PHARMACEUTICAL COMPOSITION AND METHOD FOR TREATING HYPOGONADISM

FIELD OF THE INVENTION

The present invention is directed to a pharmaceutical composition comprising testosterone in a gel formulation, and to methods of using the same.

BACKGROUND OF THE INVENTION

A. Testosterone Metabolism in Men

Testosterone is the major circulating androgen in men. More than 95% of the 6-7 mg of testosterone produced per day is secreted by the approximately 500 million Leydig cells in the testes. Two hormones produced by the pituitary gland, luteinizing hormone ("LH") and follicle stimulating hormone ("FSH"), are required for the development and maintenance of testicular function.

The most important hormone for the regulation of Leydig cell number and function is LH. In eugonadal men, LH secretion from the pituitary is inhibited through a negative-feedback pathway by increased concentrations of testosterone through the inhibition of the release of gonadotropin-releasing hormone ("GRH") by the hypothalamus. FSH promotes spermatogenesis and is essential for the normal maturation of sperm. FSH secretion from the pituitary normally is inhibited through a negative-feedback pathway by increased testosterone concentrations.

Testosterone is responsible primarily for the development and maintenance of secondary sex characteristics in men. In the body, circulating testosterone is metabolized to various 17-keto steroids through two different pathways. Testosterone can be metabolized to dihydrotestosterone ("DHT") by the enzyme 5α-reductase. There are two forms of 5α-reductase in the body: one form is found predominately in the liver and non-genital skin while another form is found in the urogenital tract of the male and the genital skin of both sexes. Testosterone can also be metabolized to estradiol ("E_2") by an aromatase enzyme complex found in the liver, fat, and the testes.

Testosterone circulates in the blood 98% bound to protein. In men, approximately 40% of the binding is to the high-affinity sex hormone binding globulin ("SHBG"). The remaining

60% is bound weakly to albumin. Thus, a number of measurements for testosterone are available from clinical laboratories. The term "free" testosterone as used herein refers to the fraction of testosterone in the blood that is not bound to protein. The term "total testosterone" or "testosterone" as used herein means the free testosterone plus protein-bound testosterone. The term "bioavailable testosterone" as used herein refers to the non-SHBG bound testosterone and includes that weakly bound to albumin.

The conversion of testosterone to DHT is important in many respects. For example, DHT binds with greater affinity to SHBG than does testosterone. In addition, in many tissues, the activity of testosterone depends on the reduction to DHT, which binds to cytosol receptor proteins. The steroid-receptor complex is then transported to the nucleus where it initiates transcription and cellular changes related to androgen action. DHT is also thought to lower prostate volume and inhibit tumor development in the prostate. Thus, given the importance of DHT and testosterone in normal body functioning, researchers frequently assess and report androgen concentrations in patients as total androgen ("DHT +T") or as a ratio of DHT to testosterone ("DHT/T ratio").

The following table from the UCLA-Harbor Medical Center summarizes the hormone concentrations in normal adult men range:

Table 1: Hormone Levels in Normal Men

Hormone	Normal Range
Testosterone	298 to 1043 ng/dL
Free testosterone	3.5 to 17.9 ng/dL
DHT	31 to 193 ng/dL
DHT/T Ratio	0.052 to 0.33
DHT + T	372 to 1349 ng/dL
SHBG	10.8 to 46.6 nmol/L
FSH	1.0 to 6.9 mIU/mL
LH	1.0 to 8.1 mIU/mL
E_2	17.1 to 46.1 pg/mL

There is considerable variation in the half-life of testosterone reported in the literature, ranging from 10 to 100 minutes. Researchers do agree, however, that circulating testosterone has a diurnal variation in normal young men. Maximum levels occur at approximately 6:00 to 8:00 a.m. with levels declining throughout the day. Characteristic profiles have a maximum testosterone level of 720 ng/dL and a minimum level of 430 ng/dL. The physiological significance of this diurnal cycle, if any, however, is not clear.

B. Hypogonadal Men and Current Treatments for Hypogonadism

Male hypogonadism results from a variety of patho-physiological conditions in which testosterone concentration is diminished below the normal range. The hypogonadic condition is sometimes linked with a number of physiological changes, such as diminished interest in sex, impotence, reduced lean body mass, decreased bone density, lowered mood, and energy levels.

Researchers generally classify hypogonadism into one of three types. Primary hypogonadism includes the testicular failure due to congenital or acquired anorchia, XYY Syndrome, XX males, Noonan's Syndrome, gonadal dysgenesis, Leydig cell tumors, maldescended testes, varicocele, Sertoli-Cell-Only Syndrome, cryptorchidism, bilateral torsion, vanishing testis syndrome, orchiectomy, Klinefelter's Syndrome, chemotherapy, toxic damage from alcohol or heavy metals, and general disease (renal failure, liver cirrhosis, diabetes, myotonia dystrophica). