The acute effects of transdermal testosterone precursor administration on serum steroid hormone levels in females

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THE ACUTE EFFECTS OF TRANSDERMAL TESTOSTERONE PRECURSOR ADMINISTRATION ON SERUM STEROID HORMONE LEVELS IN FEMALES

by

Mark Anthony Rasmussen

Bachelor of Science
University of Wisconsin, Green Bay
1998

A thesis submitted in partial fulfillment of the requirement for the

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Department of Kinesiology (Exercise Physiology)
College of Health Sciences

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Examination Committee Chair

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Graduate College Faculty Representative
ABSTRACT

The Acute Effects of Transdermal Testosterone Precursor Administration on Serum Steroid Hormone Levels in Females

by

Mark Anthony Rasmussen

Dr. Lawrence Golding, Examination Committee Chair
Distinguished Professor of Kinesiology
University of Nevada, Las Vegas

Most governing sports authorities have banned the use of anabolic steroids as ergogenic aids. Recently, a transdermal testosterone precursor dietary supplement (AndrosteDERM®) has become available; athletes can apply the cream to their skin with the belief that it will increase serum testosterone levels, muscle mass, and strength. The purpose of this study was to measure the effects of one milliliter of AndrosteDERM® applied to the inner surface of the upper arm on the serum levels of androstenedione, and free and total testosterone in female subjects. Serum levels were measured before application and every 45 minutes thereafter for six hours. Serum androstenedione, and free and total testosterone levels were analyzed using radioimmunoassay. Although the trend seemed to indicate that serum levels did not rise after application, several subjects had physiologically impossible values, which appear to be due to methodological errors. That information, along with the initial small number of subjects made the use of statistical treatment unwise and inferences about the population impossible.
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CHAPTER 1

INTRODUCTION

Athletes often use ergogenic aids in an attempt to gain an edge over their competitors. An ergogenic aid can be defined as any substance or technique used to improve athletic performance (Brooks, Fahey, White, & Baldwin, 2000). In ancient times, athletes and soldiers would consume specific animal parts to obtain the strength, speed, or agility associated with that animal (Brooks et al., 2000). Today, the use of ergogenic aids in sports is universal. Dietary supplements are some of the most popular and widely used of all ergogenic aids. The use of dietary supplements, including amino acids, caffeine, creatine, and protein powders is prevalent in almost every sport today, in both males and females.

Recently, dietary supplementation of androstenedione, a precursor to the hormone testosterone, has gained interest as a method to improve performance by increasing muscle mass and strength. Many of the manufacturers of dietary supplements have started to market new products known as testosterone precursors, or “prohormones”, with androstenedione being the most popular. Prohormones are marketed with the claim that their use will cause an increase in endogenous testosterone production, acting in much the same way as anabolic steroids (McArdle, Katch, & Katch, 1999). When anabolic steroids are added to athletes’ training, increases in muscle size and strength and gains in
total and lean body mass have been reported (Stamford & Moffatt, 1974; Friedel, Dettori, Hannan, Patience, & Plymate, 1991; Bhasin, Storer, Berman, Callegari, Clevenger, Phillips, Bunnell, Tricker, Shirazi, & Casaburi, 1996).

To date, very little research has been conducted on the effectiveness of testosterone precursor supplementation. Most of the research has investigated oral androstenedione supplementation, with mixed results. Some studies have shown an increase in testosterone levels in the body (Earnest, Olson, Broeder, Breuel, & Beckham, 2000; Leder, Longcope, Catlin, Ahrens, Schoenfeld, & Finkelstein, 2000), while most others have not (Ballantyne, Phillips, MacDonald, Tarnopolsky, & MacDougall, 1999; Brown, Kohut, Franke, Jackson, Vukovich, & King, 1999; Brown, Reifenrath, Uhl, Sharp, & King, 1999; King, Sharp, Brown, Reifenrath, & Uhl, 1999; King, Sharp, Vukovich, Brown, Reifenrath, Uhl, & Parsons, 1999; Parsons, Sharp, Brown, Reifenrath, Uhl, & King, 1999; Quindry, Brittingham, Panton, Breuel, Earnest, Olson, & Broeder, 1999; Rasmussen, Volpi, Gore, & Wolfe, 2000; Reifenrath, Sharp, Brown, Uhl, & King, 1999; Wallace, Lim, Cutler, & Bucci, 1999). None of the studies have investigated the effects of testosterone precursor supplementation in females.

Recently, new methods of testosterone precursor delivery have been developed, which are touted to be superior to oral ingestion. Sublingual and transdermal versions of testosterone precursors are now available. The benefits of these new delivery methods include absorption directly into the bloodstream, thus bypassing the rapid initial breakdown in the liver, and faster action because of the absorption directly into the bloodstream. One such product is the transdermal version AndrosteDERM®, which contains the testosterone precursors androstenedione and androstenediol.
Purpose of the Study

The purpose of this study was to evaluate the acute effects of transdermal testosterone precursor supplement (AndrosteDERM®) administration on the serum steroid hormone levels of androstenedione and free and total testosterone in females.

Need for the Study

Testosterone precursor supplements are very popular and widely used by athletes of both sexes, in many different sports. Studies to date have been inconclusive as to whether or not these supplements have any ergogenic effects. To date, no studies have investigated the effects of testosterone precursor administration in females. The effects of a transdermal delivery method also have not been tested.

Limitations

1. Since the sample size was small, seven subjects, and homogeneous, college-aged females, the generalization of the results should be done with caution.

2. Because some of the results obtained from the radioimmunoassay procedure are in question, the concentrations of serum androstenedione and free and total testosterone may not be exact.

3. AndrosteDERM® is one of several different types of testosterone precursors. The results obtained from using another type of testosterone precursor or another delivery system may be different.
Assumptions

1. Participants had normal baseline androstenedione and free and total testosterone levels for females.

2. AndrosteDERM® was absorbed into the bloodstream in 6 hours.

Statement of Hypotheses

Null Hypotheses

$H_0$: Acute AndrosteDERM® administration will not increase serum androstenedione levels within six hours of application.

$H_0$: Acute AndrosteDERM® administration will not increase serum free testosterone levels within six hours of application.

$H_0$: Acute AndrosteDERM® administration will not increase serum total testosterone levels within six hours of application.

Alternate Hypotheses

$H_1$: Acute AndrosteDERM® administration will increase serum androstenedione levels within six hours of application.

$H_1$: Acute AndrosteDERM® administration will increase serum free testosterone levels within six hours of application.

$H_1$: Acute AndrosteDERM® administration will increase serum total testosterone levels within six hours of application.
Anabolic Steroids and Testosterone

Anabolic steroids are synthetic derivatives of the male sex hormone testosterone. Anabolic steroids can be defined as steroids whose function is to stimulate protein synthesis (Ariel, 1972). Because anabolic steroids have inseparable anabolic and androgenic properties, they should correctly be called anabolic-androgenic steroids. It is the anabolic effects, such as anticatabolism, enhanced neural conduction, and increased skeletal muscle mass that are desired by those who use them, and the androgenic, or masculinizing, effects that lead to the undesirable side effects (Fahey, 1998; Blue and Lombardo, 1999). Since the 1940’s attempts have been made to synthesize a steroid that is completely anabolic, without any of the unwanted androgenic side effects (Blue and Lombardo, 1999). To date, all attempts have failed. The anabolic and androgenic effects of these drugs are not due to different actions, but instead result from the interaction of the drug with the same receptor molecule in different tissues (Wilson, 1988).

Testosterone is produced endogenously in the interstitial cells of Leydig in the testes (Blue and Lombardo, 1999). Like all steroid hormones, testosterone is synthesized from cholesterol. Loebel and Kraemer (1999) indicated that the rate-limiting step in the synthesis of testosterone is the conversion of cholesterol to pregnenolone, which takes
place in the mitochondria. According to Lamb (1989) and Hough (1990), the testes secrete between 4 and 10 mg/day of testosterone in normal adult males. Mean testosterone production in males is approximately 8 mg/day (George, 1988). The plasma concentration of testosterone in males is approximately 3-9 ng/ml, of which, 95% or more is secreted by the testes (Gard, 1998). In females, the plasma testosterone concentration is 0.2-0.7 ng/ml (Gard, 1998), all of which is produced by the ovaries and the adrenal glands (Schänzer, 1996), primarily the adrenal glands (Laycock & Wise, 1996).

Testosterone (Figure 1) is secreted after receiving a signal from luteinizing hormone, which is secreted from the pituitary gland (Alèn & Rahkila, 1988). Loebel and Kraemer (1999) state that the hypothalamus secretes luteinizing hormone-releasing hormone, which causes luteinizing hormone to be released from the anterior pituitary gland. The amount of luteinizing hormone secreted from the pituitary gland is controlled by the amount of testosterone in the blood via a negative feedback loop (Brooks, 1983).

Testosterone is responsible for the normal growth and development of the male sex organs and maintenance of secondary sexual characteristics, including facial, pubic, chest, and axillary hair growth, enlargement of the larynx, sperm production, and alterations in body musculature and fat distribution (Fahey, 1998; Blue & Lombardo, 1999). Testosterone is also responsible for the greater muscle mass in men when compared to women (Wynn, 1975). In both males and females, testosterone increases libido (Goodman, 1994). Testosterone in females is responsible for some secondary sexual characteristics, including pubic and axillary hair growth (George, 1988).
The most active form of testosterone is the free, unbound form, which accounts for 2% of the total testosterone in the body (Loebel & Kraemer, 1999). Testosterone bound to albumin accounts for 38% of the total testosterone; the remaining 60% is bound to sex hormone-binding globulin (Loebel & Kraemer, 1999). It is the testosterone that is not bound to sex hormone-binding globulin that is available for metabolism (Loebel & Kraemer, 1999).

Testosterone is rapidly metabolized by the enzyme 5β-reductase (Goodman, 1994) in the liver, into various 17-keto steroids, mainly androstenedione and then to either androsterone or one of its two isomers, epiandrosterone or etiocholanone (George, 1988). The liver is the main site of testosterone degradation, with the subsequent release of water soluble sulfates (Goodman, 1994) and testosterone glucuronide (Hackney, 1998) into the blood for excretion in the urine (Goodman, 1994). When taken orally,
testosterone is absorbed into the portal blood circulation and is rapidly degraded by the liver, leading to insignificant amounts reaching systemic circulation (Wilson & Griffin, 1980). Testosterone that is injected intramuscularly is rapidly absorbed from the injection site and degraded (Wilson & Griffin, 1980). Therefore, modifications have been made to the testosterone molecule to alter its metabolism and get around these complications. In order for serum testosterone levels to be maintained (during replacement therapy), testosterone esters are injected intramuscularly, or slowly metabolized oral derivatives are ingested (Wilson & Griffin, 1980).

Three general types of modifications have been created, esterification of the 17β-hydroxyl group, alkylation of the 17α-position, and a modification of the ring structure (Wilson & Griffin, 1980). Esterification of the 17β-hydroxyl group prolongs the duration of time the steroid is active, thus augmenting the effects (Wilson & Griffin, 1980). The more carbon molecules in the acid ester, the more prolonged the action of the anabolic steroid (Wilson & Griffin, 1980). Forbes (1985) stated that nitrogen balance studies have demonstrated that intramuscular anabolic steroids have more prolonged and greater effects. A 17α-methyl or -ethyl group is common on most orally active anabolic steroids (Wilson & Griffin, 1980). The 17α-alkylation of an orally active anabolic steroid slows the breakdown by the liver; for this reason, all 17α-alkylated anabolic steroids are toxic to the liver (George, 1988). Some substitutions made to the 2, 9, and 11 carbons of the testosterone molecule (ring structure) also appear to enhance anabolic activity (Wilson & Griffin, 1980). It could be reasoned that because exogenous testosterone must be modified to exhibit any sustained effects on the body, an unmodified
precursor to testosterone such as androstenedione or androstenediol would meet a similar fate as unmodified testosterone (rapid metabolism by the liver).

Medicinally, anabolic steroids have been used to treat a variety of disorders in the body including male hypogonadism, certain anemias, bone marrow failure, breast cancer in postmenopausal women, hereditary angioneurotic edema, osteoporosis, and disorders where catabolism and negative nitrogen balance are favored, such as wasting illnesses and patients recovering from severe trauma or surgery (Wilson & Griffin, 1980; Wilson, 1988; Hough, 1990). Haupt and Rovere (1984) report that anabolic steroids were first given to German troops during World War II to increase their aggression. At about the same time, anabolic steroids were also used for their ability to restore positive nitrogen balance in starvation victims (Ryan, 1981). Hough (1990) indicated that athletes first used anabolic steroids in 1953. In 1954, a Russian team physician told Dr. John Zeigler, a physician for the United States Weightlifting team, that the Russian weightlifters were using anabolic steroids to augment their training (Street, Antonio, & Cudlipp, 1996). One year later, Dr. Zeigler developed methandrostenolone (Dianabol) and introduced it to the United States weightlifting team (Street et al., 1996). In 1968, the International Olympic Committee placed anabolic steroids on its list of banned substances (Hough, 1990). Since that time anabolic steroids use has become very popular in sports. They were initially used mainly by power athletes, those involved in football, weightlifting, and field events (shot put and hammer throw). Now, their use is seen in sports that do not rely as heavily on power, including baseball and tennis. Some (Haupt & Rovere, 1984) believe that anabolic steroid use by athletes has reached almost epidemic proportions in most sports. Athletes use anabolic steroids in the belief that they will increase body and
muscle mass, muscular strength and aggressiveness (Hough, 1990; Fahey, 1998). Wade (1972) stated that winning at the Olympics has become a question of which country has the best doctors and chemists.

**Anabolic Steroid Research**

Since athletes first used anabolic steroids, there has been great debate whether they actually improve performance (Wagner, 1991). In an attempt to determine whether or not anabolic steroids improve performance, researchers have studied their effects, with mixed results. The majority of anabolic steroid research was performed during the 1970's. Most early studies were of poor quality and had mixed results (Wagner 1991; Blue & Lombardo, 1999). The scientific studies are fraught with methodological problems that make interpretation difficult and comparisons between studies confusing (Lombardo, 1993). Ryan (1981) stated that conclusions were drawn that were not justified by the results obtained.

A great deal of criticism has been made involving early anabolic steroid studies (American College of Sports Medicine, 1987). The studies that demonstrated no increases in muscular strength have been criticized because of the use of inexperienced participants, lack of dietary (calories and protein) control, low-intensity training, and non-specific measuring of strength (American College of Sports Medicine, 1987). The studies that have demonstrated increases in muscular strength have been criticized for low numbers of participants, improper statistical designs, inadequate execution, and unsatisfactory reporting of the results (American College of Sports Medicine, 1987). A meta-analysis of the studies from 1966 to 1990 performed by Elashoff, Jacknow, Shain,
& Braunstein (1991) assessed the effects of anabolic steroids on human muscular strength. Thirty studies were reviewed in which subjects received more than one dose of the anabolic steroid studied and in which changes in muscular strength were measured. Of the 30 studies, 14 were determined to be of poor quality for various reasons (Elashoff et al., 1991). All of the studies differed in regards to the length of time of anabolic steroid administration, the intensities of strength training programs, the pre-training levels of strength in the participants, and especially the dosages of anabolic steroids administered (Alén & Häkkinen, 1987; Lombardo, 1993).

The weight training experience of the participants is an important factor to consider (Lombardo, 1993). Alén and Häkkinen (1987) explained that anabolic steroids do not accelerate the physical development of athletes in the very early phase of strength training because the strength training itself is enough of a stimulus to produce substantial gains during that phase. Inexperienced weightlifters will make large gains at the beginning of any weight training program; these large gains are not significantly increased by anabolic steroids in the early part of their training (Lombardo, 1993).

Blue and Lombardo (1999) stated that at therapeutic doses, anabolic steroids do not enhance muscle strength or athletic performance. Endogenous testosterone production is depressed, and the net effect is negligible (Blue & Lombardo, 1999). According to Percy (1978), Lamb (1984 and 1989), Taylor (1987), Elashoff et al. (1991), and (Wagner, 1991) one obvious flaw with past anabolic steroid research, is that the amount of the anabolic steroids administered in most clinical studies has been well below the massive doses taken by athletes. The dosages of anabolic steroids taken by most athletes are 10 to 200 times greater than normal therapeutic doses, in an attempt to
maximize the anabolic effects (Street et al., 1996; Blue & Lombardo, 1999). Casaburi, Storer, and Bhasin (1996) stress that the doses of anabolic steroids used in studies are low considering, 1) all testosterone derivatives are less potent than testosterone; 2) approximately 7mg of testosterone are produced daily in healthy males; 3) the absorption of oral anabolic steroids is erratic and incomplete; 4) some of the orally administered anabolic steroid is metabolized by the liver during the first pass through. Taylor (1987) and Wilson (1988) noted that scientific research also does not involve the administration of anabolic steroids in the stacking and cyclical fashions utilized by many athletes. Lamb (1989) and Wagner (1991) stated that it is medically unethical to administer anabolic steroids in the dosages used by athletes. Very few definitive, controlled clinical trials with supraphysiological doses of anabolic steroids have been conducted (Bardin, Swerdloff, & Santen, 1991).

Further research has also been hampered by the problems of conducting a true double-blind study (Lamb, 1989; Wagner, 1991). A double blind study to investigate the effects of anabolic steroids would be ideal, but many athletes can recognize when they are on the anabolic steroid compared to the placebo (Lamb, 1989). In a study by Freed, Banks, Longson, and Burley (1975), all thirteen participants guessed correctly when they were on the steroid and the placebo portions of the study. Lamb (1984) and Taylor (1987) concluded that because of the lack of a satisfactory placebo and the usually low steroid doses that are used in research studies, great care should be considered when evaluating scientific evidence that deals with anabolic steroid use in athletes. The studies that appear in the literature are of limited value in assessing the effects of the drugs used (Fahey & Brown, 1973; Taylor, 1987).
Studies that Found Ergogenic Benefits

Papanicolaou and Falk (1938) were some of the first to note that androgenic hormones (testosterone propionate) have a stimulating effect on the muscles, producing hypertrophy after long-term administration. During the 1960’s and 70’s, researchers attempted to perform studies that investigated the effects of anabolic steroids on muscle strength and body composition (Taylor, 1987). The difficulties encountered during this process have already been mentioned. In most studies in which trained athletes took steroids in relatively low doses for short periods of time and also used weight training and proper diet, a significant increase in skeletal muscle mass and muscle strength was noticed in many subjects (Taylor, 1987). Despite the problems with the scientific data, it is commonly accepted on the part of athletes, coaches, sport physicians, and the public that anabolic steroids improve athletic performance (Bardin et al., 1991). While many of the studies were not double-blinded or placebo controlled, it has to be concluded that anabolic steroids, in high doses combined with heavy resistance training will result in increased body weight and muscle size (MacDougall, 1983).

Johnson and O’Shea (1969) studied the effects of an anabolic steroid on strength development. In what was planned to be a double blind study (only twelve men were willing to take the steroid and thus became the experimental group), twelve pairs of participants were fed protein supplements and trained with weights for six weeks. During the first three weeks, all participants were treated identically with the same weight training program and protein supplements. During the last three weeks of the study, the experimental group received 10 mg/day of methandrostenolone. The results indicated that both dynamic and static strength and body weight increased significantly in the
that both dynamic and static strength and body weight increased significantly in the experimental group. The increased body weight occurred with no significant changes in subcutaneous adipose tissue, leading the investigators to believe that the weight gained was lean body mass and not body fat. Surprisingly, maximum oxygen uptake increased significantly, even though the training program was not designed to promote cardiovascular fitness. The researchers concluded that the combination of anabolic steroids, high protein intake, and heavy weight training accelerated protein synthesis and increased static and dynamic muscle strength and body weight.

O’Shea and Winkler (1970) studied the effects of an anabolic steroid (oxandrolone) on the physical factors involved with sport-specific training in highly-advanced swimmers and weightlifters. Double blind testing was not used for this study because of the small number of participants. During the first six weeks of the eleven-week study, subjects were given 10 mg/day of oxandrolone (treatment period) and a protein supplement; during the final five-week washout period, no anabolic steroids were administered. The results of this study indicated that body weight increased in all participants. An increase in protein utilization was also demonstrated in eight of the eleven participants. In the group of swimmers, no improvement was noted in performance (swimming speed), while muscle strength improved dramatically in the group of weightlifters. The investigators concluded that muscular strength is increased when anabolic steroids are combined with a high protein diet and heavy weight training. The investigators also considered that because the weightlifters knew they were receiving anabolic steroids, the degree to which the strength increases can be attributed to the steroids rather than psychological effects cannot be distinguished.
Ward (1970) investigated the effects of an anabolic steroid (10 mg/day of methandrostenolone), in conjunction with heavy weight training on maximum strength (standing press, squat, and bench press), maximum oxygen uptake, lean body mass, and blood volume in one experienced weightlifter. Maximum strength in all three exercises increased significantly (35 lbs, 12.73% in the standing press, 50 lbs, 10.75% in the squat, and 35 lbs, 9.09% in the bench press). Maximum oxygen uptake (in the absence of cardiovascular training) increased 15.66% when measured in aerobic capacity, and 12.97% when measured in aerobic power. Lean body mass increased 18 lbs, while body fat decreased 9 lbs (4%). Blood volume was not changed. The author concluded that anabolic steroids may be beneficial in increasing muscle strength, lean body mass, and possibly aerobic endurance.

O'Shea (1971) studied the effects of an anabolic steroid on the muscular strength levels of twenty male weightlifters. The participants were divided into a placebo and a steroid group (10 mg/day of methandrostenolone); the diets of all participants were augmented with a protein supplement. The weight training program employed in this study was identical for all participants, and was noted as being very intense. The results of this study indicated that maximum bench press and squat strength increased significantly in the group who received anabolic steroids. Body weight in the steroid group also increased significantly. When a skinfold measurement was taken at the waist, no increase was noted, indicating the weight gained was lean body mass, and not adipose tissue. The researchers concluded that anabolic steroids do increase dynamic strength in competitive athletes, but that this is very dependant on the type and the quality of both the training (heavy weightlifting) and the diet (high protein) consumed.
The physiological effects of the anabolic steroid, methandrostenolone, were studied by Ariel in 1972. In the first seven weeks of a fifteen-week study, the six participants trained with weights five days a week (training period). From the eighth to the eleventh week of the study, all participants were given a placebo and were told it contained 10 mg of methandrostenolone (placebo period). From the twelfth to the fifteenth week of the study, three of the participants received 10 mg of methandrostenolone (experimental group), while the other three participants continued to receive the placebo (anabolic steroid period). Significant increases in muscle strength were noticed in the experimental group between the placebo period and the anabolic steroid period. The investigator concluded that the experimental group was able to exert greater maximum muscular contractile force during the anabolic steroid period when compared to the placebo period and to the control group, whose progress did not increase significantly.

The effects of the anabolic steroid methandrostenolone on strength and aerobic capacity were studied by Bowers and Reardon (1972). Eighteen weightlifters volunteered for this six-week study, and were divided into an experimental (steroid) and a control (placebo) group. All subjects received daily protein supplementation; the steroid group received 10 mg/day of methandrostenolone for the last twenty-one days of the study. The results of the study showed that the experimental group significantly increased strength in the bench press and squat, body weight and bicep and forearm girth over the control group. No increases were seen in aerobic capacity.

Johnson, Fisher, Silvester, and Hofheins (1972) studied the effects of an anabolic steroid (methandrostenolone) on muscular strength, body weight, oxygen uptake and
spermatogenesis. All subjects began an identical seven-week weight-training program; at
the end of the fourth week, 24 of the 31 subjects were randomly placed into either the
control (placebo) group, or the treatment (10 mg/day of the anabolic steroid) group. In
addition, all subjects supplemented their diet with a protein supplement. The results
showed no significant increase in maximum oxygen uptake, and no changes in sperm
count. In the treatment group, strength increased and the gains in body weight were
significant over the placebo group. The investigators concluded that anabolic steroid
supplementation does significantly improve muscle size and strength when accompanied
with a high protein diet and intensive weight training.

Ward (1972) investigated the effects of an anabolic steroid on maximum strength
and lean body mass. Sixteen healthy male weight trainers volunteered for the five-week
study. The participants were divided into an experimental group (10 mg/day of
methandrostenolone) and a placebo group. The results indicated that the experimental
group improved in maximum strength and gained more lean body mass than the control
group. The experimental group had a significant increase in body mass, and a significant
reduction in body fat, with no increase in body weight above the control group. The
author concluded that the use of anabolic steroids may produce significant increases in
strength and lean body mass in athletes involved in a heavy weight training program
beyond what would result from heavy weight training alone.

Ariel (1973) investigated the effect on an anabolic steroid on maximum skeletal
muscle contractile force. Six male weightlifters volunteered for this eight-week double
blind study. The participants were divided into an experimental group (10 mg/day of a
placebo from weeks two through four and the steroid from weeks four through eight) and
a placebo group (placebo administered weeks two through eight). The results indicated that the experimental group was able to exert greater maximum muscle contractile force during the anabolic steroid period when compared to the placebo period and the control group, who did not significantly improve performance. The rate of progress of the experimental group was higher during the anabolic steroid period when compared to the control group.

Brown and Tait (1973) interviewed a number of athletes who were self-administering various dosages and types of anabolic steroids. All reports of strength and weight increases were subjective and reported by the athletes themselves. The weight gained was highly variable; three athletes gained no weight, while one gained 40 and another 50 lbs. Seven athletes gained strength, while two did not. Most of the athletes who reported strength gains said they were dramatic; one increased his maximum deadlift 110 lbs, another gained 90 lbs on his maximum squat. It was concluded that anabolic steroids may increase strength development when accompanied by a healthy diet and a heavy weight training program.

Ariel (1974a) investigated the prolonged effects of an anabolic steroid on muscle strength following cessation of treatment and a 15-week detraining period. College-aged male participants were divided into two groups, the experimental group who received 15 mg of methandrostenolone daily for four weeks, or the control group who received nothing. Following the four weeks of anabolic steroid treatment, the drug was removed and weight training ceased (15 weeks of detraining). Maximum strength was tested in the bench press, military press and the squat in all participants. Following the 15 weeks of detraining, muscle strength was tested again in all participants. In the bench press, the
experimental group lost a not statistically significant 6.6 kg of strength, while the control group lost a significant 19.9 kg. In the military press, the experimental group lost a not statistically significant 4.76 kg of strength, while the control group lost a significant 9.52 kg. In the squat, the experimental group lost 8.44 kg (not statistically significant), while the experimental group lost 15.19 kg, which again was significant. This study indicated that participants who were involved in strength training without anabolic steroids lost a significant amount of strength following detraining, while the participants who trained with anabolic steroids were better able to maintain their strength levels following detraining.

Ariel (1974b) investigated the residual effects of anabolic steroid administration on muscular force following cessation. Ten male weight lifters were divided into two groups, one who received the anabolic steroid (15 mg of methandrostenolone daily) first, followed by an identical placebo (Group 1), or one who received the placebo first, then the anabolic steroid (Group 2). All participants underwent an identical weight training protocol. The results indicated that anabolic steroids enhanced muscular strength, and the rate of increase of muscular strength. Following the administration of the anabolic steroid, there was a dramatic decrease in the muscular strength gained. Group 1 showed significant strength gains while using the anabolic steroid, but following cessation of the steroid, no significant gains in muscular strength were found. During the placebo period in Group 2 (the first part of the study), significant gains in three of the four weight training exercises were demonstrated; during the anabolic steroid period for Group 2, significant gains in muscular strength were seen in all participants in all weight training exercises.
O'Shea (1974) studied the effects of stanozolol (an anabolic steroid) on adrenal, liver, and muscle function in men. Eighteen weightlifters were equally divided into two groups, a treatment (stanozolol) and a placebo group, for the five-week study. Participants in the treatment group were given 8 mg/day of stanozolol; all participants supplemented their diets with a protein supplement and participated in an identical weight training program. When the biochemical data was evaluated, no significant alterations were noted in regards to adrenal, liver, or muscle function. When body weight and muscle strength were measured, the treatment group increased significantly in regards to muscle strength, but the body weight gained was not significant. The investigator theorized that anabolic steroids, when supplemented with a high protein intake and an intense weight training program, promote significant increases in dynamic muscular strength.

The purpose of the Stamford and Moffatt (1974) study was to approximate the actual conditions under which athletes use anabolic steroids. The controlled conditions included the administration of more than the recommended dosage of anabolic steroids, a high protein diet, and an intense weight training program. Twenty-four male weightlifters all underwent an identical weight training program, for four weeks. After the initial four week period, the participants were divided into four groups, Group 1 received 20 mg of methandrosteneolone and a protein supplement daily, Group 2 received an identical placebo and a protein supplement, Group 3 received only the protein supplement, and Group 4 received nothing, serving as the control. The results indicated that Group 1 had significant increases in bench press strength, static strength, and body weight compared to Groups 2, 3, and 4. It was concluded that 20mg of
methandrostenolone (a dose higher than the recommended therapeutic dose) can effectively accelerate strength and muscle mass gains in experienced weightlifters.

Freed, Banks, Longson, and Burley (1975) tested thirteen male weightlifters, who ingested a high protein diet, exercised regularly, and took either 10 or 25 mg/day of methandrostenolone or a placebo, in a crossover design, to see what effect it had on athletic performance. The athletes chose a low dose (10 mg/day) or a high dose (25 mg/day) of methandrostenolone and were tested for two consecutive six-week sessions, with the anabolic steroid and the placebo given in a random order. The investigators insisted on some previous weightlifting experience, and only experienced weightlifters were included in the study, this was because when inexperienced participants begin a weight training program, the initially rapid strength gains caused by neuromuscular adaptations (Häkkinen, 1994) would theoretically mask any benefits that would occur due to anabolic steroid supplementation. Athletic performance was measured as percentages of maximum weight lifted in six strength training exercises, including the squat and the bench press. Improvements in strength were significantly greater during the anabolic steroid supplementation period than during the placebo period. Body weight also was measured, and increased significantly during the anabolic steroid period. The weight gain was attributed to water retention and not muscle mass. The investigators reasoned that anabolic steroids improve athletic performance only when given in combination with weight training and a high protein diet. It was theorized that while using anabolic steroids, athletes are less susceptible to fatigue, allowing longer, more frequent and harder training sessions.
Win-May and Mya-Tu (1975) conducted a study to assess the effects of anabolic steroids on various measures of physical fitness, including anthropometry, balance, coordination and flexibility tests, static and dynamic strength, and cardio-respiratory endurance. Thirty-one male university students were randomly divided into either the anabolic steroid group, who received 5 mg/day of methandrostenolone for three months, or the control group, who received a placebo. The results of this investigation showed a significant increase in the anthropometric measurements (body weight, and six body circumferences) in the steroid group, compared to the placebo group. Static and dynamic strength was also shown to increase in the steroid group. No increases were noted in balance, coordination, flexibility or cardio-respiratory endurance with the administration of the anabolic steroid. The investigators also subjectively noted that muscle hypertrophy resulted from the administration of the anabolic steroid. It was concluded that anabolic steroids have an effect on some areas of physical fitness.

Wynn (1975) cited the powerful effects of anabolic steroids in anorexic patients. First, an anorexic girl consumed 3000 calories and a high amount of protein daily for 10 days. Only a weak positive nitrogen balance was noticed. During the following period, when the anabolic steroid methandrostenolone was administered, the positive nitrogen balance increased at least threefold, and remained elevated for the 34-day administration period. The anabolic steroid period was also associated with a significant weight gain. When the anabolic steroid was removed, the positive nitrogen balance ceased and no further weight gain was noticed. Another case of the strong effects of anabolic steroids involved a teen-age boy who was also anorexic. This time on a 4000 calorie a day diet, containing high quantities of protein, a modest positive nitrogen balance was noticed.
When the anabolic steroid methandrostenolone was administered, the magnitude of the positive nitrogen balance increased threefold, and remained elevated for the whole period of anabolic steroid treatment. Again, the positive nitrogen balance associated with the anabolic steroid resulted in significant weight gain. When the anabolic steroid treatment ceased, the weight gain halted and nitrogen balance returned to zero.

Hervey (1975) and (1976) studied the effects of the anabolic steroid methandrostenolone on body composition and muscular strength in untrained men. Using a crossover, double-blind design, the participants were randomly given the steroid (100 mg/day) or a placebo. The two treatment periods (weight training and placebo or weight training and steroid administration) were separated by five weeks of abstinence from weight training. The results indicated that while on the steroid, the participants gained significantly more weight than during the placebo administration period. On the basis of total body potassium measurements and the significant increase in thigh muscle mass width, the weight gained was determined to be in the lean body compartment. The increases noticed in muscular strength were not significantly different during either steroid or placebo administration. It was concluded that the widely held belief that anabolic steroids cause weight gain (lean body mass gain) in young men undergoing weight training is true, although, the belief that anabolic steroids improve strength and performance was not shown to be true during this study.

Studies with anabolic steroids since 1975 have tended to be more carefully carried out, better controlled, and more sophisticated (George, 1988).

Tahmindjis (1976) studied the effects of self-administration of anabolic steroids in twenty male weightlifters. In all participants, body weight was shown to increase,
ranging from 1.5 to 13.2% of body weight, with an average of a 5.7% increase. Muscle strength was also shown to increase dramatically when the participants were using the anabolic steroid compared to when they were not. The smallest improvement in muscular strength (combination of bench press and squat strength) noticed while using anabolic steroids was 100% greater than the improvement without anabolic steroids; the largest improvement was 380%, the average improvement was 195%.

Hervey, Knibbs, Burkinshaw, Morgan, Jones, Chettle, and Vartsky (1981) studied the effects of methandienone on muscular strength and body composition in competitive weightlifters. A 100 mg/day dose (a dose sometimes quoted as being as high as some athletes take) of the anabolic steroid or an identical placebo were administered for six weeks, and then alternated in a double-blind, crossover fashion. During the steroid treatment period, body weight, total body potassium and nitrogen, muscle size, and leg strength increased dramatically when compared to the placebo period.

Alèn, Häkkinen and Komi (1984) investigated the changes in body composition, muscle fiber characteristics, and isometric and dynamic force output of the leg extensor muscles in five experimental (self-administration of very high doses of anabolic steroids) and six control (no anabolic steroids) power athletes during twenty-four weeks of a strength training program. Following the twenty-four weeks of anabolic steroid self-administration, a six-week period without any anabolic steroids followed, and the same measurements were taken again. The results indicated that anabolic steroids significantly increased body mass (average of 11.25 pounds), lean body mass (average 17 pounds) and mean muscle fiber area in the experimental power athletes, while no significant changes were noted in any of the measurements in the control power athletes. Maximum
isometric force also significantly improved in the experimental group. During the six
weeks without anabolic steroids, significant changes were noticed in the experimental
power athletes in body composition and force output. Significant increases were seen in
dynamic and isometric force, while body mass was significantly decreased. The
researchers noted that this decrease in body mass could reflect the elimination of fluid
that was retained during the anabolic steroid period. An interesting point that was noted
during this study was that during the first six weeks, similar increases in all
measurements were noticed in both groups. While the study continued, the increases in
the experimental group far surpassed those of the control group. The researchers
concluded that the achievements in muscular strength might depend on the duration of
the steroid use and the training methods utilized.

Alèn and Håkkinen (1985) performed a case study on an elite bodybuilder who
self-administered extremely high doses of anabolic steroids over a one year period.
During one twenty-six and one twenty-four week steroid self-administration cycle
(separated by four weeks without anabolic steroids) total body mass, lean body mass,
mean fiber area of the vastus lateralis muscle, total thigh girth, and maximum leg
extension strength all increased significantly. No changes were noticed in maximum
oxygen uptake or maximum anaerobic power. It was concluded that strength training
combined with the use of extremely large doses of anabolic steroids might have increased
protein synthesis, which resulted in muscle hypertrophy. The authors explained that low
receptor affinities characterize most of the anabolic steroids so that high doses must be
administered to obtain anabolic effects greater than those due to a natural training
stimulus.
Forbes (1985) analyzed four of his own subjects, along with ten studies that measured changes in lean body mass before and after anabolic steroid administration. His four subjects demonstrated significant increases in lean body mass, and decreases in body fat during anabolic steroid administration. It was determined that the magnitude of the increases in lean body mass were related to the total amount of the anabolic steroid administered. With low doses of anabolic steroids, increases in lean body mass were only one to two kilograms. When large doses of anabolic steroids were administrated the results indicated a progressive increase in lean body mass. Using the available information, the author developed a dose-response curve. The x-intercept of the regression line for the dose response curve was 2535 mg of total steroid dose; this represented the threshold for a significant effect on lean body mass. Forbes (1985) also calculated that between the ages of 13 to 20, males have produced 15,330 mg of testosterone more than females (6mg/day x 365 days x 7 years). This number represents the upper limit of the dose response curve. The corresponding upper limit of lean body mass gain was calculated to be 19 kilograms.

Häkkinen and Alen (1986) investigated the effects of self-administration of multiple anabolic steroids and strength training on the physiological performance and physical health of an elite bodybuilder. This study was noted as being of great value in assessing the effects of anabolic steroids in athletes. It investigated the self-administration of multiple anabolic steroids, in the dosages often taken by athletes, addressing two of the major criticisms of anabolic steroid research. The results of this study indicated an extraordinary increase in serum testosterone during anabolic steroid
supplementation. This rise in testosterone was associated with large gains in fat-free mass, mean muscle fiber area, and maximal strength.

Alén and Häkkinen (1987) investigated the effects of six months of self-administered anabolic steroid supplementation on serum hormone levels and maximal force development in four top-level Finnish strength athletes (experimental group). Five other top-level Finnish strength athletes who did not supplement their training with anabolic steroids served as a control group. The investigators commented on the need for a study of this design, examining strength development in elite strength athletes who are self-administering very high doses of multiple anabolic steroids while involved in heavy prolonged weight training. All participants were also given a protein supplement. The results indicated that total body mass and lean-body mass increased significantly (an average of 12 and 22 pounds, respectively) in the experimental group. Muscular strength and mean serum testosterone also increased significantly in the experimental group. No significant changes were seen in the control group.

Griggs, Kingston, Jozefowicz, Herr, Forbes, and Halliday (1989) investigated the effects of testosterone on muscle mass and protein synthesis in the absence of a weight-training program in nine normal male participants. The participants were administered 3 mg/kg/week of testosterone enanthate for twelve weeks. Muscle mass (estimated by creatinine excretion) increased in all nine participants on average 20 %. Muscle protein synthesis was also shown to increase on average 27 % in all participants. No significant increases in muscle fiber diameter were seen. The investigators concluded that testosterone increases muscle mass by increasing muscle protein synthesis.
Friedel, Dettori, Hannan, Patience, and Plymate (1991) examined the effects of supraphysiological doses of anabolic steroids on body composition and muscular strength. Thirty male participants were divided into one of four groups who received either, (1) 100 mg/week of testosterone enanthate (served as a replacement dose comparison), (2) 300 mg/week testosterone enanthate, (3) 100 mg/week nandrolone decanoate, or (4) 300 mg/week nandrolone decanoate, for six weeks. The results indicated that body weight significantly increased in groups 2, 3, and 4, but not group 1. No changes were found in skinfold thickness or percent body fat, therefore, the weight gained was theorized to be lean body mass. Muscular strength significantly increased in all groups except in group 1. The greatest improvements in strength were noticed in group 4. Serum testosterone increased threefold in group 2, while no significant increase was noted in group 1. The researchers concluded that high doses of anabolic steroids (groups 2, 3, and 4) produced significant increases in body weight (lean body mass), and muscular strength. In group 1, the absence of any changes, and the maintenance of normal serum testosterone levels, indicated that 100 mg/week was the same as a replacement dose of testosterone.

A study conducted by Forbes, Porta, Herr, and Griggs (1992) looked at the changes in body composition caused by testosterone administration. Seven untrained subjects were given 3 mg/kg of intramuscular testosterone enanthate for a total of twelve weeks. The participants were told to stick to their normal daily routine (diet and exercise). Lean body mass increased progressively throughout the study. The average increase in lean body mass was 7.5 kg (range 5.7 – 11.9 kg). The average change in body weight was 4.1 kg, thus, on average, 3.4 kg of body fat was lost during the study. The
participants retained the increased lean body mass for at least five to six months following the study, demonstrating that the increases in lean body mass are maintained following cessation of the steroid administration. The investigators concluded that the changes in body composition were the result of the testosterone supplementation. Urinary creatinine was measured and indicated that most, if not all, of the increased lean body mass was muscle tissue.

Welle, Jozefowicz, Forbes, and Griggs (1992) investigated the effects of testosterone on basal metabolic rate (BMR) and lean body mass in normal men and men with muscular dystrophy, for periods lasting three and twelve months. The participants were divided into a control (no anabolic steroid) and experimental (testosterone injections) group in both the normal men and men with muscular dystrophy. No significant changes were demonstrated in either BMR or lean body mass in the control groups. The testosterone injections significantly increased plasma testosterone levels for the study period (average threefold increase). In normal men, after three months, BMR was shown to increase 7%, while men with muscular dystrophy demonstrated a 13% increase in BMR; after twelve months, the BMR had still increased a total of 9% in men with muscular dystrophy. Lean body mass (using urinary creatinine excretion) also increased significantly (approximately 10.5%) after three months, and remained elevated after twelve months. The men with muscular dystrophy who were treated with testosterone lost body fat (average 6 kg) during the treatment, while those who received the placebo actually gained body fat during the study.

Bhasin, Storer, Berman, Callegari, Clevenger, Phillips, Bunnell, Tricker, Shirazi, and Casaburi (1996) looked at the effects of supraphysiologic doses of testosterone on
muscle size and strength in normal men. The men were randomly assigned to one of four
groups, a placebo and no exercise group, a placebo and exercise group, a testosterone and
no exercise group, and a testosterone and exercise group. The participants received either
600 mg/week of testosterone enanthate or a placebo for 10 weeks. The 600 mg/week
dose of testosterone enanthate is six times higher than the usual replacement dose
administered to hypogonadal men. Before and after the treatment, body composition (fat
free mass) using underwater weighing, muscle size using magnetic resonance imaging,
and upper and lower body strength using the bench press and squat, respectively, were
measured. The intake of energy and protein and the exercise (strength training) program
were standardized in an attempt to control for some of the factors that have been
criticized in many of the previous studies conducted on the effects of anabolic steroids on
performance. Free and total testosterone increased significantly in the two groups who
received testosterone, but not in either placebo group. The men in the testosterone and no
exercise group had a significant increase in body weight, coupled with a 3.2 kg average
increase in lean body mass. The testosterone and exercise group had an average increase
of 6.1 kg in body weight and an average increase in lean body mass of 6.1 kg, indicating
that the weight gained was lean body mass. No significant changes were seen in either of
the placebo groups for body composition. When muscle size was analyzed, the mean
cross sectional area of the arm and leg muscles in the placebo groups did not significantly
change. Both of the groups that received testosterone had significant increases in cross
sectional area of the arm and leg muscles, with the testosterone and exercise group
greater than the testosterone and no exercise group. The men in the testosterone and no
exercise group had significant increases in maximum squat and bench press strength of
19% and 10%, respectively, almost identical to the increases seen in the placebo and exercise group. In the testosterone and exercise group, the increases in maximum squat and bench press strength were 38% and 22%, respectively, twice as great as the testosterone and no exercise and the placebo and exercise groups. The investigators concluded that supraphysiological doses of testosterone increase lean body mass, muscle size and strength in normal men.

Bhasin, Storer, Berman, Yarasheski, Clevenger, Phillips, Lee, Bunnell, and Casaburi (1997) investigated the effects of replacement doses of testosterone on lean body tissue and muscle size in hypogonadal males while on a protein and energy controlled diet. Participants were given testosterone enanthate (100 mg/week) for 10 weeks. Body weight increased on average 4.5 kg, with lean body mass increasing approximately 5 kg. Cross-sectional area of the triceps and quadriceps, as measured by magnetic resonance imaging, both increased significantly. Muscle strength also increased approximately 11 kg in the bench press, and 31 kg in the squat, even though the participants were not involved in a strength training program. The investigators concluded that testosterone replacement in hypogonadal men increases lean body mass and muscle size.

Kadi, Eriksson, Holmner, and Thornell (1999) found that the mean area of the muscle fibers in high-level power athletes using anabolic steroids were larger than in similar athletes who did not use anabolic steroids. The researchers concluded that the use of anabolic steroids and strength training cause an increase in the size of the muscles both by hypertrophy and hyperplasia. It was proposed that anabolic steroid use causes the activation of satellite cells, resulting in an increase in the number of muscle fibers.
Sattler, Jaque, Schroeder, Olson, Dube, Martinez, Briggs, Horton, and Azen (1999) investigated the effects on body weight, lean body mass, muscle cross-sectional area, and muscle strength during nandrolone decanoate supplementation, combined with weight training in men with human immunodeficiency virus. Participants were divided into either a group who received the anabolic steroid (600 mg/day) (A), or a group who received the anabolic steroid (600 mg/day) combined with weight training (B). Body weight rose on average 4.0 kg and 3.2 kg, and lean body mass (measured by dual energy x-ray absorptiometry) increased on average 5.2 kg and 3.9 kg in Group B and A, respectively. Muscle cross-sectional area of the thigh, quadriceps and hamstrings, measured by magnetic resonance imaging, increased significantly in both groups. Muscle strength also increased significantly in both groups, with more significant increases in Group B.

Taylor, Brooks and Ryan (1999) demonstrated that short-term administration of anabolic steroids (nandrolone decanoate) resulted in increased mass and contractile force in immobilized and nonimmobilized rabbit skeletal muscle. The authors concluded that anabolic steroid administration would result in muscle hypertrophy without weight training or a high protein diet.

It has been concluded that in inexperienced weightlifters, neither total and lean body mass, muscular strength, nor aerobic strength are likely to be influenced by administration of therapeutic doses of anabolic steroids in the absence of an exercise program that promotes anabolism (Wright, 1980). Even when anabolic steroids are administrated during strength training and protein supplementation, strength and body weight may not increase more than with training alone (Wright, 1980; Lamb, 1984 and
1989). For experienced weightlifters, therapeutic doses of anabolic steroids appear to enhance the normal increases in body mass that occur with weight training (Wright, 1980; Lamb, 1984 and 1989). The effects on body composition are not as clear as the effects on body weight because of factors such as lack of dietary control, different anabolic steroids and dosages, different training programs and durations, and individual differences in body composition and metabolic rates (Wright, 1980).

Haupt and Rovere (1984) concluded that anabolic steroids will consistently show increases in muscular strength if the following criteria are met: 1) anabolic steroids are administered to athletes who have trained with weights immediately before administration, and who continue weight training during the treatment period; 2) athletes maintain a high protein diet; and 3) strength changes are measured by the one repetition maximum (1RM) technique for the exercise that the athlete trains.

**Studies that Found No Ergogenic Benefits**

While most believe that anabolic steroid supplementation increases muscular size and strength, and total and lean body mass, not every study has come up with those conclusions.

Samuels, Henschel, and Keys (1942) first investigated the effects of anabolic steroids on a measure of muscular performance, grip strength. Participants ingested 50 mg of methyl testosterone daily. No improvements in grip strength, beyond a training effect, were noticed in the participants. The authors concluded that no strength improvements are obtained with supplementation of 50 mg/day of methyl testosterone.
The effects of an anabolic steroid (Nibal), either taken alone or in combination with exercise (non-weight training workouts), were studied by Fowler, Gardner, and Egstrom (1965). Participants were placed into one of four groups, a placebo group, an anabolic steroid group, a placebo and exercise group, and an anabolic steroid and exercise group. Of interest to the investigators were the effects of the anabolic steroid on strength, physical working capacity, a series of anthropometric measurements, and motor performance. The results of this study showed that there were no significant differences in any of the measurements between the groups that did not exercise and who received either the placebo or the anabolic steroid. The only improvements in performance were noted in both exercising groups, with no significant differences between the two groups. The investigators concluded that anabolic steroid supplementation in normal doses does not increase muscle size or strength, and would be of no use to athletes.

Casner, Early, and Carlson (1971) studied the effects of an anabolic steroid (stanozolol) on body weight, body composition, and muscle strength in normal, untrained, young men. Twenty-seven men were placed into one of four groups: steroid and normal daily activity, steroid and weight training, placebo and normal daily activity, and placebo and weight training. The participants in the two steroid groups received 2 mg of stanozolol three times a day on days 1-21 and 28-49 of the 56-day experiment. The results indicated no significant increases in muscle strength in any group. An increase in body weight was noted for both groups receiving the anabolic steroids, but was theorized to be fluid retention. The investigators concluded that anabolic steroids cause an increase in body weight, however, the weight gained may have been due to fluid retention and not muscle mass and therefore was of no benefit to athletes.
In a study conducted by Fahey and Brown (1972), the effects of an anabolic steroid (nandrolone decanoate) on strength, body composition, and endurance were studied in college aged males with limited weightlifting experience. Participants were placed into either the treatment group (1 mg/kg of nandrolone decanoate at week two, five, and seven of a nine week program) or the placebo group. While the treatment group gained more weight, and increased maximum muscular strength compared to the placebo group, none of the differences were statistically significant. It was concluded that the anabolic steroid, in the usual recommended dosage, did not enhance strength, lean body mass or aerobic capacity. The investigators did acknowledge that the results could have been due to the fact that adequate protein intake (protein powder supplementation) seems to be necessary for anabolic steroids to be effective.

The effects of an anabolic steroid on strength development, anthropometric measurements, body weight, and aerobic power were investigated by Stremme, Meen, and Aakvaag (1974). During the eight-week experiment, twenty-one men were divided into a treatment group which received the anabolic steroid at a dosage of 75 mg/day for the first four weeks, and 150 mg/day for the last four weeks, or a placebo group. Throughout the experiment, all participants were given a protein supplement and underwent the same weight training program. The results indicated that for the strength development and anthropometric measurements, the steroid group showed gains over the control group, but the differences were not statistically significant (the only significant increase over the control group was in right thigh circumference). The steroid group showed no improvement in aerobic power, and actually showed a slight decrease, while the placebo group showed an increase in aerobic power. It was concluded that this
anabolic steroid (mesterolone) does not improve muscular strength or aerobic power in normal healthy men.

Golding, Freydinger, and Fishel (1974) examined the effects of an anabolic steroid (methandrostenolone) and a protein supplement on muscle size, strength, and body composition in male weightlifters. During a twelve-week study, 40 participants were divided into one of four groups, a protein and no steroid group, a steroid and no protein group, a steroid and protein group, and a no steroid and no protein group. All subjects were told that they were taking anabolic steroids and a protein supplement. The anabolic steroid groups received 10 mg/day of methandrostenolone. The type, frequency, and intensity of training were not controlled for; all subjects maintained their normal training protocol. No significant differences were found between the groups in skeletal measurements, body weight, body composition, static strength, or dynamic strength measured with the bench press and arm curl. It was concluded that strength athletes supplementing their training with 10 mg/day of methandrostenolone, either in the presence or absence of additional protein, did not increase body weight, muscular size or strength compared to a placebo.

Loughton and Ruhling (1977) investigated the effects of an anabolic steroid (methandrostenolone) and protein supplementation on cardiovascular endurance and muscular strength while participating in a seven-week endurance and weight training program. Twelve males (six trained athletes, and six untrained non-athletes) were divided into one of four groups, a trained steroid group, an untrained steroid group, a trained placebo group, and an untrained steroid group. The participants in the steroid groups received 10 mg of the steroid daily for the first three weeks and 5 mg of the
steroid daily for the last three weeks; all participants received additional protein supplementation. Again, no significant differences were observed in any of the four groups for any of the factors that were measured (strength, oxygen uptake, anthropometric measurements) with the exception of body weight and bench press which increased significantly in both steroid groups. The investigators concluded that anabolic steroid administration resulted in no significant changes in muscle size or strength, or any aerobic adaptations to exercise beyond those found with normal training.

Crist, Stackpole, and Peake (1983) studied the effects of anabolic steroids on neuromuscular power and body composition in nine (including one woman) experienced weightlifters, receiving protein supplementation. Experienced weightlifters were used in this study because novice weightlifters might have gained strength so rapidly from weight training alone that any results from anabolic steroid administration may have been masked. All subjects were randomly assigned to one of three groups and were given the three treatments (1 ml of placebo, 100 mg/ml testosterone cypionate, and 100 mg/ml nandrolone decanoate) in a double blind, crossover fashion. No significant differences were seen in seven of the ten peak torque measurements obtained from an isokinetic dynamometer. Mean lean body mass and body fat were also unaffected by administration of either anabolic steroid. The investigators concluded that anabolic steroids do not produce any significant changes in body composition or neuromuscular power. The investigators theorized that the dosage or duration of steroid administration might have been inadequate to produce any significant improvements in the performance measurements of this study.
While the difficulty in assessing anabolic steroid research has already been discussed, Haupt and Rovere (1984) explain that the studies that did not show a significant increase in muscle strength usually had some similarities including 1) the use of inexperienced weightlifters; 2) the use of anabolic steroids other than methandrostenolone; and 3) the use of strength testing techniques other than one repetition maximums (1RM) for weightlifting exercises.

Controversy as to the effects of anabolic steroids on performance has persisted since the 1970's, primarily because of misinformation from the medical and scientific communities. From anecdotal evidence and self-administration, athletes had concluded that anabolic steroids increased muscular size and strength, and thus improved performance. The medical and scientific communities reached much the opposite conclusion. Brown and Tait (1973) stated that in order to discourage steroid use, a number of physicians and sports scientists resorted to the same scare tactics that proved so ineffective in curbing the use of recreational drugs. Taylor (1987) concluded that by failing to distinguish between the effects of anabolic steroids on trained and untrained subjects, the scientific community came to faulty conclusions in the mid-1970's. The conclusions resulted in the publication of official position statements that anabolic steroid use did not enhance athletic performance, and a halt in research on the effects of anabolic steroids on athletes (Taylor, 1987).

The original American College of Sports Medicine (1977) position statement on the use of anabolic steroids stated that administration to young, healthy people (below age 50) in medically approved therapeutic doses, does not bring about any significant improvements in strength, endurance, lean body mass, or body weight. Any positive
effects come about because the users have been led to expect some positive effects (placebo effect). Also, the report stated that there is no conclusive evidence that extremely large doses of anabolic steroids aid or hinder athletic performance. The position statement concluded that because of the failure of studies to show improvements in muscular strength, lean body mass, or body weight after therapeutic doses of anabolic steroids, it is obvious that any benefits are likely to be small and not worth the health risks (American College of Sports Medicine, 1977).

The medical community's unconditional stance on anabolic steroids cost them their credibility and alienated athletes (Wright, 1980; Taylor, 1987). Some excellent examples came from (Ward, 1972) where Alan Ryan, the team physician at the University of Wisconsin and a former president of the American College of Sports Medicine, stated that the use of anabolic steroids is a complete waste of time and money, and Daniel Hanley, the official doctor to the United States Olympic team, emphasized that steroids have no effect on muscle strength. Athletes continued to believe that anabolic steroids improved performance, and therefore used them even though the medical community denied their role in improving performance (Taylor, 1987; Blue & Lombardo, 1999; Taylor et al., 1999).

Anabolic Steroid and Endurance

Because anabolic steroids are used medicinally to stimulate erythropoiesis (Wilson & Griffin, 1980; Lombardo, 1993) and treat certain anemias (Lamb, 1989), supplementation in athletes could theoretically increase the oxygen carrying capacity of blood, thus increasing maximal oxygen uptake (VO₂ max) (Lombardo, 1993).
(1988) indicated that part of the weight gain noticed while using anabolic steroids may be increased blood volume and hematocrit.

O’Shea (1970) studied the effects of anabolic steroid administration in swimmers. The intent of the study was to determine the influence, if any, of anabolic steroids during an activity that demands cardiovascular endurance and speed. Oxygen uptake, static strength and maximum performance (speed) were measured before and after steroid supplementation in fifteen college male swimmers. Eleven participants were divided into either Group A (who received 10 mg of methandrostenolone (anabolic steroid) for the first three weeks, followed by three weeks of placebo) or Group B (who received the placebo for the first three weeks followed by three weeks of methandrostenolone). Four participants served as controls in Group C. The results indicated that anabolic steroid administration had no effect on static strength, speed, or oxygen uptake. The author noted that all subjects in this study had very high levels of cardiovascular fitness, and statistically significant improvements in this group of people would be very difficult. It was concluded that anabolic steroid treatment alone (in the absence of a high protein diet) is ineffective in improving athletic performance.

Johnson, Roundy, Allsen, Fisher, and Silvester (1975) studied the effects of anabolic steroids on endurance training exclusively. The investigators stated that previous studies which showed no relationship between anabolic steroid supplementation and changes in endurance performance involved strength training exclusively. Maximum oxygen uptake (VO₂ max), mile-run time, and body composition (skinfold thickness) were measured in subjects who administered an anabolic steroid (or a placebo) and a protein powder, and who participated in a supervised running program over a three-week
period. The results of the study showed no significant differences between the group supplemented with anabolic steroids, and the placebo group. Thus, the investigators concluded that anabolic steroids do not appear to have any positive effects on endurance exercise performance or body composition (percent body fat).

Looking at the results of other studies, utilizing strength training exclusively, only two had demonstrated an increase in maximum oxygen uptake (Johnson & O’Shea, 1969; Ward, 1970) while many others demonstrated either no ergogenic benefit (Bowers & Reardon, 1972; Fahey & Brown, 1973; Hervey et al., 1976; Johnson et al., 1972; Johnson et al., 1975; Win-May & Mya-Tu, 1975), or a significant decrease in aerobic performance (Stromme et al., 1974) following anabolic steroid administration.

In reviewing all current literature, Lamb (1989) and Haupt and Rovere (1984) concluded that there is no scientific support to justify anabolic steroids as ergogenic aids for improving aerobic performance over what regular aerobic training would do. Lombardo (1993) explained that, instead, endurance athletes might administer anabolic steroids in an attempt to improve recovery time, allowing more frequent, longer, and higher intensity workouts.

Summary of Anabolic Steroid Research

The American College of Sports Medicine Position Stand on the Use of Anabolic-Androgenic Steroids in Sports (1987) concluded that 1) anabolic steroids in the presence of an adequate diet can contribute to increases in body weight, often in the lean mass compartment; 2) the gains in muscular strength achieved through high-intensity exercise and proper diet can be increased by the use of anabolic steroids in some individuals; and,
3) anabolic steroids do not increase aerobic power or capacity for muscular endurance. Lombardo, (1993) concluded that based on the available scientific evidence and the overwhelmingly consistent anecdotal reports of athletes, anabolic steroids will increase strength and lean body mass in subjects – especially experienced weight lifters – who perform high-intensity exercises and consume adequate diets.

To date, no controlled studies of the effects of anabolic steroids as ergogenic aids have been performed on females (MacDougall, 1983; Lombardo, 1993). It is theorized that even greater increases in muscle mass and strength may be noticed in females using anabolic steroids compared to males because of the lower circulating level of testosterone in females (MacDougall, 1983; George, 1988; Lombardo, 1993).

Possible Mechanism of Action

While there is general agreement that anabolic steroid use enhances performance by increasing muscular size and strength, the exact mechanism for this is unclear. Possible mechanisms include 1) an increase in protein synthesis; 2) inhibition of the catabolic effects of glucocorticoids (anticatabolic effects); 3) effects on the central nervous system (CNS); and, 4) the placebo effect (American College of Sports Medicine, 1987; Lombardo, 1993).

Increased Protein Synthesis

Testosterone (and anabolic steroids) is lipid soluble and readily diffuses into the cell (George, 1988). Rogozkin (1979) demonstrated the existence of androgen receptors in skeletal muscle treated with anabolic steroids. Anabolic steroids combine with an
androgen receptor in the cytoplasm in skeletal and cardiac muscles, the prostate gland, skin, and various areas of the brain (Wright, 1980; Lamb, 1984; Wilson, 1988; Lombardo, 1993). The hormone receptor complex interacts with receptor sites within the chromosomes to promote the transcription of genes, and the synthesis of messenger RNA molecules (Wright, 1980; Lamb, 1984; Wilson, 1988; Lombardo, 1993). The increased RNA synthesis translates into the synthesis of more protein in the ribosomes of the cells (Wright, 1980; Lamb, 1984; Lombardo, 1993). Rogozkin (1979), Hough (1990), and Blue and Lombardo (1999) suggest that anabolic steroids increase skeletal muscle actin and myosin protein synthesis. Hough (1990) states that strength training without anabolic steroids increases the number of steroid receptor sites in muscle, while strength training combined with anabolic steroids not only causes an increase in the number of receptor sites but also saturates them.

In some tissues, testosterone is first metabolized to estradiol and 5α-dihydrotestosterone via the aromatase and 5α-reductase enzymes, respectively, in order to be effective (George, 1988; Bardin et al., 1991). Estradiol and 5α-dihydrotestosterone act via the estrogen and androgen receptors, respectively (Bardin et al., 1991). Casaburi et al. (1996) and Wilson (1988) explain that in healthy men, the androgen receptors in most tissues are fully saturated or might even be down regulated by the androgen levels. Thus, if androgens are to be effective in healthy men, a mechanism other than the androgen receptor must be involved, presumably with a dose-response relationship (Bardin et al., 1991). Since the receptors are not entirely specific, some of the effects of supraphysiological doses of anabolic steroids are mediated via estrogen and progesterin receptors (Bardin et al., 1991).
Anticatabolic Effects

Anabolic steroids have their most pronounced effects in athletes who are in a chronic catabolic state caused by training (Haupt & Rovere, 1984). It has been hypothesized that the anabolic response in muscle could be due to a blocking of catabolism induced by glucocorticoids binding to their receptors, slowing protein breakdown (Wright, 1980; Haupt & Rovere, 1984; Mellion, 1984; Blue & Lombardo, 1999). Anabolic steroids competitively inhibit and displace glucocorticoids (Wright, 1980). Anabolic steroids can convert a negative nitrogen balance to a positive one, given adequate protein intake (Haupt & Rovere, 1984; Lamb, 1989). Wilson (1988) stated that at supraphysiological doses, anabolic steroids act as glucocorticoid antagonists and promote a positive nitrogen balance independent of the androgen receptor.

Boone, Lambert, Flynn, Michaud, Rodriguez-Zayas, and Andres, (1990) demonstrated that anabolic steroid users had significantly lower increases in creatine kinase concentration, and a faster clearance rate following identical strength training programs when compared to a control group who did not use anabolic steroids. This indicates a decrease in skeletal muscle damage following exercise. Cortisol concentration significantly increased following the strength training program in the control group, while no significant increase was observed in the anabolic steroid group, indicating possible suppression of glucocorticoids. The researchers concluded that anabolic steroid users have a diminished creatine kinase response and an altered stress response to a bout of exercise.
Effects on the CNS

Another theory on how anabolic steroid work deals with the androgenic (CNS) action of the drugs (Brooks, 1983; Lamb, 1989; Lombardo, 1993). It has been theorized that anabolic steroids make athletes more aggressive and competitive, allowing them to train harder, for a longer period of time, and to recover quicker from workouts. Thus, the increases in performance are a result of more intense and a greater volume of training. Hervey (1975) stated that if steroids are taken during training, fatigue is halted and that the extra work performed during each training session provides the real ergogenic benefits. Taylor (1987) stated that while taking anabolic steroids, athletes experience a euphoric state with diminished fatigue, allowing them to train more intensely. Some athletes take anabolic steroids in the hope that they will decrease healing time after a muscle injury like a pull or a strain (Mellion, 1984). Although these authors (Hervey, 1975; Brooks, 1983; Taylor, 1987) have theorized that anabolic steroids work solely through the CNS, Lamb (1989) has stated that the only evidence to support this theory consists of subjective sensations of greater strength gains in athletes undergoing steroid treatment compared to a placebo.

The Placebo Effect

The placebo effect (psychological mechanisms) has been suggested as a possible mechanism of action for anabolic steroids by some authors (Ryan, 1981; Lombardo, 1993). Ariel and Seville (1972) demonstrated the psychological (placebo) effect of anabolic steroid supplementation in fifteen varsity male athletes undergoing weight training. Four months prior to the actual study, the participants exercised with weights
five days a week. All participants were told of the benefits and dangers of anabolic steroid use, and that some would receive the anabolic steroids during the study while others would not. Six participants were given what they were told were anabolic steroids, but were actually placebos. The results of the study indicated that the strength gains measured in four weight training exercises were greater during the placebo (experimental) period than during the pre-placebo period. The experimenters concluded that taking the placebo supplied the psychological benefits needed to enhance strength gains above and beyond what would be expected. This study demonstrated the powerful psychological effect that cannot be excluded from any ergogenic aid research. In contrast, Wilson (1988) suggested that if this were a significant phenomenon, one would expect the effects on muscle strength to be more consistent in the double blind studies of athletic performance, when actually the results are inconsistent. In reality, athletes do not care whether the improvements in muscular strength are caused by the anabolic effects of steroids on muscle mass or psychologically induced improvements, as long as there are improvements (Lamb, 1989).

Other Mechanisms

Another possible mechanism for the effects of anabolic steroid usage is related to the increased secretion of other anabolic hormones. Anabolic steroids may also stimulate growth hormone secretion in adults (Alén & Rahkila, 1988). Hough (1990) noted that during anabolic steroid administration, growth hormone secretion is between 5 and 60 times greater than baseline measurements.
Alèn et al. (1984), Häkkinen and Alen (1986), and Bardin et al. (1991) have suggested that anabolic steroids may only be effective in large doses. Because relatively low receptor affinities characterize most of the anabolic steroids, high doses of these drugs should be administered in order to obtain any anabolic effects greater than those due to the natural training stimulus (Alèn et al., 1984; Häkkinen & Alen, 1986). The effects of anabolic steroids may be uniform, but of a small magnitude so that they are statistically difficult to demonstrate (Bardin et al., 1991). However, even small effects could be a benefit to a top athlete. As the caliber of the athlete increases, the effects on athletic performance become more difficult to assess (Wilson, 1988). The effects may be related to the log of the dose, and benefits on performance may be observed at 10 or more times the replacement dose for hypogonadal men (Bardin et al., 1991). Studies showing the maximal effects of androgens in animals are only observed at supraphysiological doses support this hypothesis (Bardin et al., 1991).

Bardin et al. (1991) have hypothesized that there is genetic polymorphism among individuals. Only a small subset will show a large response to the administration of anabolic steroids. Studies in inbred mice show that some strains have as much as a 20-fold greater response to anabolic steroids than others (Bardin et al., 1991). Hough (1990) has stated that individual differences in the responses to anabolic steroids are well known.

Steroid Alternatives

In 1990, the federal government passed the Anabolic Steroid Control Act (Yesalis & Wright, 1993), listing anabolic steroids as Schedule III controlled substances. With the passage of this law, the Drug Enforcement Agency (DEA) controls the manufacture,
importation, distribution and dispensing of anabolic steroids (Yesalis & Wright, 1993). Possession of anabolic steroids without a legitimate prescription is now a criminal offense. When this law went into effect, some athletes and bodybuilders were unable to find a source to supply them with anabolic steroids. Many athletes turned to dietary supplements with hopes that their use would produce results similar to anabolic steroid administration.

Dehydroepiandrosterone

While many different supplements have been marketed to athletes as steroid alternatives, the most popular have been "testosterone precursors", such as dehydroepiandrosterone (DHEA) and androstenedione. Testosterone precursors are administered with the belief that their use will increase endogenous testosterone production in the body, and therefore, produce effects similar to those seen when anabolic steroids are administered (McArdle et al., 1999). The use of testosterone precursors has increased in popularity since 1996 when they were first sold as dietary supplements in the USA (Brooks et al., 2000). DHEA was the first testosterone precursor marketed to athletes. Brown et al. (1999c), Wallace et al. (1999), and Welle, Jozefowicz, and Statt (1990) determined that supplementation with DHEA does not increase serum testosterone or protein metabolism. Thus, DHEA was not found to be an effective alternative to anabolic steroid usage. Recently, androstenedione was marketed as the newest version of testosterone precursor purported to increase serum testosterone concentrations and act like anabolic steroids in the body.
Androstenedione and Androstenediol

Androstenedione (Figure 2) is a steroid hormone produced endogenously in the gonads and adrenal glands of all humans (Leder et al., 2000). The production of androstenedione is approximately 3.4 mg per day in females and 1.4 mg per day in males (Horton & Tait, 1966). Androstenedione is synthesized from dehydroepiandrosterone and can be converted into testosterone via the enzyme 17β-hydroxysteroid dehydrogenase (Leder et al., 2000). Androstenedione serves as the immediate precursor to testosterone in the endogenous production of testosterone (Wallace et al., 1999). Androstenedione is characterized as a weak androgen in humans, with only minimal amounts being converted into testosterone in males (Wallace et al., 1999). In males, a greater amount is converted into estrogen hormones (Wallace et al., 1999). In females, as much as 60% of their testosterone may come from the peripheral conversion of androstenedione to testosterone (Horton & Tait, 1966). Androstenedione also serves as an immediate precursor to the hormone estrone (E1), which can then be converted into the more metabolically active estrogen, estradiol (E2) (Longcope, Kato & Horton, 1969). This conversion of androgens to estrogens takes place to a greater extent in males when compared to females (Longcope et al., 1969). While previous studies (Longcope et al., 1969; Edman, C.D., Aiman, E.J., Porter, J.C., & MacDonald, P.C., 1978; Kley, Deselaers, Peerenboom, & Krüskemper, 1980) have found that adipose tissue contains the enzyme aromatase, which aromatizes androgens to estrogens, Matsumine, Hirato, Yanaihara, Tamada, & Yoshida (1986) found that skeletal muscle in both males and females can also convert androgens into estrogens. Edman et al. (1978) stated that the skin and the brain can also aromatize androgens into estrogens. This peripheral
aromatization of androgens (including androstenedione) is the main source of estrogens in males, and represents a substantial amount of the estrone produced in females (Longcope et al., 1969). In males (Thomson, Wallace, & Cook, 1989) and females (Diagnostic Products Corporation, 1999a), androstenedione exhibits a diurnal variation, where it is highest in the morning; it also has cyclical variation during the female menstrual cycle, where it is highest near the middle of the cycle (Diagnostic Products Corporation, 1999).

![Androstenedione Chemical Structure](image)

**ANDROSTENEDIONE**

Figure 2: Androstenedione Chemical Structure

Androstenediol (Figure 3) is another steroid hormone that can be converted to testosterone in the body. According to Ballantyne et al. (2000), androstenediol can also be synthesized from dehydroepiandrosterone and converted into testosterone via the action of the enzyme 3β-hydroxysteroid dehydrogenase (Earnest et al., 2000). The most common pathway for the synthesis of testosterone involves the degradation of
pregnenolone to 17α-hydroxy pregnenolone, to dehydroepiandrosterone, to androstenediol, and finally to testosterone (Ballantyne et al., 2000). Testosterone can also be synthesized by another pathway involving the conversion of pregnenolone to progesterone and the subsequent formation of 17-hydroxy pregnenolone and androstenedione, which can be converted to estrone (then to estradiol) or testosterone (Ballantyne et al., 1999).

![Androstenediol Chemical Structure](image)

**ANDROSTENEDIOL**

Figure 3: Androstenediol Chemical Structure

Androstenedione and Androstenediol Studies

Early studies with androstenedione and other testosterone precursors (including androstenediol) were completely unrelated to their possible use as ergogenic aids. Instead, the early studies were conducted in an attempt to determine if weaker androgens could be converted into more potent ones. In general, the results indicated that human blood does contain the enzymes necessary to convert weaker androgens (like...
androstenedione) into stronger ones (like testosterone) (Mahesh & Greenblatt, 1962; Horton & Tait, 1966; Blaquier, Forchielli, & Dorfman, 1967).

Mahesh and Greenblatt (1962) found that orally administered dehydroepiandrosterone and androstenedione could be converted into testosterone in females. Dehydroepiandrosterone ingestion resulted in a 3-4-fold increase in plasma testosterone, while androstenedione ingestion caused a 4-7-fold increase in plasma testosterone.

Horton and Tait (1966) found that in females, a significant amount of testosterone is derived from androstenedione (as high as 60% peripheral conversion of androstenedione to testosterone). In males, plasma androstenedione conversion to testosterone is less than 0.3%. A negligible amount of plasma testosterone comes from androstenedione in males. One of the major findings of this study was that only 1.8% of orally administered androstenedione and 5.9% of intravenous androstenedione enters the plasma as testosterone.

Blaquier et al. (1967) demonstrated that androgen transformation and interconversions could take place in human blood. Small amounts of dehydroepiandrosterone were converted to androstenedione and testosterone. Larger amounts of androstenedione were converted to testosterone, but the androgen that was most readily (highest percentage) converted into testosterone was androstenediol. The researchers concluded that human blood can convert less active androgens into more biologically active ones.

The first reported use of androstenedione as an ergogenic aid came from East Germany, where athletes began administering it as early as 1981 (Franke & Berendonk,
1997). The androstenedione was not taken orally; instead, it was administered via a nasal spray (Franke & Berendonk, 1997).

Other than the original early studies performed on testosterone precursors, no data was available to assess the possible ergogenic benefits of their use. As a result, a number of studies have recently been performed to test whether these dietary supplements increase serum concentrations of testosterone and muscle size and strength (Ballantyne et al., 1999; Brown et al., 1999a; Brown et al., 1999b; King et al., 1999a; King et al., 1999b; Parsons et al., 1999; Quindry et al., 1999; Reifenrath et al., 1999; Wallace et al., 1999; Earnest et al., 2000; Leder et al., 2000; Rasmussen et al., 2000). To date almost all studies have investigated the orally administered versions of the testosterone precursor dietary supplements, with androstenedione being the most often used.

The results of the majority of the studies indicate that orally administered testosterone precursors do not increase serum testosterone concentrations or muscle size and strength (Ballantyne et al., 1999; Brown et al., 1999a; Brown et al., 1999b; King et al., 1999a; King et al., 1999b; Parsons et al., 1999; Quindry et al., 1999; Reifenrath et al., 1999; Wallace et al., 1999; Wallace et al., 1999; Rasmussen et al., 2000). Wallace et al. (1999) explained that the bioavailability of oral testosterone precursors is only approximately 5%, due to the effects of digestion, liver metabolism, and tissue enzyme activity. Horton and Tait (1966) found that 89% of orally administered androstenedione is converted to testosterone glucuronide and excreted in the urine.

Wallace et al. (1999) conducted a study to compare the effects of short-term dehydroepiandrosterone (DHEA) versus androstenedione supplementation on body composition, muscular strength, and hormonal levels in middle-aged male weightlifters.
Participants were randomly divided into groups who received either 100 mg/day of a placebo, androstenedione, or dehydroepiandrosterone. No significant changes were observed in any measure of muscular strength (bench press or leg press), body composition (total body mass, lean body mass, or fat mass) or hormonal levels (testosterone or androstenedione). These results indicated that supplementation with either androstenedione or dehydroepiandrosterone did not increase lean body mass or muscular strength levels compared to the placebo.

Quindry et al. (1999) administered either 200 mg/day of a placebo, androstenedione, or androstenediol to 50 middle-aged men in combination with heavy resistance weight training, for a 12-week period. Both the androstenedione and androstenediol groups demonstrated increases in serum androstenedione of 183% and 62%, respectively. At one- and two-month periods, the androstenedione group demonstrated a significant increase in total testosterone, which returned to normal by the end of the treatment period. No changes in serum testosterone levels were found in the androstenediol group. Estrogen concentrations were significantly increased in the androstenedione group at each measurement (1, 2, and 3 months). The androstenediol group only had significantly increased estrogens at the end of the treatment period (3 months). The investigators concluded that the main effects of oral androstenedione or androstenediol administration in middle-aged men are significant increases in estrogen-related hormones and a down-regulation in testosterone synthesis within one month of continuous androstenedione use.

The acute (Parsons et al., 1999) and long-term (Brown et al., 1999b), (King et al., 1999a), (Reifenrath et al., 1999) effects of an anabolic supplement, AN6 (containing
androstenedione), have been investigated in college-aged males. Participants received either a placebo, DHEA, or AN6. The results of acute administration indicated that serum androstenedione was increased following the intake of DHEA and AN6. Neither serum testosterone nor estradiol were increased following acute administration of AN6 (Parsons et al., 1999). In the long-term studies, serum testosterone and estradiol concentrations (King et al., 1999a), body composition and muscle strength (Reifenrath et al., 1999), and glucose tolerance, insulin action and blood lipids (Brown et al., 1999b) were examined in a group of college-aged males undergoing 8 weeks of resistance training supplemented with either a placebo, DHEA, or AN6. Serum testosterone was unaffected by any supplement. Serum estradiol and estrone levels were elevated with AN6 supplementation. The investigators concluded that AN6 does not increase serum testosterone, but increased androgen conversion to estrogens. AN6 also did not enhance changes in muscle strength or body composition during strength training. The AN6 supplement was shown to reduce serum HDL cholesterol, increasing the risk for coronary artery disease. In addition, insulin sensitivity was not increased with strength training during AN6 supplementation.

Brown et al. (1999a) investigated the effects of placebo, androstenedione (300 mg/day), or androstenediol (300 mg/day), combined with herbal extracts, on serum sex steroid levels in middle-aged men. Serum testosterone was unchanged in any group; serum androstenedione and estradiol were significantly elevated in the androstenedione and androstenediol groups. The researchers concluded that androstenedione and androstenediol do not increase serum testosterone levels.
King et al. (1999b) investigated the acute and long-term effects of oral androstenedione supplementation in combination with a weight training program. Thirty healthy young men volunteered for the study. First, the short term effects of androstenedione supplementation were investigated in ten participants in a crossover, double-blind fashion. The participants received either 100 mg of androstenedione or a placebo. Blood samples were drawn every 30 minutes for six hours and analyzed for the androstenedione and for free and total testosterone concentrations. The results indicated that ingestion of androstenedione increased the serum androstenedione concentration up to 325-350%. However, serum concentrations of free and total testosterone were unaffected. The effects of androstenedione during strength training were investigated in the other 20 participants, who were randomly assigned into a group who received either 300 mg of androstenedione (treatment group) or a placebo (control group) daily for 6 of the 8 weeks of the weight training program. The serum androstenedione concentration was significantly elevated in the treatment group. Even though serum free testosterone concentrations were significantly higher in the treatment group before and following the treatment, the androstenedione supplementation did not significantly alter free testosterone concentrations in either group. Total testosterone was also unaffected in either group following treatment. Serum estradiol and estrone concentrations were significantly elevated in the treatment group after supplementation and when compared to the control group. Weight training caused a significant increase in muscle strength in both groups, with no significant differences between the two groups. Mean cross-sectional area of the type II muscle fibers increased similarly in both groups. The investigators concluded that supplementation with androstenedione, both long- and short-
term, did not significantly alter serum testosterone concentrations, muscle size, or muscle strength in healthy young males. Serum estradiol and estrone concentrations were significantly elevated following androstenedione supplementation, indicating aromatization of androstenedione or of testosterone, possibly derived from the exogenous androstenedione supplementation.

Ballantyne et al. (1999) investigated the effects of androstenedione supplementation in healthy young males during normal activity and while undergoing weight training. First, participants ingested 200 mg of androstenedione daily for 2 days; blood samples were drawn every three hours for twelve hours on the second day and twenty-four hours after ingestion of the last pill on the third day of the experiment. At least two weeks later, the same subjects, in a crossover design, were administered either androstenedione or a placebo at a dosage of 200 mg per day for two days. Blood samples were drawn just before, immediately after, and 90 minutes after a weight training workout. The results demonstrated that androstenedione supplementation elevated serum androstenedione levels 2-3-fold. There were no significant differences in testosterone levels at any time of the day during the supplementation periods. While estradiol concentrations were higher during supplementation, the differences were not statistically significant. Following exercise, free and total testosterone levels were elevated during both conditions, with no significant differences as a result of androstenedione supplementation. Serum estradiol concentrations were also elevated following exercise, and remained significantly elevated for at least 90 minutes after exercise with androstenedione supplementation. During both conditions, serum testosterone was well within the normal range and displayed a normal circadian rhythm. The researchers
concluded that androstenedione supplementation had no effect on plasma testosterone during a twenty-four hour period. The researchers also concluded that exercise interacted with androstenedione supplementation to exaggerate the conversion of androstenedione to estradiol. According to this study, androstenedione supplementation appears to be of no benefit to the male athlete.

Rasmussen et al. (2000) investigated the effects of five days of oral androstenedione supplementation (100 mg/day) on muscle protein synthesis and serum hormone levels. The results indicated that plasma androstenedione concentrations increased three-fold, causing a significant increase in serum estradiol concentrations. Plasma testosterone levels did not change during the investigation. An increase in muscle protein breakdown was noticed during androstenedione supplementation. Muscle protein synthesis was also slightly elevated during the supplementation, but the change was not different from the control condition, nor was it as large as the increased muscle protein breakdown. In other words, the net protein balance was negative during androstenedione supplementation, indicating protein catabolism. This trend towards increased protein breakdown was theorized to have been a consequence of the increased estradiol levels.

The authors concluded that oral androstenedione supplementation did not stimulate muscle protein anabolism or increase serum testosterone levels. Instead, it was theorized that oral androstenedione was aromatized to estradiol and probably reduced and conjugated for excretion by the liver. Previous studies have shown that 89% of orally administered androstenedione was converted to testosterone glucuronide and excreted in the urine (Horton & Tait, 1966). Androstenedione was shown to have no direct anabolic effects on muscle.
Only two published studies indicated that testosterone precursor dietary supplements do increase serum testosterone concentrations, and therefore might contain ergogenic effects (Earnest et al., 2000; Leder et al., 2000).

Earnest et al. (2000) set out to determine if androgenic hormone precursors for testosterone could be transformed into testosterone. Eight male participants were treated in a crossover fashion with either 200 mg of androstenedione, androstenediol, or a placebo. Blood samples were drawn every 30 minutes for 90 minutes. Analysis of the data revealed that the mean area under the curve (AUC) for serum androstenedione was greater during androstenedione administration than during androstenediol or placebo administration. During androstenedione administration, a significant increase in free and total testosterone was demonstrated when compared to the placebo group, but not the androstenediol group. The investigators concluded that androstenedione, and possibly androstenediol, when taken orally, can increase testosterone concentrations in healthy young men.

Leder et al. (2000) investigated the effects of oral androstenedione administration on serum androstenedione, estradiol, estrone, and testosterone levels in healthy young men. Participants were randomly divided into groups that received either 100 mg/day or 300 mg/day of androstenedione, or placebo (0 mg/day) for seven days. The results indicated that the mean changes in AUC for serum testosterone in the groups receiving 0, 100, and 300 mg/day of androstenedione were -2%, -4%, and 34%, respectively. The change in the 300 mg/day group was significant compared to the placebo and 100 mg/day groups. In the 100 mg/day group, the mean AUC for androstenedione, estrone, and estradiol increased 72%, 74%, and 42%, respectively. In the 300 mg/day group, the
mean AUC for androstenedione, estrone, and estradiol increased 697%, 196%, and 128%, respectively. Mean daily baseline serum testosterone and estrone concentrations did not change, while mean daily serum androstenedione and estradiol increased significantly over the seven day period in the 300 mg/day group. In the 100 mg/day group, no subjects had serum testosterone concentrations that exceeded the upper limit of normal, but 4 of 14 subjects in the 300 mg/day group had levels that exceeded the upper limit of normal. Twelve of 15 and 10 of 14 participants in the 100 mg/day and 300 mg/day groups, respectively, had estradiol levels above the upper limit of normal for males. The investigators concluded that higher doses of orally administered androstenedione can increase serum testosterone and estrogen levels.

Even though Blaquier et al. (1967) found that androstenediol is more readily converted into testosterone than any other androgen precursor, few studies (Brown et al., 1999a; Quindry et al., 1999; Earnest et al., 2000) have investigated the possible ergogenic effects of this substance. Two of the studies, Brown et al. (1999a) and Quindry et al. (1999), concluded that androstenediol does not increase serum testosterone concentrations, and therefore is not effective as an ergogenic aid. Earnest et al. (2000) concluded that androstenediol possibly has ergogenic effects.

Only one study published to date has investigated an alternate delivery method (transdermal) of a testosterone precursor, androstenedione (Stoppe & Krause, 1986). Stoppe and Krause (1986) administered percutaneous (transdermal) testosterone, androstenedione (1.25 mg/m² body surface area), and estradiol to the skin of healthy young men to investigate the effects on serum steroid hormone levels. Testosterone and estradiol administration led to short peaks in their respective serum concentrations.
Androstenedione did not alter the serum levels of any of the hormones measured. It was concluded that precursor steroids, including androstenedione, are rapidly metabolized to other compounds before they could reach the blood in any sizable amount.

While most studies have investigated whether androgen precursor supplements can raise serum testosterone levels, one study looked at the urinary metabolites of androgen precursor supplementation, and whether ingestion of these products could lead to a positive drug test for anabolic steroids. Uralets and Gillette (1999) investigated the excretion products of the orally administered androgen precursors androstenedione, androstenediol, and norandrostenedione. After oral administration of androstenedione, all participants had significantly higher concentrations of androsterone and etiocholanolone in their urine. Androsterone and etiocholanolone are two of the major urinary metabolites of testosterone (Schänzer, 1996). Urinary testosterone and epitestosterone concentrations also rose briefly, with testosterone rising faster than epitestosterone. This rapid rise in testosterone, coupled with the fact that most men respond to androstenedione administration with a greater rise in urinary testosterone (T) relative to epitestosterone (E), could cause the T/E ratio to rise over 6, which would result in a positive drug test for the use of anabolic steroids. The urinary metabolites disappeared in less than 24 hours. The researchers explained that it is uncertain whether the ingestion of androstenedione would cause an increase in serum testosterone, because androstenedione, like other anabolic steroids has a high rate of metabolic inactivation when administered orally. Androstenediol administration caused an increase in urinary testosterone ten times greater than that of androstenedione. Epitestosterone also rose to levels greater than were seen during androstenedione administration; thus the T/E ratio...
was not as high as in androstenedione administration. The urinary metabolites
disappeared after 20 hours. The main excretion products of norandrostenedione are
norandrosterone and noretiocholanolone, the same metabolites as the anabolic steroid
nandrolone (nortestosterone), because norandrostenedione is rapidly converted to
nortestosterone. The concentrations of the major metabolites in the first urine samples
were as high as 100,000 ng/ml, higher than seen with the injectable (parenteral) anabolic
steroid nandrolone. Other than the unusually high first urine output, the urinary
metabolites of norandrostenedione are identical to those of nandrolone. Norandrosterone
and noretiocholanolone are detectable in a urine sample up to 10 days after a single 50
mg dose. This study indicated that supplementation with androstenedione and
norandrostenedione could cause the user to fail a drug test for anabolic steroids.

When all of the available data on androstenedione administration in males is
analyzed, most studies have concluded that orally administered androstenedione is not
converted to testosterone; it is instead aromatized into estrone, which can then be
converted into estradiol. Therefore, androstenedione and the other popular testosterone
precursors have no performance enhancing actions in males.

To date, no studies have assessed the possible ergogenic benefits of
androstenedione supplementation in females. Mahesh & Greenblatt (1962) found that
ingestion of androstenedione did increase testosterone levels for a short time. Horton &
Tait (1966) found that as much as 60% of the testosterone in females may come from the
peripheral conversion of androstenedione to testosterone. It is theorized that, because
females have a much lower circulating level of testosterone when compared to males,
increasing the level of testosterone could lead to greater increases in muscle mass and strength (MacDougall, 1983; George, 1988; Lombardo, 1993).

In summary, while exogenous testosterone administration has been shown to increase serum testosterone levels, thus improving athletic performance by increasing muscle size and strength, it has been concluded that the administration of testosterone precursors, including androstenedione and androstenediol, does not increase serum testosterone levels in males. All but one of the studies have looked at orally administered androstenedione. To date, no studies have investigated the possible ergogenic effects of testosterone precursor supplementation in females, or the effects of the administration of a transdermal testosterone precursor containing androstenedione and androstenediol. Therefore, the purpose of my study is to examine the acute effects of transdermal androstenedione and androstenediol administration (the dietary supplement AndrosteDERM®) on the serum steroid hormone levels of androstenedione and free and total testosterone in females.
CHAPTER 3

METHODOLOGY

Subjects

Seven healthy college-aged females volunteered to participate in this study. The participants were selected on the basis of being in general good health, not being pregnant, and no previous history of anabolic steroid, testosterone, or testosterone precursor supplementation. The average age (and standard deviation) of the participants was: 23.43 ± 4.31 years. Participants signed an informed consent (Appendix I) prior to participation. The UNLV Institutional Review Board approved the use of human subjects for this study (Appendix I).

AndrosteDERM®

AndrosteDERM® is a transdermal testosterone precursor cream produced by Medlean (Duxbury, MA). It contains the testosterone precursors androstenedione and androstenediol, and is sold without a prescription at a number of health food retailers as a dietary supplement. The recommended daily dosage of 2 ml is supposed to contain 130 mgs of a mixture of androstenedione and androstenediol. The cream is to be applied once daily to any number of locations including the inner upper arm, the inner thigh, and the back.
In order to assure purity, a 10 ml sample of AndrosteDERM® was sent to Integrated Biomolecule Corporation (Tucson, AZ) for a HPLC purity assay. The results of the assay (Appendix II) indicated that 1 ml of AndrosteDERM® contained 21.8 ± 0.26 mg of androstenedione and 44.6 ± 0.37 mg of androstenediol. These results are in agreement with the manufacturers claim that 2 ml of AndrosteDERM® contained, in total, 130 mg of a mixture of androstenedione and androstenediol.

Test Protocol

All testing was conducted in the morning, beginning between 8am and 12pm, to account for the normal diurnal variation in androstenedione levels (Diagnostic Products Corporation, 1999). Subjects were advised to go about their normal daily schedules on the day of testing; the subjects need not be fasted, and no other special preparations are necessary for proper analysis (Diagnostic Products Corporation, 1999).

Using aseptic technique, a registered nurse drew an approximately 6 ml baseline sample of blood from an antecubital vein. One ml of the AndrosteDERM® cream was applied to the inner surface of the left upper arm. Every 45 minutes for the next six hours following the initial application of the AndrosteDERM®, another approximately 6 ml sample of blood was drawn. A total of nine samples of blood, totaling approximately 54 mls, were drawn.

Blood Analysis

The collected blood samples were centrifuged at 700 xg for 12 minutes the same day; the plasma was removed and frozen at −80 °C until analysis. Serum
androstenedione and free and total testosterone concentrations were analyzed using radioimmunoassay (Diagnostic Products Inc., Los Angeles, CA) by an independent laboratory. An independent laboratory was chosen to perform the radioimmunoassay procedures because the University of Nevada, Las Vegas lacks the equipment and experienced personnel.

**Design**

The study was a 1 (Subject) X 9 (Time) within-subjects design. The factor time had nine levels: 0 minutes (baseline), 45 minutes, 90 minutes, 135 minutes, 180 minutes, 225 minutes, 270 minutes, 315 minutes, and 360 minutes post treatment. The dependent variables of interest were serum androstenedione, free testosterone, and total testosterone concentration.
CHAPTER 4

RESULTS AND DISCUSSION

Serum Androstenedione Concentration

When the serum androstenedione values calculated from the radioimmunoassay procedure were assessed, a number of the values were beyond the expected physiological level for females (Diagnostic Products Corporation, 1999a) (Table 1). All data from Table 1 appears in Figure 4. However, values such as 44.47 ng/ml, 17.03 ng/ml and 57.70 ng/ml are beyond the expected range for females (males as well) (Diagnostic Products Corporation, 1999a). Subjects who had at least one value that was beyond the expected range of 0.4 to 2.7 ng/ml (Diagnostic Products Corporation, 1999a) were excluded, leaving one subject, Subject 1 (Table 2). This made statistical analysis impractical.

Table 1: Androstenedione Raw Data For All Subjects (in ng/ml)

<table>
<thead>
<tr>
<th>Subject</th>
<th>0 min</th>
<th>45 min</th>
<th>90 min</th>
<th>135 min</th>
<th>180 min</th>
<th>225 min</th>
<th>270 min</th>
<th>315 min</th>
<th>360 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subject 1</td>
<td>1.68</td>
<td>1.66</td>
<td>1.92</td>
<td>2.03</td>
<td>1.85</td>
<td>1.61</td>
<td>1.26</td>
<td>1.26</td>
<td>1.39</td>
</tr>
<tr>
<td>Subject 2</td>
<td>3.33</td>
<td>28.83</td>
<td>2.73</td>
<td>2.60</td>
<td>2.40</td>
<td>2.44</td>
<td>2.55</td>
<td>2.74</td>
<td>2.19</td>
</tr>
<tr>
<td>Subject 3</td>
<td>44.47</td>
<td>7.76</td>
<td>8.23</td>
<td>7.82</td>
<td>9.28</td>
<td>4.52</td>
<td>7.73</td>
<td>8.84</td>
<td>9.49</td>
</tr>
<tr>
<td>Subject 4</td>
<td>4.05</td>
<td>4.00</td>
<td>7.16</td>
<td>4.77</td>
<td>8.85</td>
<td>4.63</td>
<td>7.52</td>
<td>17.03</td>
<td>3.99</td>
</tr>
<tr>
<td>Subject 5</td>
<td>19.16</td>
<td>6.98</td>
<td>6.11</td>
<td>5.75</td>
<td>5.33</td>
<td>5.91</td>
<td>6.14</td>
<td>8.25</td>
<td>6.72</td>
</tr>
<tr>
<td>Subject 6</td>
<td>4.41</td>
<td>23.39</td>
<td>3.60</td>
<td>3.56</td>
<td>3.02</td>
<td>3.97</td>
<td>3.75</td>
<td>4.77</td>
<td>4.14</td>
</tr>
<tr>
<td>Subject 7</td>
<td>7.60</td>
<td>2.80</td>
<td>2.61</td>
<td>2.40</td>
<td>2.88</td>
<td>5.62</td>
<td>4.62</td>
<td>57.70</td>
<td>3.23</td>
</tr>
</tbody>
</table>

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Figure 4: Serum Androstenedione Versus Time Plot For All Subjects

Table 2: Androstenedione Values (in ng/ml) (Exclusion of Unexpected Values)*

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Subject 1</th>
<th>Subject 2</th>
<th>Subject 3</th>
<th>Subject 4</th>
<th>Subject 5</th>
<th>Subject 6</th>
<th>Subject 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 min</td>
<td>1.68</td>
<td>1.66</td>
<td>1.92</td>
<td>2.03</td>
<td>1.85</td>
<td>1.61</td>
<td>1.26</td>
</tr>
<tr>
<td>45 min</td>
<td>1.66</td>
<td>1.66</td>
<td>1.92</td>
<td>2.03</td>
<td>1.85</td>
<td>1.61</td>
<td>1.26</td>
</tr>
<tr>
<td>90 min</td>
<td>1.92</td>
<td>1.92</td>
<td>2.03</td>
<td>1.85</td>
<td>1.61</td>
<td>1.26</td>
<td>1.39</td>
</tr>
<tr>
<td>135 min</td>
<td>2.03</td>
<td>2.03</td>
<td>1.85</td>
<td>1.61</td>
<td>1.26</td>
<td>1.39</td>
<td></td>
</tr>
<tr>
<td>180 min</td>
<td>1.85</td>
<td>1.85</td>
<td>1.61</td>
<td>1.26</td>
<td>1.39</td>
<td></td>
<td></td>
</tr>
<tr>
<td>225 min</td>
<td>1.61</td>
<td>1.61</td>
<td>1.26</td>
<td>1.39</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>270 min</td>
<td>1.26</td>
<td>1.26</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>315 min</td>
<td>1.39</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>360 min</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Values that were beyond the expected values range (Diagnostic Products Corporation, 1999a) were excluded which resulted in 6 of the 7 subjects being excluded

The data from Subject 1 (Table 2) appears in Figure 5. The serum androstenedione concentration for Subject 1 appeared to rise slightly from 45 minutes post application to 225 minutes post application. It then fell below baseline for the rest of the testing period. This could be due to absorption of the AndrosteDERM® or the
normal diurnal androstenedione variation for females (Diagnostic Products Corporation, 1999a).

![Subject 1 Androstenedione](image)

**Figure 5: Serum Androstenedione Versus Time Plot For Subject 1**

Serum Free Testosterone Concentration

When the serum free testosterone values calculated from the radioimmunoassay procedure were assessed, a number of the values were beyond the expected physiological level for females (Diagnostic Products Corporation, 1999b) (Table 3). All data from Table 3 appears in Figure 6. However, values such as 202.88 pg/ml, 63.82 pg/ml and 69.88 pg/ml are beyond the expected range for females (Diagnostic Products Corporation, 1999b). Subjects who had at least one value that was beyond the expected range of 0.06 to 2.57 pg/ml (Diagnostic Products Corporation, 1999b) were excluded, leaving one subject, Subject 1 (Table 4). This made statistical analysis impractical.
Table 3: Free Testosterone Raw Data For All Subjects (in pg/ml)

<table>
<thead>
<tr>
<th>Subject</th>
<th>0 min</th>
<th>45 min</th>
<th>90 min</th>
<th>135 min</th>
<th>180 min</th>
<th>225 min</th>
<th>270 min</th>
<th>315 min</th>
<th>360 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subject 1</td>
<td>2.15</td>
<td>0.94</td>
<td>1.01</td>
<td>0.87</td>
<td>1.28</td>
<td>1.08</td>
<td>1.30</td>
<td>1.95</td>
<td>1.77</td>
</tr>
<tr>
<td>Subject 2</td>
<td>1.47</td>
<td>5.85</td>
<td>1.43</td>
<td>1.49</td>
<td>1.33</td>
<td>1.89</td>
<td>1.92</td>
<td>3.34</td>
<td>1.86</td>
</tr>
<tr>
<td>Subject 3</td>
<td>202.88</td>
<td>26.16</td>
<td>34.49</td>
<td>24.89</td>
<td>43.60</td>
<td>9.24</td>
<td>36.59</td>
<td>39.13</td>
<td>45.13</td>
</tr>
<tr>
<td>Subject 4</td>
<td>6.12</td>
<td>14.96</td>
<td>26.64</td>
<td>17.33</td>
<td>63.82</td>
<td>6.35</td>
<td>20.64</td>
<td>12.35</td>
<td>7.42</td>
</tr>
<tr>
<td>Subject 5</td>
<td>22.09</td>
<td>17.56</td>
<td>13.82</td>
<td>5.06</td>
<td>6.69</td>
<td>7.58</td>
<td>17.63</td>
<td>13.75</td>
<td>6.82</td>
</tr>
<tr>
<td>Subject 6</td>
<td>6.53</td>
<td>69.88</td>
<td>4.28</td>
<td>3.19</td>
<td>3.88</td>
<td>5.87</td>
<td>3.93</td>
<td>6.23</td>
<td>6.24</td>
</tr>
<tr>
<td>Subject 7</td>
<td>9.88</td>
<td>2.70</td>
<td>1.79</td>
<td>2.12</td>
<td>3.38</td>
<td>8.10</td>
<td>8.23</td>
<td>89.92</td>
<td>9.06</td>
</tr>
</tbody>
</table>

Figure 6: Serum Free Testosterone Versus Time Plot For All Subjects
Table 4: Free Testosterone Values (in pg/ml) (Exclusion of Unexpected Values)*

<table>
<thead>
<tr>
<th>Time</th>
<th>Subject 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 min</td>
<td>2.15</td>
</tr>
<tr>
<td>45 min</td>
<td>0.94</td>
</tr>
<tr>
<td>90 min</td>
<td>1.01</td>
</tr>
<tr>
<td>135 min</td>
<td>0.87</td>
</tr>
<tr>
<td>180 min</td>
<td>1.28</td>
</tr>
<tr>
<td>225 min</td>
<td>1.08</td>
</tr>
<tr>
<td>270 min</td>
<td>1.30</td>
</tr>
<tr>
<td>315 min</td>
<td>1.95</td>
</tr>
<tr>
<td>360 min</td>
<td>1.77</td>
</tr>
</tbody>
</table>

* Values that were beyond the expected values range (Diagnostic Products Corporation, 1999b) were excluded, which resulted in 6 of the 7 subjects being excluded.

The data from Subject 1 (Table 4) appears in Figure 7. The serum free testosterone concentration for Subject 1 declined sharply from application to 45 minutes post application. It then began to rise, reaching near baseline concentration at 315 minutes post application.

![Subject 1 Free Testosterone](image)

Figure 7: Serum Free Testosterone Versus Time Plot For Subject 1
Serum Total Testosterone Concentration

When the serum total testosterone values calculated from the radioimmunoassay procedure were assessed, a number of the values were beyond the expected physiological level for females (Diagnostic Products Corporation, 1999c) (Table 5). All data from Table 5 appears in Figure 8. However, values such as 3290.03 ng/dl, 225.42 ng/dl and 130.06 ng/dl are beyond the expected range for females (Diagnostic Products Corporation, 1999c). Subjects who had at least one value that was beyond the expected range of 0 to 81 ng/dl (Diagnostic Products Corporation, 1999c) were excluded, leaving two subjects, Subjects 1 and 2 (Table 6). This made statistical analysis impractical.

Table 5: **Total Testosterone Raw Data For All Subjects (in ng/dl)**

<table>
<thead>
<tr>
<th>Subject</th>
<th>0 min</th>
<th>45 min</th>
<th>90 min</th>
<th>135 min</th>
<th>180 min</th>
<th>225 min</th>
<th>270 min</th>
<th>315 min</th>
<th>360 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>29.88</td>
<td>75.48</td>
<td>30.78</td>
<td>36.86</td>
<td>33.59</td>
<td>38.27</td>
<td>45.26</td>
<td>56.17</td>
<td>57.79</td>
</tr>
<tr>
<td>3</td>
<td>3290.03</td>
<td>49.16</td>
<td>93.68</td>
<td>89.18</td>
<td>240.25</td>
<td>84.63</td>
<td>195.96</td>
<td>240.36</td>
<td>256.29</td>
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<td>4</td>
<td>46.90</td>
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<td>79.05</td>
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<td>84.51</td>
</tr>
<tr>
<td>5</td>
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<td>50.79</td>
<td>52.49</td>
<td>96.00</td>
<td>93.05</td>
<td>65.97</td>
</tr>
<tr>
<td>6</td>
<td>62.77</td>
<td>333.09</td>
<td>47.37</td>
<td>53.59</td>
<td>69.78</td>
<td>97.31</td>
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<td>86.72</td>
<td>97.35</td>
<td>868.62</td>
<td>51.29</td>
</tr>
</tbody>
</table>

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**Figure 8: Serum Total Testosterone Versus Time Plot For All Subjects**

**Table 6: Total Testosterone Values (in ng/dl) (Exclusion of Unexpected Values)**

<table>
<thead>
<tr>
<th>Subject 1</th>
<th>0 min</th>
<th>45 min</th>
<th>90 min</th>
<th>135 min</th>
<th>180 min</th>
<th>225 min</th>
<th>270 min</th>
<th>315 min</th>
<th>360 min</th>
</tr>
</thead>
</table>

* Values that were beyond the expected values range (Diagnostic Products Corporation, 1999c) were excluded which resulted in 5 of the 7 subjects being excluded.

The data from Subjects 1 and 2 (Table 6) appears in Figure 9. The serum total testosterone concentration for Subject 1 declined sharply from baseline to 45 minutes post application. The general trend for the rest of the testing period was to rise, reaching a peak level at 315 minutes post application. For Subject 2, the total testosterone concentration rose sharply from baseline to 45 minutes post application. It then declined to approximately baseline levels at 90 minutes post application. From 90 minutes post
application, the general trend for Subject 2 was a steady increase in total testosterone, reaching a peak concentration at 360 minutes post application. Whether serum total testosterone levels continued to rise after that point is unknown. This increase in total testosterone could be caused by the peripheral conversion of androstenedione or androstenediol supplied via AndrosteDERM®.

![Subject 1 and 2 Total Testosterone](image)

Figure 9: Serum Total Testosterone Versus Time Plot for Subjects 1 and 2

While serum androstenedione, free and total testosterone levels were analyzed using radioimmunoassay, much of the data received (including baseline levels) was beyond the physiological levels possible for females, indicating a methodological error. Therefore, the data for most of the subjects had to be eliminated from statistical analysis. While the general trend did not indicate a rise in serum androstenedione, free or total testosterone following application, too few subjects remained following elimination to run the appropriate statistical analysis.
CHAPTER 5

SUMMARY AND RECOMMENDATIONS

Summary

Testosterone is an anabolic hormone; it promotes the building of new tissue, such as skeletal muscle, by increasing protein synthesis (anabolic action) and decreasing protein breakdown (anticatabolic action) (Blue & Lombardo, 1999). The serum concentration of testosterone in males is ten times that of females (Gard, 1998), which Wynn (1975) states is responsible for the greater amount of muscle mass seen in males.

Anabolic steroids are synthetic versions of the hormone testosterone. Because anabolic steroids have powerful tissue building effects, it is believed that their use will cause the accretion of skeletal muscle mass and strength, although, the exact mechanism at work remains unknown.

With the scheduling of anabolic steroids as controlled substances in 1990 (Yesalis & Wright, 1993), the possession of anabolic steroids without a legitimate medical prescription became a criminal offense. Many athletes and bodybuilders turned to legal dietary supplements in hopes that their use would produce effects similar to those of anabolic steroids. Some of the most popular dietary supplements purported to have “muscle building” effects similar to anabolic steroids are testosterone precursors (or prohormones), with androstenedione and androstenediol being two of the most popular. In the human body, both androstenedione and androstenediol serve as immediate
precursors to the hormone testosterone (Wallace et al., 1999; Earnest et al., 2000).

Androstenedione can be converted to testosterone via the hormone 17β-hydroxysteroid dehydrogenase (Leder et al., 2000). Androstenediol can be converted into testosterone via the enzyme 3β-hydroxysteroid dehydrogenase (Earnest et al., 2000). Thus, it is theorized that if these testosterone precursor supplements are administered, they will be converted into testosterone, increasing the serum testosterone concentration. It is believed that by increasing the endogenous production of testosterone in the body, results similar to those seen during anabolic steroid administration (increased muscle mass and strength) will be recognized.

Recently, a number of studies (Ballantyne et al., 1999; Brown et al., 1999a; Brown et al., 1999b; King et al., 1999a; King et al., 1999b; Parsons et al., 1999; Quindry et al., 1999; Reifenrath et al., 1999; Wallace et al., 1999; Earnest et al., 2000; Leder et al., 2000; Rasmussen et al., 2000) have been conducted on males to determine if the use of oral testosterone precursors, including androstenedione and androstenediol, will increase serum testosterone levels, and produce anabolic effect seen while using anabolic steroids. The results of the majority of the studies indicate that orally administered testosterone precursors do not increase serum testosterone concentrations or muscle size and strength (Ballantyne et al., 1999; Brown et al., 1999a; Brown et al., 1999b; King et al., 1999a; King et al., 1999b; Parsons et al., 1999; Quindry et al., 1999; Reifenrath et al., 1999; Wallace et al., 1999; Rasmussen et al., 2000). Only two published studies indicated that supplementation with testosterone precursor dietary supplements does increase serum testosterone concentrations, and therefore, may have ergogenic value (Earnest et al., 2000; Leder et al., 2000).
It has been determined that the bioavailability of oral testosterone precursors is only approximately 5%, due to the effects of digestion, liver metabolism, and tissue enzyme activity (Wallace et al., 1999). Thus, alternate methods for testosterone precursor delivery have been developed in an attempt to increase their bioavailability. Transdermal and sublingual versions of androstenedione and androstenediol have been marketed with claims stating better results than those seen with oral testosterone precursors.

Only one study published to date has investigated an alternate delivery method (transdermal) of a testosterone precursor, androstenedione (Stoppe & Krause, 1986). Stoppe and Krause (1986) administered percutaneous (transdermal) testosterone, androstenedione (1.25 mg/m² body surface area), and estradiol to healthy young men in order to investigate the effects on serum steroid hormone levels. Testosterone and estradiol administration led to short peaks in their respective serum concentrations. Androstenedione did not alter the serum levels of any of the hormones measured, including androstenedione and testosterone. It was concluded that precursor steroids, including androstenedione, are rapidly metabolized to other compounds before they can reach the blood in any sizable amount. In the case of androstenedione, it would be metabolized in the liver to such products as androsterone, etiocholanone, 5α- and 5β-androstandiols (Uralets & Gillette, 1999), and testosterone glucuronide (Horton & Tait, 1966) to be excreted in the urine.

To date, no studies have ever been conducted on females to determine whether testosterone precursor administration may behold ergogenic benefits. If testosterone precursor dietary supplements do increase serum testosterone levels, and thus hold
ergogenic benefits, females should exhibit a greater response to their administration because of much lower serum testosterone concentrations and the fact that as much as 60% of testosterone in females comes for the peripheral conversion of androstenedione (Horton & Tait, 1966).

Mahesh and Greenblatt (1962) found that orally administered dehydroepiandrosterone and androstenedione could be converted into testosterone in females. Ingestion of 100 mgs of androstenedione caused a 4-7-fold increase in plasma testosterone. The researchers concluded that androstenedione could be converted into testosterone in the human body. The present study was designed to determine whether the acute administration of a transdermal testosterone precursor supplement in females (AndrosteDERM®) would elevate serum androstenedione and free and total testosterone levels above baseline over the six hour testing period.

In the present study, the data obtained as a result of the radioimmunoassays may not be correct, making the results obtained questionable. While all blood collection was performed properly, an error appeared to have occurred somewhere between sampling and the final results. Some of the subsequent data obtained was not physiologically possible for men or women. Proper interpretation of the data is unwarranted without retesting all of the blood samples. Conclusions and generalizations cannot be made at this time.

Recommendations

Based on the current study, the following recommendations are offered for future research:
1. Conduct the study with a larger number of subjects. For monetary reasons, this study could only use seven subjects, and because of apparent errors that occurred somewhere after the blood collection, a number of the subjects had to be eliminated from statistical analysis because of unexpected values. This left either one or two subjects depending on the dependant variable. An increase in the number of subjects could potentially offset a similar problem in a similar study.

2. Conduct each sample in duplicate (or triplicate) during the radioimmunoassay procedure. Errors occurred somewhere after collection of the blood samples. If the errors were in the radioimmunoassay, performing duplicate (or triplicate) analyses on each sample could potentially document that error.

3. Increase the testing period beyond six hours. More blood sample could have been collected 12, and possibly 24, hours post application in order to test for a sustained increase in androstenedione, free or total testosterone.

4. Either in males or females, a study with a longer duration could be conducted. Instead of the acute effects of a one time application of AndrosteDERM®, the effects of application of AndrosteDERM® for one week or longer could determine whether continued usage will elevate androstenedione, free or total testosterone levels, and if the increase is sustained for a period of time.

5. Analyze other metabolites or hormones. A urine analysis could detect metabolites that may cause a failed drug test for anabolic steroids. Analyzing serum estrogen levels (estrone and estradiol) could determine if the androstenedione is being converted into testosterone or estrogen hormones.
APPENDIX I

IRC APPROVAL/INFORMED CONSENT
DATE: March 28, 2001

TO: Mark Rasmussen
Kinesiology
M/S 3034

FROM: Dr. Carl Reiber
UNLV Biomedical Sciences Institutional Review Board

RE: Status of Human Subject Protocol Entitled:
"The Acute Effects of AndrostederM Administration on Serum Steroid Levels
and Grip Strength"

OPRS# 504s0800-062

This memorandum is official notification that the UNLV Biomedical Sciences Institutional Review Board approved the protocol for the project listed above and work on the project may proceed. This approval is effective March 27, 2001 and will continue for a period of one year.

Should the use of human subjects described in this protocol continue beyond a year from the approval date, it will be necessary to request an extension.

If you have any questions or require any assistance, please contact the Office for the Protection of Research Subjects at 895-2794.

cc: OPRS File
University of Nevada Las Vegas
Informed Consent Statement

THE ACUTE EFFECTS OF TRANSDERMAL TESTOSTERONE PRECURSOR ADMINISTRATION ON SERUM STEROID HORMONE LEVELS IN FEMALES

Information
You are invited to participate in a research study investigating the acute effects of a transdermal testosterone precursor supplement, AndrosteDERM®, on the serum steroid hormone levels of androstenedione and free and total testosterone.

Procedures
You will be asked to report to the Sports Injury Research Center for one six-hour testing session.

As listed on the packaging, AndrosteDERM® is an all-natural, non-prescription, nutritional product (this product is labeled as a dietary supplement by the FDA, not a food or a drug) that can be purchased at health food stores. It is a transdermal (absorbed through the skin) version of the testosterone precursors androstenedione and androstenediol, used for performance enhancement in sports requiring power.

Dietary supplements are regulated under a different set of regulations than those covering foods and drugs (over-the-counter and prescription). Under the Dietary Supplement Health and Education Act of 1994 (DSHEA), the manufacturer of the dietary supplement is responsible for ensuring that it is safe before it is marketed. The FDA is then responsible for taking action against any unsafe dietary supplement after it reaches the market, not before. In most cases, the manufacturers of dietary supplements do not need to register with the FDA or get FDA approval before producing or selling their products.

The procedures of the study will be explained to you upon your arrival to the Sports Injury Research Center. At this time, you will be asked to sign the informed consent and participation in the study will begin. Using aseptic technique, a registered nurse will draw a baseline blood sample (approximately 6 ml) from an antecubital vein (front of the elbow). One (1) ml (21.8 mg of androstenedione and 44.6 mg of androstenediol, a total of 66.4 mg) of AndrosteDERM® will be applied to the inner surface of your left upper arm. Blood samples of 6-ml each will be drawn every 45 minutes for six hours from the initial application of AndrosteDERM®. The total amount of blood drawn will be approximately 54 mls.
THE ACUTE EFFECTS OF TRANSDERMAL TESTOSTERONE PRECURSOR ADMINISTRATION ON SERUM STEROID HORMONE LEVELS IN FEMALES

Benefits of Participation
1. Satisfaction from participating in a research project and contributing to the body of human performance literature.
2. An increased understanding of the acute effects of testosterone precursor supplementation on serum steroid hormone levels.

Risks
In this experiment, the risk to the participant is minimal. A registered nurse will perform all of the blood draws using aseptic technique. There is always a risk of infection when a blood sample is drawn. The risk is minimized with the use of disinfectants, gloves, aseptic technique, and sterile equipment. There is the risk of bruising associated with any venipuncture. Bruising usually resolves in 7-10 days. This risk is minimized by using correct technique for blood drawing. Sterile, one time use, disposable needles will be used. Universal blood and body fluid precautions will be carried out at all times. Used needles will be disposed immediately after use in a sharps container. The risk of skin redness or irritation following the application of the topical cream is also possible. The possible effects are mitigated with the one-time application of a low dosage of AndrosteDERM®.

The researcher and a registered nurse will be monitoring you during the study. If you feel any discomfort during the study, notify the researcher or the registered nurse and the study will be stopped immediately. If any other problems should arise, the researcher will address the situation.

There are no known risks associated with the acute administration of a testosterone precursor. Most studies conducted on testosterone precursors have been performed on males, with no side effects being reported with their acute (one-time) use. With chronic use (eight weeks), a lowering of HDL cholesterol was reported, although, the serum concentration did not reach a level that is considered a risk factor for cardiovascular disease (King et al., 1999). No negative side effects were reported in males who received either 100 or 300 mg of androstenedione daily for seven days (short-term); the authors indicated that long-term use could be hazardous, particularly in women and children (Leder et al., 2000).

Some potential side effects associated with the chronic use of testosterone precursors in males include elevated estrogen levels, which may cause gynecomastia or other feminizing effects, and may increase the risk for cardiovascular disease, and prostate and pancreatic cancer (King et al., 1999).
women, the chronic use of testosterone precursors could elevate serum testosterone levels, possibly causing hirsutism or virilization (Leder et al., 2000).

In the only known study on testosterone precursor administration in females, the female participants ingested 100mg of androstenedione (Mahesh and Greenblatt, 1962). Peak testosterone levels were noticed 60 minutes after administration and had begun declining 90 minutes following administration (Mahesh and Greenblatt, 1962). No side effects were reported with the one-time administration of the androstenedione, or the transient rise in testosterone (Mahesh and Greenblatt, 1962). In the only known study ever to investigate the transdermal administration of a testosterone precursor, when the androstenedione was administered to the male participants, no rise in serum androstenedione, testosterone, or estradiol was reported (Stoppe and Krause, 1986).

The potential for side effects associated with the use of testosterone precursors in females is mitigated by the one-time (acute, not chronic) administration of a low dose (66.4 mg) of the testosterone precursor.

The effects of an acute (one-time) dose of AndrosteDERM® on the menstrual cycle are unknown. Because serum testosterone levels may be elevated with the application of AndrosteDERM®, the date when menses occurs may be effected; it may be either earlier or later than expected. Again, the effects on the menstrual cycle are unknown.

Contact
If you have any questions at any time about the study, or if you experience adverse effects as a result of participation in this study, you may contact the researchers: Mark Rasmussen at 895-2780 or Dr. John Young at 895-4626. For questions about your rights as a research participant, you may contact the UNLV Office for the Protection of Research Subjects at 895-2794.

Participation
Your participation in this study is voluntary. You may refuse to participate in this study or in any part of this study. At any time you may withdraw without prejudice to your relations with UNLV or the director of this study. If you withdraw, you should understand that your data would be destroyed. You may ask any questions about this study prior to its beginning or at any time during the investigation.

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THE ACUTE EFFECTS OF TRANSDERMAL TESTOSTERONE PRECURSOR ADMINISTRATION ON SERUM STEROID HORMONE LEVELS IN FEMALES

Confidentiality
No reference will be made in written or oral materials that could link you to this study. All data will be placed in a locked cabinet in the Sports Injury Research Center at UNLV.

Consent
I have read and understand the above information. My questions have been answered by the director of the study. I agree to participate in this study.

________________________________________  _______________________
Subject’s Signature                      Date

________________________________________  _______________________
Researcher’s Signature                  Date
THE ACUTE EFFECTS OF TRANSDERMAL TESTOSTERONE PRECURSOR ADMINISTRATION ON SERUM STEROID HORMONE LEVELS IN FEMALES

QUESTIONNAIRE

Participant Initials (first, middle, last) __________
Participant Number (leave blank) __________

SECTION 1: General Information

1. Name:

2. Age:

SECTION 2: Medical History

1. Because androgen levels could change during this study, it would be inappropriate to include any women who are, or might be pregnant. Are you, or is there any chance that you might be pregnant?

2. The use of anabolic steroids and/or testosterone precursors (including androstenedione) can cause changes in the androgen levels in the body, which would confound the results of this study. Are you currently using, or have you ever used anabolic steroids and/or testosterone precursors?

3. The use of certain medications, over-the-counter medicines, or dietary supplements could interfere with the results of this study. Are you currently taking any? If yes, please list.

4. The use of healthy subjects is necessary for this study; certain disorders could interfere with the results. Have you ever been diagnosed or hospitalized with a chronic disease? If yes, please explain.

5. Certain allergies could potentially compromise your participation in this study. Do you have any allergies? If yes, please list the substances you are allergic to and the type of reaction to the substance.
Human Subjects Protocol

THE ACUTE EFFECTS OF TRANSDERMAL TESTOSTERONE PRECURSOR ADMINISTRATION ON SERUM STEROID HORMONE LEVELS IN FEMALES

Name: Mark Rasmussen & Dr. John Young
Department: Kinesiology
Title of Study: The Acute Effects of Transdermal Testosterone Precursor Administration on Serum Steroid Hormone Levels in Females

1. SUBJECTS:

Ten healthy UNLV female students will be recruited for participation in this study. An informed consent form and questionnaire (see attached forms) will be used to alert the test investigator of any conditions that may affect participation in the study. The questionnaire will be administered to the participants to survey medical history and any past or present ergogenic aid use.

2. PURPOSE, METHODS, AND PROCEDURES:

Purpose
The purpose of this study is to examine whether administration of the over-the-counter dietary supplement and testosterone precursor, AndrosteDERM®, will affect the steroid hormones androstenedione and testosterone (free and total). Research has shown that the compounds androstenedione and androstenediol are precursors to the hormone testosterone in the body. AndrosteDERM® contains the testosterone precursors androstenedione and androstenediol. Dietary supplement companies recently marketed these and similar compounds as ergogenic aids that are supposed to increase serum testosterone levels in the body, thus producing the benefits of higher testosterone in the body, including increased muscle strength and muscle hypertrophy. To date, research on testosterone precursor supplementation has been performed on oral versions in males, and has had mixed results. A new and supposedly superior version of testosterone precursors, transdermal (as in the supplement AndrosteDERM®), has been developed and is on the market. To date, no research has been performed on AndrosteDERM®. Evaluation of the effects of a new form of supplement delivery (transdermal) may provide clear answers as to whether or not testosterone precursor supplements raise serum testosterone levels and thus, may enhance physical performance.

