, 1994). Both chronic and acute exercise decrease fatigue, anger, confusion, tension, and depression, while they increase vigor and quality of life. Collectively, acute and chronic exercise improves mood states, whereas only chronic exercise elevates testosterone levels. Thus concluding that, exercise coupled with some form of ART could potentially help restore testosterone levels quickly and efficiently while benefiting from mood alterations immediately.

#### Research on Androstenedione

Androstenedione may be one type of ART, but the conversion of this supplement into testosterone is not well studied. The supplement was first synthesized in 1935 (Philips, 1997). In 1936, Dr. Charles Kochakian discovered that it possessed both anabolic and androgenic effects (Colgan, 2000). The effects of androstenedione

supplementation were first studied in humans in 1962. Mahesh & Greenblatt (1962) looked at plasma testosterone levels before and after oral consumption of 100 mg of androstenedione in two women. Although testosterone levels rose considerably in these subjects, the applicability of these results to males was limited.

The next study to take place compared the rate of conversion of oral and injected radio labeled androstenedione to testosterone in both males and females ages 21-33 years (Horton and Tait, 1966). It was found that approximately 5.9% of injected androstenedione was converted into plasma testosterone in contrast to 1.8% of orally consumed androstenedione. Forty percent of intravenous androstenedione was converted into urinary testosterone and excreted. In contrast 89% of orally administered androstenedione was converted to urinary testosterone and excreted indicating that little testosterone enters general circulation after oral consumption.

In a more recent study, 20 college-aged men either consumed 100 mg of androstenedione or a placebo every day for 8 wk while completing a resistance training program (King et al., 1999). There was no difference in fitness between the experimental and control group in this study. In addition, and more importantly, testosterone levels did not increase in the experimental group, whereas the estrogen concentration did. These authors suggested their findings may illustrate a ceiling effect in young, healthy males for the conversion of androstenedione into testosterone. Since the subjects in this study had normal testosterone levels, perhaps the androstenedione supplement could not be converted into testosterone. Instead, the supplement may have been shunted into the less commonly used estrogen pathway (Greenspan & Baxter, 1994).

Data from Mahesh and Greenblatt (1962) support the notion of a ceiling effect. Since their subjects were women, with lower testosterone levels than men, androstenedione supplementation had better potential for and was readily converted to testosterone. In short, the putative testosterone ceiling had not been reached in these subjects. Two additional studies support the ceiling hypothesis (Rasmussen, Volpi, Gore,

& Wolfe, 2000; Wallace, Lim, Cutler, & Bucci, 1999). In both studies, the subjects were young males (28-52 y) who had normal testosterone levels. Despite consuming 100-300 mg of androstenedione per day, there was no acute change in testosterone levels in this subject cohort. Estrogen levels, however, did significantly increase. Researchers concluded that since the subjects already had normal testosterone levels, the androstenedione supplement was converted to estrogen.

### Summary

Androstenedione, a natural precursor to testosterone may help alleviate symptoms of andropause. Current literature suggests that androstenedione may not elevate testosterone levels in young men with normal testosterone levels. These data may show a “ceiling effect.” In short, androstenedione may not boost testosterone in males with normal levels, because there is no deficiency in the hormone. Instead, it is converted to estrogen and eliminated from the body. In older men, however, those with low testosterone levels, androstenedione may elevate testosterone levels because the ceiling had not been reached. Consequently, if androstenedione were to allow older males to produce normal ranges of testosterone once again, the possibility of increasing mood states along with exercise performance should coexist.

## Chapter 3

### METHODS

This chapter describes the subjects used in this study and outlines the methodology of data collection.

#### Subjects

Twenty-five males between the ages of 55 and 85 volunteered to participate in this study. Their descriptive characteristics are reported in Appendix A. Subjects were recruited from the Heart Health Institute in Newington, NH. Prior to participation, subjects were screened for current or former injuries, such as orthopedic limitations, that could interfere with completion of the study. All subjects were asked to complete a physical activity readiness questionnaire (PAR-Q). They were ineligible to be tested if their questionnaire answers could not satisfy the requirement that they were healthy individuals. The only test question that was accepted with a “yes” answer was in regards to a doctor prescribing a diuretic or water pill, all others had to be answered “no” or the subject was excluded from the study.

Each subject was also screened for a history of prostate cancer and the use of testosterone replacement therapy. Although nine out of twenty-five subject had previous cardiac events, no subject had any cardiac events within the past five years. Nor had any recently experienced any shortness of breath or chest pain with activity. All subjects had been exercising for at least three times a week for a minimum of three months consistently. Before the first test, subjects read and signed an informed consent form that had been approved by the Human Subjects Committee at Ithaca College (Appendix B).

All were given a full explanation of the study along with the benefits, risks of participation, and participation requirements.

### Method of Data Collection

#### Brief Overview

Each subject reported to the Heart Health Institute, Newington, NH on two occasions. The first visit consisted of a submaximal bike test and a submaximal strength test. Each subject returned for a second testing day during which he received the experimental intervention, provided three saliva samples, completed three POMS-short forms, and performed the prescribed exercise program.

#### Submaximal Testing Day

Upon arriving at the Heart Health Institute, Newington, NH subjects were weighed on a pre-calibrated scale (Detecto, Webb City, MO). Target heart rate range was calculated using the Karvonen method as follows:  $[(220 - \text{age} - \text{Resting Heart Rate}) \times 70\%] + \text{Resting Heart Rate}$  (American College of Sports Medicine, Guidelines for Exercise Testing and Prescription, 2001). Borg's RPE scale was then explained as a subjective table that allows subjects to rate their perceived exertion (Appendix C). Next the subject was fitted to his correct seat height on the cycle ergometer (True Fitness Technology, Inc. model 750R, O'Fallon, MO) with legs at a full extension, leaving a soft bend in the knee. Once fitted, a standard YMCA protocol (American College of Sports Medicine, Guidelines for Exercise Testing and Prescription, 2001) was used to assess submaximal fitness. This test uses two to four, 3 min stages of continuous exercise. Workload was increased each stage by 10 W to 30 W, as determined from the heart rate (HR) response. The test was terminated when the subject attained approximately 70% of

his age-predicted maximum HR. HR and RPE were taken towards the end of the second minute and again at the end of each stage to assess intensity and to determine if a steady state HR was obtained.

Fifteen minutes following the submaximal bike test, maximum chest and leg strength were assessed with 10 repetition maximum tests conducted on the Nautilus Chest Press and Leg Extension machines (Independence, VA). The initial load was set at 100% of the load normally lifted by the subject during their exercise regimen. If 10 repetitions were completed, then another set of 10 repetitions were completed at higher workload (10 to 20 lbs greater depending on the ease of completion) and so on until the subject could not complete 10 repetitions. There was 5 min of rest between trials and no subject attempted more than three trials in a day. If the maximum was not found in three trials, the subject was retested 48 hours later. If the subject could not complete ten repetitions at the initial load, then he completed additional trials every 5 min at progressively lighter loads until the 10 repetition maximum was measured.

#### Intervention Day

A single blind research design was used and subjects were randomly placed into either a treatment group or a control group. No significant differences were seen between groups prior to testing. Submaximal YMCA testing data were used to predict maximum MET levels for each subject. Individual exercise prescriptions were written for each subject. Workload was set at approximately 70% of his predicted maximum capability of the chest press, leg extension, and cardiovascular equipment. Metabolic equations (American College Of Sports Medicine, Guidelines for Exercise Testing and Prescription, 2001) used to determine workload for the cardiovascular equipment are:

Treadmill:  $METS = [(m / \text{min} \times 0.1) + 3.5] / 3.5$

Bike:  $METS = [(Watts \times 6.1 \times 2.0 / \text{Weight in kg}) + 3.5] / 3.5$

Rower:  $METS = [(Watts \times 6.1 \times 3.0 / \text{Weight in kg}) + 3.5] / 3.5$

Upon arrival to the Heart Health Institute, Newington, NH, each subject rinsed his mouth with water and then provided a small quantity of saliva, about 0.50 ml, which was used to measure baseline testosterone levels (Bricaire et al., 1991; Chen, Bookstein, & Meldrum, 1991; Heinonen, 1992; Yen, 1991). Additional saliva samples were taken at minutes 60 and 120 during the testing protocol. Each sample was transferred into a small, pre-labeled tube that was sealed and placed in dry-ice until all samples were collected for the testing session. Within three hours of initial data collection, the saliva samples were stored in a -80 °C freezer. After completion of all testing, the samples were packed on dry ice and shipped by UPS overnight to Ithaca College, where they were analyzed using a Steroid Hormone ELISA Assays kit (Immuno Biological Laboratoreis, Hamburg, Germany). Detailed procedures are shown in Appendix D.

After the initial saliva sampling, the treatment group consumed 200 mg of Ultimate Nutrition Androstenedione (Farmington, CT), where as the control group ingested a flour placebo. Next they completed a POMS-short form (see Appendix E) before exercise started. Additional POMS forms were completed immediately after the workout was finished and at 180 min after treatment. After the first POMS was completed, subjects were given their individual workout card. The card outlined the exercise regimen, which consisted of 20 min walking on the treadmill (True Fitness Technology, Inc. model 7500, O'Fallon, MO), 15 min of cycle ergometry (True Fitness Technology, Inc. model 750R, O'Fallon, MO), 10 min of rowing (Concept II,

Farmington, CT), and one set of 12 repetitions for the leg extension and chest press, respectively. RPE was recorded at the end of the work bout for each piece of equipment. Average RPE was then assessed for each individual on all four workout modes. All RPE scores were then combined and averaged as a whole for each group.

### Statistics

A 2 x 3 (group x time) analysis of variance (ANOVA) with repeated measures on the second factor was used to examine differences in salivary testosterone levels between groups over time. A 2 x 3 (group x time) analysis of variance (ANOVA) with repeated measures on the second factor was also used to observe variation in mood states between groups over time. The dependent variable was mood state (A- Anger-Hostility, V- Vigor-Activity, F- Fatigue-Inertia). A t-test was used to assess the differences in RPE between groups using a single composite score for all exercises performed. A t-test was also used to determine any initial difference in predicted  $\dot{V}O_2$  max levels between the groups. A significance level was at  $p < 0.05$  for all tests. The statistical analyses were run using the SPSS/PC 10.0 program for an IBM compatible computer. A detailed description of analyses is presented in the next chapter.



## RESULTS

In this chapter the results and statistical analyses of data are presented. Sections in this chapter include the following: (a) subject characteristics (b) testosterone levels (c) profile of mood states and (d) rating of perceived exertion.

### Subject Characteristics

The mean  $\pm$  SE for age, weight, and 70 % of VO<sub>2</sub> max for the control group were  $66.6 \pm 8.2$  y,  $92.1 \pm 13.9$  kg, and  $19.95 \pm 1.4$  ml<sup>-1</sup>·kg<sup>-1</sup>·min<sup>-1</sup>, respectively. The corresponding values for the treatment group were  $65.3 \pm 8.1$  y,  $89.9 \pm 16.5$  kg, and  $19.25 \pm 0.9$  ml<sup>-1</sup>·kg<sup>-1</sup>·min<sup>-1</sup>, respectively. There were no significant differences in these variables between the groups. Details for subject characteristics and all raw data can be seen in Appendix A.

### Testosterone Levels

Means and standard deviations for testosterone levels are reported in Table 2. No significant interaction was found between Group and Time in levels of testosterone. A 2x3 ANOVA revealed no significant main effect for Group,  $F(1,69) = 3.480$ ,  $p = 0.066$ , indicating there was no significant difference in the level of testosterone between the control group and the treatment group (see Appendix F, Table F-1). There was no significant main effect for Time,  $F(1,69) = 1.449$ ,  $p = 0.242$ , indicating there was no significant difference in testosterone levels over the three time points (Figure 3).

### Profile of Mood States

#### Anger

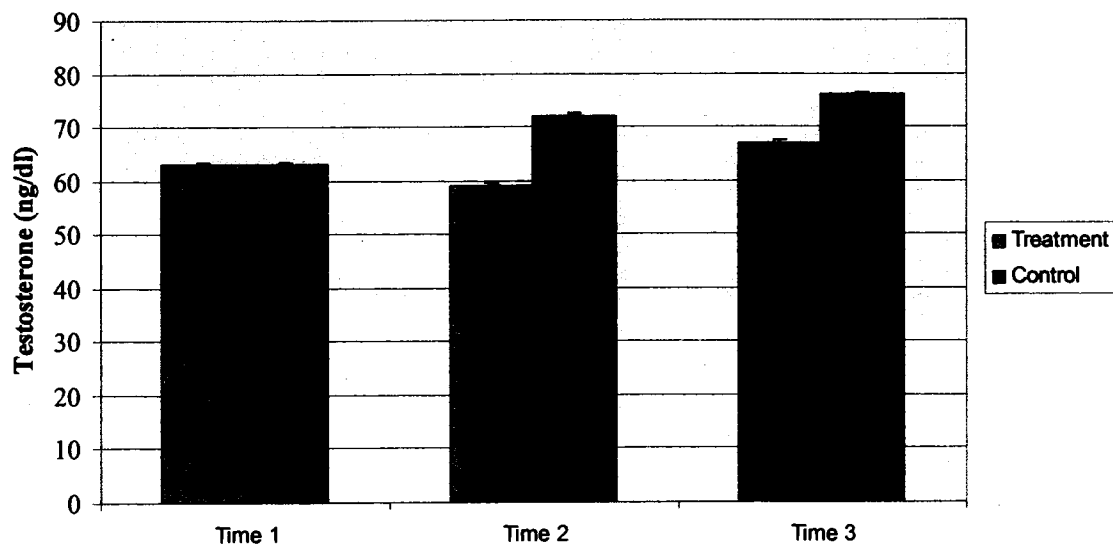
No significant interaction was found between Group and Time for anger (Appendix F, F-2). A 2x3 ANOVA revealed no significant main effect for Group,  $F(1,69)$

Table 2

Mean + SD for Selected Variables by Group and Time

Variable	<u>Control Group</u>		<u>Treatment Group</u>	
	<u>M</u>	<u>SD</u>	<u>M</u>	<u>SD</u>
Testosterone Levels (ng/dl)				
Time 1	63.00	0.17	63.00	0.11
Time 2	72.00	0.23	59.00	0.22
Time 3	76.00	0.21	67.00	0.13
POMS Score – Anger				
Time 1	00.69	0.00	01.64	1.07
Time 2	00.08*	0.28*	00.64*	1.28*
Time 3	00.00*	1.29*	00.29*	2.04*
POMS Score – Vigor				
Time 1	09.15	5.33	10.71	2.94
Time 2	09.77	4.99	10.21	3.09
Time 3	08.38	5.01	10.79	4.56
POMS Score – Fatigue				
Time 1	02.00	0.95	01.79	1.83
Time 2	03.54*	2.26*	03.86*	2.96*
Time 3	01.08*	1.75*	01.14*	1.70*

Note. Time 1 = 0 min or pre-treatment, Time 2 = 60 min post-treatment, Time 3 = 120 min post-treatment. \* = significant difference from Time 1.

**Mean and  $\pm$  SD for Testosterone Over Time**

**Figure 3.** Mean and  $\pm$  SD for testosterone across time. Treatment = Treatment Group ( $n = 13$ ); Control = Control Group ( $n = 12$ ). Time 1 = 0 min or pre-treatment, Time 2 = 60 min post-treatment, Time 3 = 120 min post-treatment.

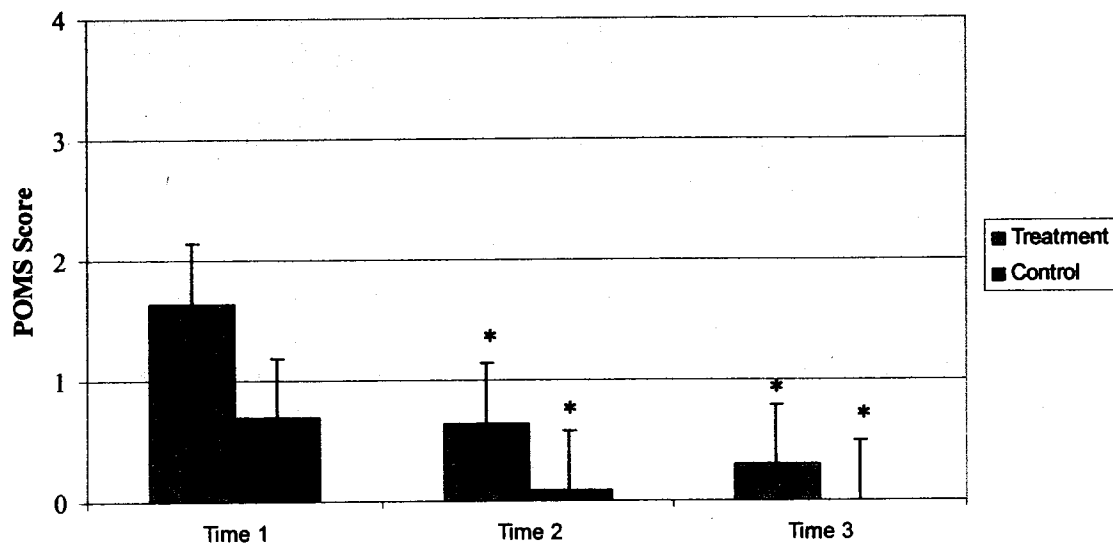
= 1.750,  $p = 0.190$ , indicating there was no significant difference in anger between treatment and control (Appendix F, Table F-2). There was a significant main effect for Time,  $F(1,69) = 4.017$ ,  $p = 0.022$ , indicating there was a significant difference in anger across time for both groups (Figure 4). The Tukey HSD test revealed that anger levels decreased significantly from pretreatment to 60 min post-treatment and from pretreatment to 180 min post-treatment. Means and standard deviations for anger are reported in Table 2.

### Vigor

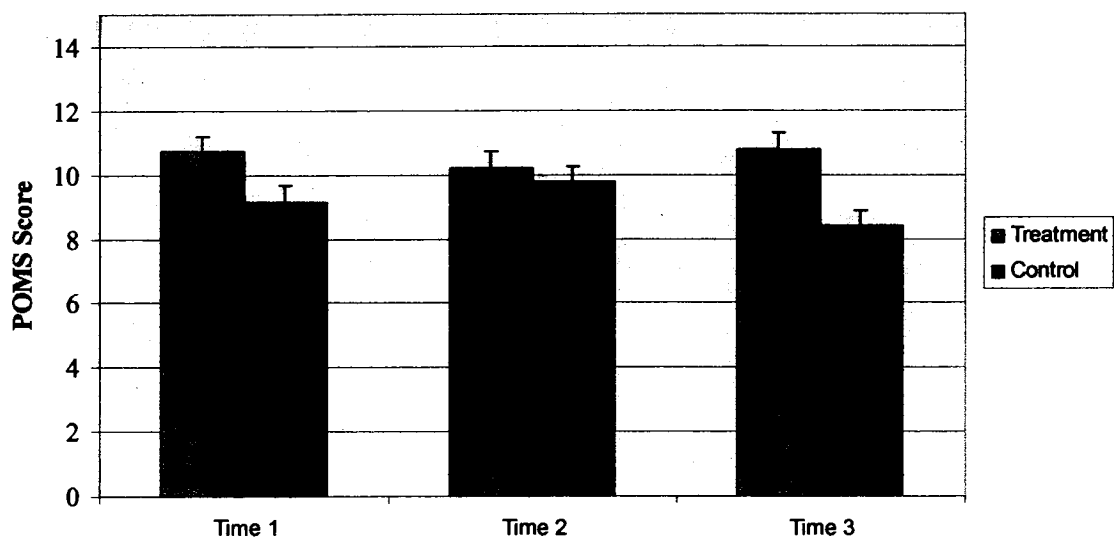
No significant interaction was found between Group and Time in vigor (Appendix F, Table F-3). A 2x3 ANOVA revealed no significant main effect for Group,  $F(1,69) = 1.396$ ,  $p = 0.241$ , indicating there was no significant difference in vigor between the treatment and control groups (Appendix F, Table F-3). There was no significant main effect for Time,  $F(1,69) = 0.085$ ,  $p = 0.919$ , indicating there was no significant change in vigor over time (Figure 5). Means and standard deviations for vigor are reported in Table 2.

### Fatigue

No significant interaction was found between Group and Time (Appendix F, Table F-4). A 2x3 ANOVA revealed no significant main effect for Group,  $F(1,69) = 0.106$ ,  $p = 0.749$ , indicating there was no significant difference in fatigue between the treatment and control groups (Appendix F, Table F-4). There was a significant main effect for Time,  $F(1,69) = 11.196$ ,  $p = 0.000$ , indicating there was a significant change in fatigue across time (Figure 6) in both groups. The Tukey HSD test revealed that fatigue levels significantly increased from pre-treatment to 60 min post-treatment, and

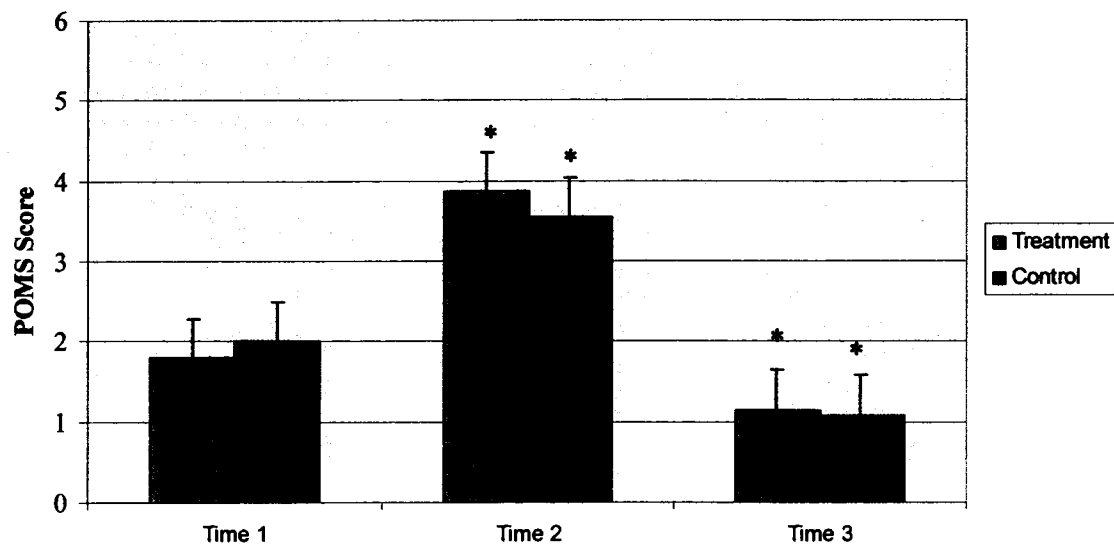
**Mean and  $\pm$  SD for Anger Score Across Time**

**Figure 4.** Mean and  $\pm$  SD for anger score across time. Treatment = Treatment Group ( $n = 13$ ); Control = Control Group ( $n = 12$ ). Time 1 = 0 min or pre-treatment, Time 2 = 60 min post-treatment, Time 3 = 180 min post-treatment. \* = significant difference from Time 1.

**Mean and  $\pm$  SD for Vigor Score Across Time**

**Figure 5.** Mean and  $\pm$  SD for vigor score across time. Treatment = Treatment Group ( $n = 13$ ); Control = Control Group ( $n = 12$ ). Time 1 = 0 min or pre-treatment, Time 2 = 60 min post-treatment, Time 3 = 180 min post-treatment.

### Mean and $\pm$ SD for Fatigue Score Across Time



**Figure 6.** Mean and  $\pm$  SD for fatigue score across time. Treatment = Treatment Group ( $n = 13$ ); Control = Control Group ( $n = 12$ ). Time 1 = 0 min or pre-treatment, Time 2 = 60 min post-treatment, Time 3 = 180 min post-treatment. \* = significant difference from Time 1.

## Chapter 5

### DISCUSSION

Androstenedione is produced naturally within the human body, and serves a precursor to testosterone (Johnson & Everitt, 1984). Based on this biology, supplement manufactures claim that oral consumption of androstenedione elevates serum testosterone levels. Numerous studies have tested but not confirmed these claims (Greenspan & Baxter, 1994; Horton & Tait, 1966; King et al., 1999; Rasmussen, Volpi, Gore, & Wolfe, 2000; Wallace, Lim, Cutler, & Bucci, 1999). Since the subjects in these studies were between the ages of 18 and 55, and had testosterone levels within the normal range of 376 to 617 ng·dl<sup>-1</sup> (<http://www.weymouthelclinic.co.uk/wellman/paper.html>), it is possible that the ingested supplement could not be converted to testosterone, because a testosterone ceiling may have been reached. This may explain the increase in estrogen levels found in the subjects of the aforementioned studies. If consumed androstenedione cannot be converted to testosterone, it may be eliminated via the less commonly used estrogen pathway (Horton & Tait, 1966). To determine if the conversion of orally consumed androstenedione to testosterone is limited by initial testosterone level, this study examined the effects of the supplement in older males, who typically have lower testosterone levels than the 18 to 55 y old subjects in the aforementioned studies. Testosterone levels in my subjects ranged from 22.6 to 85.4 ng·dl<sup>-1</sup>, which is below normal and less than the range of 376 to 617 ng·dl<sup>-1</sup> reported in the aforementioned studies. Despite the low initial levels of testosterone and the consumption of the recommend dosage of androstenedione, the product did not alter serum testosterone levels. Therefore, it seems unlikely a ceiling effect limits the conversion of orally consumed androstenedione to testosterone. Indeed, the collective data from all studies that have examined androstenedione suggest that orally consumed androstenedione is not converted to testosterone.



The secondary purpose of this study was to determine if androstenedione supplementation affected aspects of exercise performance reported by RPE and POMS between the groups. It was thought that the supplement could conceivably improve exercise performance and the perception of exercise performance if it elevated testosterone levels (Craig, Brown, & Everhart, 1989; Hurel et al., 1999; Izquierdo et al., 2001; Suominen & Rahkila, 1991; Young & Ismail, 1979). Although we did not measure changes in exercise performance by tracking HR or weight lifted, RPE is a valid measure of exercise intensity (Foster, 2001; Goss et al., 2003; Herman, Nagelkirk, Pivarnik, & Womack, 2003; Kang, Hoffman, Walker, Chaloupka, & Utter, 2003). There were no differences in RPE between the groups, showing that the supplement did not alter exercise intensity or perception of exercise effort. The RPE data are supported by the POMS data, which included analyses of anger, vigor, and fatigue. There was no difference in these variables between the groups. Androstenedione, therefore, did not alter exercise performance or the perception of exercise performance. A likely explanation for these findings is that the supplement did not alter testosterone levels as previously described.

We did note that there were significant differences across time in anger and fatigue in both groups. Anger decreased significantly during and after exercise, whereas fatigue increased during exercise and decreased after relative to baseline. These data concur with other studies (Annesi, 2002; Fox, 1999; Hansen, Stevens, & Coast, 2001; Lane & Lovejoy, 2001). The drop in fatigue and anger, relative to baseline, after exercise suggests that the short bout of exercise improved mood, which is also consistent with the literature (Bartholomew & Miller, 2002; DiLorenzo et al., 1999; Rehor, Dunnagan, Stewart, & Cooley, 2001). Therefore, it can be claimed that exercise is beneficial in altering mood state in a positive manner with only a short bout of exercise.

We also noted that short-term exercise did not independently increase testosterone levels. This finding agrees with other studies in which the effect of short-term exercise

on testosterone in middle-aged and older men and women was examined (Borst, Vincent, Lowenthal, & Braith, 2002; Smilios, Pilianidis, Karamouzis, & Tokmakidis, 2003; Zhou, Liu, Jin, He, Zhao, & Wang, 2000). Although single bouts of activity do not significantly alter testosterone levels, several months of regular exercise does increase the resting concentration of this hormone (Hakkinen et al., 2002; Izquierdo et al., 2001).

#### Summary

Single dose androstenedione supplementation in older males did not affect testosterone levels even though the subject cohort had below normal initial testosterone levels. These results suggest that there is no ceiling effect limiting the conversion of orally consumed androstenedione to testosterone. These data, when combined with the data from the other studies on the effects of androstenedione supplementation, suggest that orally consumed androstenedione is not converted to testosterone. In addition, androstenedione supplementation did not alter RPE or POMS during an acute bout of exercise. Collectively, these data show that older males will not benefit from androstenedione supplementation.

## Chapter 6

### SUMMARY, CONCLUSIONS, AND RECOMMENDATIONS

#### Summary

This study examined the effects of androstenedione and its purported effect as a natural precursor to testosterone. Secondly, this study focused on the effects of androstenedione on exercise responses. Twenty five older male subjects volunteered to participate in this study. Each subject reported to the testing site on two occasions. The first visit consisted of a submaximal bike test and a submaximal strength test. Each subject returned for a second testing day during which he received the experimental intervention, provided three saliva samples, completed three POMS-short forms, and completed an exercise program.

No significant differences were seen in testosterone levels over time in either the control or treatment group. No significant interaction ( $p < 0.05$ ) was found between group and time in regards to anger, vigor, or fatigue. There were however, significant changes in anger and fatigue over time in both groups, indicating this was due to exercise and not androstenedione treatment. The Tukey HSD test revealed that anger significantly decreased at both 60 and 180 min post-treatment, whereas fatigue increased significantly from pre-treatment to 60 min post-treatment, and decreased significantly from pre-treatment to 180 min post-treatment (Table 2).

Therefore, it appears that a single dose of androstenedione supplementation is not effective in increasing testosterone levels or enhancing mood state in older men. No ceiling effect was found to occur, suggesting that older males would not benefit

from androstenedione supplementation. It can also be speculated that short-term exercise does not alter testosterone independently in older men.

### Conclusions

The results of this study yielded the following conclusions:

1. Androstenedione does not significantly alter testosterone levels in older men.
2. Androstenedione does not significantly alter mood state or exercise perception.
3. Single bouts of exercise do not significantly affect testosterone levels.
4. Single bouts of exercise can decrease anger, affect fatigue and generally improve mood in older men.

### Recommendations

The following recommendations for further study were made after the completion of this investigation:

1. Different types and different brands of androstenedione should be tested.
2. A future study in older men should measure circulating and urinary androstenedione levels to determine the route of supplemental androstenedione removal.
3. A similar study using a more conventional ART supplement (e.g. testosterone) should be completed to determine if ART can impact exercise performance.

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## APPENDICES

## APPENDIX A

### RAW DATA

subj.	age (yrs)	ht(cm)	wt(kg)	70% Vo2 (ml/kg/min)	group	time	testosterone (ng/dl)	POMS Anger	POMS Vigor	POMS Fatigue	RPE
1	85	182.88	70.91	18.55	T	1	56.90	0	5	0	N/A
2	70	185.42	88.18	18.55	C	1	68.40	0	15	0	N/A
3	55	180.34	70.00	28.70	T	1	66.00	0	13	0	N/A
4	66	177.80	89.09	17.50	T	1	68.00	0	10	3	N/A
5	73	177.80	102.27	17.50	C	1	64.00	0	11	4	N/A
6	70	190.50	96.36	14.00	T	1	69.90	0	10	0	N/A
7	60	175.26	99.32	22.05	T	1	60.00	0	5	3	N/A
8	61	172.72	88.64	18.55	C	1	85.40	0	13	0	N/A
9	73	180.34	80.45	17.50	T	1	67.30	0	17	0	N/A
10	76	175.26	90.45	24.50	C	1	70.80	0	2	0	N/A
11	57	177.80	114.09	12.25	T	1	70.30	0	11	2	N/A
12	66	170.18	76.36	23.10	C	1	69.00	0	9	2	N/A
13	62	167.64	120.91	17.15	T	1	70.20	4	13	1	N/A
14	55	172.72	101.36	22.40	C	1	65.20	1	3	2	N/A
15	62	187.96	106.36	17.85	C	1	60.40	2	6	2	N/A
16	58	182.88	120.45	19.25	C	1	74.40	4	16	6	N/A
17	68	180.34	91.82	18.20	T	1	73.80	1	11	5	N/A
18	65	180.34	75.00	19.95	C	1	73.70	0	12	4	N/A
19	61	177.80	83.18	27.30	T	1	64.30	0	10	0	N/A
20	63	177.80	92.73	19.60	T	1	39.80	6	9	2	N/A
21	64	180.34	69.55	18.90	T	1	45.80	4	12	3	N/A
22	85	175.26	73.64	13.30	C	1	22.60	0	0	0	N/A
23	68	167.64	86.36	18.90	C	1	35.30	0	10	0	N/A
24	68	172.72	84.55	23.80	C	1	69.40	2	12	2	N/A
25	59	172.72	103.18	22.40	C	1	64.60	0	10	4	N/A
1	85	182.88	70.91	18.55	T	2	28.20	0	10	1	13.25
2	70	185.42	88.18	18.55	C	2	52.40	0	19	1	12.75
3	55	180.34	70.00	28.70	T	2	44.50	0	13	0	12.5
4	66	177.80	89.09	17.50	T	2	59.10	0	10	6	12.5
5	73	177.80	102.27	17.50	C	2	9.40	1	11	5	12
6	70	190.50	96.36	14.00	T	2	24.10	0	4	9	11
7	60	175.26	99.32	22.05	T	2	53.10	0	6	6	12.75
8	61	172.72	88.64	18.55	C	2	73.30	0	14	4	12.75
9	73	180.34	80.45	17.50	T	2	56.10	0	15	0	11.5
10	76	175.26	90.45	24.50	C	2	78.60	0	9	2	12
11	57	177.80	114.09	12.25	T	2	84.10	0	13	6	13.5
12	66	170.18	76.36	23.10	C	2	77.80	0	4	4	13.25
13	62	167.64	120.91	17.15	T	2	85.00	0	6	8	12.75
14	55	172.72	101.36	22.40	C	2	79.00	0	8	6	12
15	62	187.96	106.36	17.85	C	2	102.30	0	4	4	12
16	58	182.88	120.45	19.25	C	2	83.30	0	14	5	11.5
17	68	180.34	91.82	18.20	T	2	87.30	0	10	6	13.25
18	65	180.34	75.00	19.95	C	2	86.00	0	10	8	11.75
19	61	177.80	83.18	27.30	T	2	62.00	0	11	2	12
20	63	177.80	92.73	19.60	T	2	44.20	0	9	4	11.75
21	64	180.34	69.55	18.90	T	2	77.20	3	12	2	12.25