Procedures
Each participant will report to the Sports Injury Research Center for one six-hour testing session. Prior to testing, each participant will be asked to complete an informed consent form and an information questionnaire (see attached forms). Participants will be informed of the specific protocol of the study, but will remain naïve as to the theoretical
THE ACUTE EFFECTS OF TRANSDERMAL TESTOSTERONE PRECURSOR ADMINISTRATION ON SERUM STEROID HORMONE LEVELS IN FEMALES

question. Following the introduction, specific instructions will be given prior to the start of testing.

Using aseptic technique, a registered nurse will draw a baseline blood sample (approximately 6-ml) from an antecubital vein (front of the elbow). Next, 1 ml of AndrosteDERM® will be applied to the inner surface of the left upper arm. Every 45 minutes for the next six hours, another 6-ml sample of blood will be drawn via venipuncture. The total number of venipunctures will be 9; the total amount of blood drawn will be approximately 54 mls.

3. RISKS:

For the proposed experiment the risk to the participant is minimal. A registered nurse will draw all blood samples (using aseptic technique), and be present throughout the entire testing period. The participant will also be allowed to stop testing at any time, for any reason. There is always the risk of bruising when blood samples are drawn. This risk is minimized by using correct technique for blood drawing. There is a risk of infection when blood samples are drawn. The risk is minimized with the use of disinfectants, aseptic technique, sterile equipment, and latex gloves. Sterile, one time use, disposable needles will be used. Universal blood and body fluid precautions will be carried out at all times. Used needles will be disposed immediately after use in a sharps container. The risk of skin redness or irritation following the application of the topical cream is also possible. The possible effects are mitigated with the one-time application of a low dosage of AndrosteDERM®.

Because this is a one-time single dose of AndrosteDERM®, the potential risks associated with testosterone precursor use are minimal. AndrosteDERM® is a testosterone precursor that contains androstenedione and androstenediol, natural products found in all humans. It is labeled as a dietary supplement and is available over-the-counter at health food stores. It does not require a doctor's prescription.

There are no known risks associated with the acute administration of a testosterone precursor. Most studies conducted on testosterone precursors have been performed on males, with no side effects being reported with their acute (one-time) use. With chronic use (eight weeks), a lowering of HDL cholesterol was reported, although, the serum concentration did not reach a level that is considered a risk factor for cardiovascular disease (King et al., 1999). No negative side effects were reported in males who received either 100 or 300 mg of androstenedione daily for seven days (short-term); the authors indicated that long-term use could be hazardous, particularly in women and children (Leder et al., 2000).
THE ACUTE EFFECTS OF TRANSDERMAL TESTOSTERONE PRECURSOR ADMINISTRATION ON SERUM STEROID HORMONE LEVELS IN FEMALES

Some potential side effects associated with the chronic use of testosterone precursors in males include elevated estrogen levels, which may cause gynecomastia or other feminizing effects, and may increase the risk for cardiovascular disease, and prostate and pancreatic cancer (King et al., 1999). In women, the chronic use of testosterone precursors could elevate serum testosterone levels, possibly causing hirsutism or virilization (Leder et al., 2000).

In the only known study on testosterone precursor administration in females, the female participants ingested 100mg of androstenedione (Mahesh and Greenblatt, 1962). Peak testosterone levels were noticed 60 minutes after administration and had begun declining 90 minutes following administration (Mahesh and Greenblatt, 1962). No side effects were reported with the one-time administration of the androstenedione, or the transient rise in testosterone (Mahesh and Greenblatt, 1962). In the only known study ever to investigate the transdermal administration of a testosterone precursor, when the androstenedione was administered to the male participants, no rise in serum androstenedione, testosterone, or estradiol was reported (Stoppe and Krause, 1986).

The potential for side effects associated with the use of testosterone precursors in females is mitigated by the one-time (not chronic) administration of a low dose (66.4 mg) of the testosterone precursor. The effects of an acute (one-time) dose of AndrosteDERM® on the menstrual cycle are unknown.

4. BENEFITS:

There are no known benefits or improvements for the participant as a result of this investigation. However, this study has the potential to contribute to the body of scientific knowledge on the subject of testosterone precursor supplementation.

5. RISKS-BENEFITS RATIO: The risks are minimized in this study by using a registered nurse, and aseptic technique for all blood drawings. The participants are also allowed to stop testing at any time, for any reason. The one-time administration of a low dose of the testosterone precursor mitigates the possible side effects associated with it.

The benefits in regard to adding to the body of scientific knowledge on the subject of testosterone precursor supplementation and human performance are substantial; thus, the risk-benefit ratio for this study is weighted highly in favor of the benefits.

6. COSTS TO SUBJECTS:

There will be no costs to participants except for their time.
THE ACUTE EFFECTS OF TRANSDERMAL TESTOSTERONE PRECURSOR ADMINISTRATION ON SERUM STEROID HORMONE LEVELS IN FEMALES

7. **INFORMED CONSENT:**

Prior to agreeing to participate in this research project, each participant will be given an informed consent form (see attached informed consent). After reading the form and asking any questions, the individual can decide whether or not to participate. Those individuals who choose to participate will be assigned a number based on their informed consent; this number will be used for further identification of the participant, thus insuring participant anonymity. The informed consents will be collected and stored in a locked cabinet in the Sports Injury Research Center at UNLV. Those individuals who choose not to participate will be excused from the study.
APPENDIX II

ANDROSTEDERM® PURITY ANALYSIS
PRODUCT ANALYSIS

TO: Mark Rasmussen
COMPANY University of Nevada, Las Vegas
FAX NO: (702) 895-2780
DATE: February 16, 2001

<table>
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<th>Product Name: AndrosteDERM</th>
<th>Lot Number: 2KS1</th>
<th>P.O. Number: 2LPOCSB362</th>
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<tr>
<td>Androst-4-ene-3,17-dione</td>
<td>21.8 ± 0.26</td>
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</tr>
<tr>
<td>Androst-4-ene-3β,17β-diol</td>
<td>44.6 ± 0.37</td>
<td>mg/ml</td>
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Assay performed by HPLC, identification / quantification by external standards method. Standards obtained from Steroids Inc. Briefly, the chromatographic conditions Spherisorb ODS2 C8 (5 μm) column (12.5 cm x 4.6 mm) with a mobile phase (1.2 ml/min) of acetonitrile/1% triethylamine (2:3). Methodology employed as published by Glass, RL Johnson, El. "Comparison of high-performance liquid-chromatographic and gas-chromatographic analyses of synthetic steroids" as published in J. Liq. Chromatogr., 1993, Vol.16, No.16, pp.3543-3555

This Product Analysis is subject to our Standard Terms.

Dinesh Patel, Ph.D.
Director of Chemistry
APPENDIX III

RAW DATA
### Androstenedione Raw Data

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| 1       | 55.28107 | 55.42391 | 53.39887 | 52.60062 | 53.90723 | 55.93227 | 59.41938 | 59.34795 | 57.97412 |
| 2       | 45.52138 | 14.80128 | 48.39509 | 49.0463 | 50.22267 | 49.94387 | 48.32787 | 51.52508 |
| 3       | 8.829527 | 33.48458 | 32.65272 | 33.37535 | 30.94693 | 33.5392 | 31.6360 | 30.62768 |
| 4       | 42.78111 | 42.94597 | 34.84835 | 40.42517 | 31.91919 | 40.84951 | 33.9875 | 42.97118 |
| 5       | 20.62432 | 35.00126 | 36.90866 | 37.79699 | 38.84968 | 37.36241 | 36.82884 | 32.62331 | 35.55163 |
| 6       | 41.53853 | 17.78002 | 44.42484 | 44.57188 | 46.92043 | 43.0510 | 43.63245 | 40.42517 | 42.42921 |
| 7       | 33.79548 | 48.01697 | 48.99588 | 50.18066 | 47.58428 | 38.08503 | 40.88312 | 4.91755 | 45.98353 |

| Log      |     |     |     |     |     |     |     |     |     |
| 1       | 0.225419 | 0.221063 | 0.282817 | 0.30716 | 0.267314 | 0.20558 | 0.09922 | 0.101398 | 0.143293 |
| 2       | 0.523043 | 1.45986 | 0.435408 | 0.41555 | 0.379676 | 0.388132 | 0.406325 | 0.437458 | 0.339958 |
| 3       | 1.648069 | 0.890108 | 0.915476 | 0.894393 | 0.967493 | 0.655263 | 0.884492 | 0.946811 | 0.97723 |
| 4       | 0.607218 | 0.801581 | 0.854619 | 0.678453 | 0.946993 | 0.685513 | 0.876143 | 1.231165 | 0.600812 |
| 5       | 1.282285 | 0.843566 | 0.795699 | 0.759425 | 0.726498 | 0.778153 | 0.789124 | 0.916373 | 0.827073 |
| 6       | 0.644501 | 1.36922 | 0.556842 | 0.551998 | 0.480379 | 0.598376 | 0.574547 | 0.678453 | 0.517339 |
| 7       | 0.880627 | 0.446939 | 0.417067 | 0.380957 | 0.460135 | 0.749816 | 0.664486 | 1.781199 | 0.506949 |

| ng/ml    |     |     |     |     |     |     |     |     |     |
| 1       | 1.680423 | 1.683653 | 1.917859 | 2.028428 | 1.850607 | 1.605314 | 1.256666 | 1.262985 | 1.390992 |
| 2       | 3.334592 | 28.83101 | 2.725262 | 2.603452 | 2.397043 | 2.444172 | 2.548736 | 2.738156 | 2.187552 |
| 7       | 7.596735 | 2.798589 | 2.612885 | 2.404125 | 2.884391 | 5.621031 | 4.618359 | 57.70311 | 3.228118 |

| Std Dev  | 15.42318 | 10.81059 | 2.504433 | 2.110092 | 3.110421 | 1.583427 | 2.48603 | 19.79957 | 2.792294 |
| Std E    | 5.829414 | 4.086018 | 0.948587 | 0.79754 | 1.175629 | 0.598479 | 0.932826 | 7.483538 | 1.055626 |

*Counts - actual number received during radioimmunoassay (net count)

*% Bound - net count/maximum binding count X 100

*Log - Anti log of % Bound

*ng/ml - Actual androstenedione concentration in ng/ml
### Free Testosterone Raw Data

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| Log* | 1 | 0.332061 | -0.029059 | 0.002628 | -0.055282 | 0.10708 | 0.035138 | 0.112713 | 0.289459 | 0.246974 |
| Log* | 2 | 0.1674034 | 0.767118 | 0.195254 | 0.173624 | 0.123628 | 0.276549 | 0.2823 | 0.523594 | 0.269155 |
| Log* | 3 | 2.3072747 | 1.417581 | 1.537711 | 1.395339 | 1.639483 | 0.965811 | 1.563413 | 1.592519 | 1.65488 |
| Log* | 4 | 0.7807887 | 1.174948 | 1.425514 | 1.239891 | 1.904942 | 0.902444 | 1.317257 | 1.091739 | 0.872079 |
| Log* | 5 | 1.3441827 | 1.244543 | 1.134106 | 0.704213 | 0.825682 | 0.87955 | 1.26304 | 1.138331 | 0.833662 |
| Log* | 6 | 0.8148843 | 1.844375 | 0.831668 | 0.503996 | 0.538316 | 0.788409 | 0.594128 | 0.794346 | 0.794333 |
| Log* | 7 | 0.9849192 | 0.431348 | 0.253781 | 0.326193 | 0.526853 | 0.906538 | 0.91556 | 1.932673 | 0.926681 |

| pg/mi* | 1 | 2.148132 | 0.935279 | 1.000871 | 0.874416 | 1.279618 | 1.08427 | 1.296323 | 1.947171 | 1.785833 |
| pg/mi* | 2 | 1.4702914 | 5.849495 | 1.430286 | 1.491501 | 1.329314 | 1.89038 | 1.915578 | 3.338829 | 1.85647 |
| pg/mi* | 3 | 202.8382 | 26.18078 | 34.49142 | 24.88507 | 43.59765 | 9.242951 | 36.59427 | 39.13079 | 45.13207 |
| pg/mi* | 5 | 22.08335 | 17.56075 | 13.61778 | 5.060772 | 6.693936 | 0.79724 | 17.63208 | 13.75091 | 6.81808 |
| pg/mi* | 7 | 9.8596229 | 2.699905 | 1.793829 | 2.119302 | 3.37694 | 8.109996 | 8.233417 | 89.92352 | 9.05057 |

### Notes:
- **Counts**: actual number received during radioimmunoassay (net count)
- **% Bound**: net count/maximum binding count X 100
- **Log**: Anti log of % Bound
- **pg/mi**: Actual free testosterone concentration in pg/mL

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**Total Testosterone Raw Data**

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<th>t3</th>
<th>t4</th>
<th>t5</th>
<th>t6</th>
<th>t7</th>
<th>t8</th>
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</table>

| % Bound* |     |     |     |     |     |     |     |     |     |
| 1       | 81.042341 | 92.98501 | 83.85627 | 88.31349 | 83.36066 | 66.81551 | 68.55935 | 76.67613 | 83.68452 |
| 2       | 77.904749 | 64.35696 | 74.68870 | 76.19394 | 71.83434 | 68.67683 | 68.26078 |       |     |
| 3       | 9.181584 | 61.19955 | 47.43378 | 62.88578 | 50.41284 | 47.24717 | 46.4892 |       |     |
| 4       | 71.312504 | 68.78664 | 56.08693 | 63.6632 | 48.36515 | 70.26224 | 59.53498 | 69.45637 | 62.70559 |
| 5       | 64.885395 | 32.86214 | 66.70855 | 73.25451 | 70.14994 | 69.67775 | 60.84286 | 61.29863 | 66.32538 |
| 6       | 67.051985 | 42.65804 | 71.16718 | 69.38389 | 60.5631 | 61.24579 | 58.40399 | 54.18456 |     |
| 7       | 59.713323 | 78.78182 | 77.32347 | 74.49633 | 69.1327 | 62.32908 | 60.63809 | 28.64786 | 70.00462 |

| Log* |     |     |     |     |     |     |     |     |     |
| 1       | 1.3821095 | 1.027275 | 1.295504 | 1.225496 | 1.313223 | 1.21058 | 1.158768 | 1.511836 | 1.303606 |
| 2       | 1.475332 | 1.87757 | 1.488265 | 1.556592 | 1.526163 | 1.582881 | 1.655693 | 1.748504 | 1.761989 |
| 3       | 3.5172005 | 1.691608 | 1.971669 | 1.950276 | 2.39067 | 1.927511 | 2.292158 | 2.380867 | 2.408735 |
| 4       | 1.8711975 | 1.718533 | 2.123572 | 1.697876 | 2.352998 | 1.702403 | 2.021126 | 1.728346 | 1.926922 |
| 5       | 1.8621566 | 2.813615 | 1.807889 | 1.613488 | 1.705739 | 1.720086 | 1.982267 | 1.968725 | 1.819372 |
| 6       | 1.797794 | 2.522565 | 1.875515 | 1.729094 | 1.843708 | 1.988154 | 1.970295 | 2.141152 | 2.180085 |
| 7       | 2.0156285 | 1.506896 | 1.462603 | 1.576601 | 1.735963 | 1.938108 | 1.988351 | 2.398828 | 1.710057 |

| ng/dl* |     |     |     |     |     |     |     |     |     |
| 2       | 29.87666 | 75.48441 | 30.78117 | 38.86311 | 33.58635 | 38.27202 | 45.25777 | 56.17001 | 57.79214 |
| 3       | 3290.0346 | 49.1596 | 93.68468 | 89.18194 | 240.2538 | 84.62731 | 195.9557 | 240.3624 | 256.2921 |
| 4       | 46.902668 | 52.08347 | 132.9145 | 79.05451 | 225.4228 | 50.39875 | 104.9486 | 53.25324 | 84.51265 |
| 5       | 72.804226 | 65.05157 | 64.2672 | 41.06745 | 50.78541 | 52.4687 | 95.99896 | 93.05178 | 65.97393 |
| 6       | 62.774602 | 333.0925 | 47.37129 | 53.59122 | 68.77637 | 97.30928 | 93.38679 | 130.0624 | 151.3891 |
| 7       | 103.71143 | 32.26234 | 31.08871 | 37.72256 | 54.44559 | 86.71784 | 97.35326 | 868.6165 | 51.29283 |

Mean 516.60133 171.966 59.96879 50.11213 99.26283 60.86453 92.47893 210.5733 98.19596
Std Dev 1222.3861 237.9748 40.67712 25.47544 92.67231 29.56011 56.60517 298.4691 80.5996
Std E 462.01852 89.94603 15.37451 9.628811 35.02684 11.17267 21.39474 112.8107 30.46378

*Counts - actual number received during radioimmunoassay (net count)

*% Bound - net count/maximum binding count X 100

*Log - Anti log of % Bound

*ng/dl - Actual total testosterone concentration in ng/dl
BIBLIOGRAPHY


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Bioscience Award, University of Wisconsin, Marinette, 1994-95
Chemistry Award, University of Wisconsin, Marinette, 1994-95

Thesis Title: The Acute Effects of Transdermal Testosterone Precursor Administration on Serum Steroid Hormone Levels in Females

Thesis Examination Committee:
Chairperson, Dr. Lawrence Golding, Ph.D.
Committee Member, Dr. Mark Guadagnoli, Ph.D.
Committee Member, Dr. John Mercer, Ph.D.
Graduate Faculty Representative, Dr. Susan Silverton, M.D., Ph.D.