## APPENDIX A (cont.)

## RAW DATA

subj.	age (yr)	ht(cm)	wt(kg)	70% Vo2 (ml/kg/min)	group	time	testosterone (ng/dl)	POMS Anger	POMS Vigor	POMS Fatigue	RPE
22	85	175.26	73.64	13.30	C	2	54.30	0	0	0	11.75
23	68	167.64	86.36	18.90	C	2	77.60	0	12	1	11.25
24	68	172.72	84.55	23.80	C	2	72.80	0	12	2	11.5
25	59	172.72	103.18	22.40	C	2	86.50	0	10	4	10
1	85	182.88	70.91	18.55	T	3	63.70	0	10	1	N/A
2	70	185.42	88.18	18.55	C	3	69.40	0	17	0	N/A
3	55	180.34	70.00	28.70	T	3	52.40	0	10	0	N/A
4	66	177.80	89.09	17.50	T	3	68.60	0	12	0	N/A
5	73	177.80	102.27	17.50	C	3	12.90	0	10	2	N/A
6	70	190.50	96.36	14.00	T	3	42.10	4	7	6	N/A
7	60	175.26	99.32	22.05	T	3	83.00	0	8	2	N/A
8	61	172.72	88.64	18.55	C	3	86.30	0	15	0	N/A
9	73	180.34	80.45	17.50	T	3	68.90	0	15	0	N/A
10	76	175.26	90.45	24.50	C	3	82.40	0	6	2	N/A
11	57	177.80	114.09	12.25	T	3	78.40	0	10	2	N/A
12	66	170.18	76.36	23.10	C	3	76.30	0	3	2	N/A
13	62	167.64	120.91	17.15	T	3	75.70	0	5	0	N/A
14	55	172.72	101.36	22.40	C	3	80.00	0	5	2	N/A
15	62	187.96	106.36	17.85	C	3	89.70	0	1	2	N/A
16	58	182.88	120.45	19.25	C	3	79.80	0	15	1	N/A
17	68	180.34	91.82	18.20	T	3	84.30	0	11	4	N/A
18	65	180.34	75.00	19.95	C	3	85.90	0	10	0	N/A
19	61	177.80	83.18	27.30	T	3	64.40	0	15	0	N/A
20	63	177.80	92.73	19.60	T	3	50.90	0	9	1	N/A
21	64	180.34	69.55	18.90	T	3	66.20	0	13	0	N/A
22	85	175.26	73.64	13.30	C	3	66.70	0	0	0	N/A
23	68	167.64	86.36	18.90	C	3	93.70	0	10	0	N/A
24	68	172.72	84.55	23.80	C	3	81.20	0	9	1	N/A
25	59	172.72	103.18	22.40	C	3	88.60	0	8	2	N/A

## APPENDIX B

### Informed Consent Form

#### **The Effects of Androgen Supplementation on Testosterone Levels in Older Males**

##### **1. Purpose of the study:**

This study is being conducted to assess the change in testosterone levels during the consumption of androstenedione.

##### **2. Benefits of the study:**

This study may identify a method that will raise testosterone levels. This could help overcome symptoms such as low energy levels, muscular weakness, irritability, and lack of sexual desire. These findings could be extremely important to the millions of American's that have hypogonadism (a lack of testosterone). All testing associated with this study will be done free of charge and the results will be provided to you if you so request.

##### **3. Your Participation Requires:**

First you will attend a brief meeting where you will be given a full explanation of the details of the study. Any questions you might have will be answered at this time. Next, each subject will provide a saliva sample used to measure testosterone levels. This noninvasive, simple procedure involves the participant spitting into a cup. Once all saliva samples are obtained, the subjects will each consume a pill. Half will receive 200mg of androstenedione and half, a placebo. Androstenedione is sold over-the-counter as a dietary supplement. No acute, adverse effects have been documented, and its effects are believed to be acute. You will then continue with your regular workout protocol. Saliva samples will be taken 20 min. post treatment, 45 min. post treatment, and 75 min. post treatment. Upon completion of your workout, you will be asked to complete a short questionnaire describing your workout performance. Total participation time for each subject in this project is 2 hours.

##### **4. Risks of Participation:**

There are always slight risks involved with a testosterone study, but they are minimal. Since there is limited evidence of the product used, there has been no documentation showing any acute, adverse effects. The treatment has been hypothesized to increase testosterone levels within the body. If this is true, some effects of increased testosterone levels could be seen if taken for an extended period of time. These effects could include such effects as an increase of acne, rash, flushing, sweating, dizziness, headache, fatigue, tremors, paresthesias, anxiety, insomnia, carpal tunnel



syndrome, cramps, spasms, increased B/P, nausea, vomiting, constipation, weight gain, conjunctival edema, nasal congestion, abnormal GTT, hair loss (male pattern baldness), insomnia, an decrease in HDL Cholesterol, and a possible increase in the risk of developing prostate cancer. These effects are acute and will diminish within a few hours of ceasing treatment. However the effects occur only when testosterone levels are extremely high for an extended amount of time. Since our subjects have diminishing testosterone levels and will not be consuming androstenedione for any length of time, it is highly unlikely for any such effects to occur. Also androstenedione does not have any documentation showing adverse effects. It is not recommended that subjects continue to use these aides after the experiment has ceased.

**5. For more information:**

If you would like more information about this study at anytime prior to, during, or following the data collection, you may contact either Samantha J. McCarthy 207/698-4471 or Tom Swensen 274-3114, or email at smccart1@hotmail.com

**6. Withdrawal from the study:**

Participation in this study is voluntary and you may withdraw at any time if you so choose.

**7. Confidentiality:**

Information gathered during this study will be maintained in complete confidence. Only S. J. McCarthy and T. Swensen will have access to this information. All reporting of this information to outside parties will be done in group form. You and your name will never be associated with this information in any future disclosures.

I have read and understood the above document. I agree to participate in this study and realize that I can withdraw at anytime. I also understand that I can and should address questions related to this study at any time to the researchers involved. I also verify that I am at least 18 years of age.

\_\_\_\_\_  
Name of Subject (please print)

\_\_\_\_\_  
Signature of Subject

\_\_\_\_\_  
Date

## APPENDIX C

### BORG'S RATE OF PERCEIVED EXERTION

6	
7	Very, Very Light
8	
9	Very Light
10	
11	Fairly Light
12	
13	Somewhat Hard
14	
15	Hard
16	
17	Very Hard
18	
19	Very, Very Hard
20	

## APPENDIX D

### ELISA METHODOLOGY

#### Testosterone ELISA

Cat.-No. : RE 52151  
Size : 12x8  
Storage : 1-8 °C

Enzyme immunoassay (microtiter strips) for the quantitative determination of testosterone in human serum and plasma

#### Instructions for Use

##### 1. Preparation of Reagents

- Wash Buffer: Dilute 1:40 with distilled water (e.g. 10 ml concentrate + 390 ml dist. water). Store at room temperature for up to 8 weeks.

##### 2. Specimen Collection and Storage

- Samples: Serum, plasma. Storage for up to 24 h at 2-8 °C, longer storage at -20 °C. Avoid repeated freezing and thawing.

##### 3. Assay Procedures

- Storage of reagents at 2-8 °C. Allow reagents to react room temperature.
  - a. Pipet 25 µl of Standards, controls and samples.
  - b. Incubate 5 minutes at room temperature (18-24 °C).
  - c. Add 200 µl of Enzyme Conjugate to each well.
  - d. Incubate 60 minutes at room temperature.
  - e. Wash\* each well 3 x with 400 µl Wash Buffer.
  - f. Pipet\*\* 200 of TMB-Substrate Solution.
  - g. Incubate 15 minutes at room temperature.
  - h. Pipet\*\* 100 µl of TMB-Stop Solution.

- i. Briefly mix contents, read the optical density at 450nm (reference wave length 600-650 nm) within 30 minutes after stopping.

\*Wash procedure is essential for the assay results.

\*\*\*Stop solution should be pipetted after incubation in the same time intervals as the substrate solution.

On a semi logarithmic graph paper the concentrations of the standards (x-axis, logarithmic) are plotted against their optical densities (y-axis, linear). Patient results are read directly from the graph constructed from the standards. Samples with values above the highest standard have to be diluted with zero standard. In the case, the additional dilution factor has to be taken into account.

# APPENDIX E

## POMS SHORT FORM

NAME \_\_\_\_\_ DATE \_\_\_\_\_

SEX: Male  Female

Identification No. \_\_\_\_\_

Below is a list of words that describe feelings people have. Please read each one carefully. Then fill in ONE circle under the answer to the right which best describes HOW YOU HAVE BEEN FEELING DURING THE PAST WEEK INCLUDING TODAY.

The numbers refer to these phrases.

- = Not at all
- = A little
- = Moderately
- = Quite a bit
- = Extremely

	Not at all A little Moderately Quite a bit Extremely		Not at all A little Moderately Quite a bit Extremely		Not at all A little Moderately Quite a bit Extremely
1. Tense . . . . .	<input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>	12. Uneasy . . . . .	<input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>	23. Weary . . . . .	<input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>
2. Angry . . . . .	<input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>	13. Fatigued . . . . .	<input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>	24. Bewildered . . . . .	<input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>
3. Worn out . . . . .	<input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>	14. Annoyed . . . . .	<input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>	25. Furious . . . . .	<input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>
4. Lively . . . . .	<input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>	15. Discouraged . . . . .	<input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>	26. Efficient . . . . .	<input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>
5. Confused . . . . .	<input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>	16. Nervous . . . . .	<input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>	27. Full of pep . . . . .	<input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>
6. Shaky . . . . .	<input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>	17. Lonely . . . . .	<input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>	28. Bad-tempered . . . . .	<input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>
7. Sad . . . . .	<input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>	18. Muddled . . . . .	<input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>	29. Forgetful . . . . .	<input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>
8. Active . . . . .	<input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>	19. Exhausted . . . . .	<input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>	30. Vigorous . . . . .	<input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>
9. Grouchy . . . . .	<input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>	20. Anxious . . . . .	<input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>		
10. Energetic . . . . .	<input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>	21. Gloomy . . . . .	<input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>		
11. Unworthy . . . . .	<input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>	22. Sluggish . . . . .	<input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>		

**MAKE SURE  
YOU HAVE ANSWERED  
EVERY ITEM.**



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APPENDIX F

ANOVA SUMMARY TABLES

Table F-1

ANOVA Summary Table for Testosterone

Source	SS	df	MS	F	p
Group	0.155	1	0.115	3.480	0.066
Time	0.096	2	0.048	1.449	0.242
Group x Time	0.052	2	0.026	0.785	0.460
Error	2.279	69	0.033		

Table F-2

ANOVA Summary Table for Anger

Source	SS	df	MS	F	p
Group	2.355	1	2.355	1.750	0.190
Time	10.812	2	5.406	4.017	0.022*
Group x Time	0.466	2	0.233	0.173	0.841
Error	92.859	69	1.346		

Note. \*  $p < 0.05$

Table F-3

## ANOVA Summary Table for Vigor

Source	SS	df	MS	F	p
Group	25.855	1	25.855	1.396	0.241
Time	3.142	2	1.571	0.085	0.919
Group x Time	11.355	2	5.678	0.307	0.737
Error	1277.9100	69	18.520		

Table F-4

## ANOVA Summary Table for Fatigue

Source	SS	df	MS	F	p
Group	0.455	1	0.455	0.106	0.746
Time	96.514	2	48.257	11.196	0.000*
Group x Time	3.501	2	1.750	0.406	0.668
Error	297.404	69	4.310		

Note. \*  $p < 0.05$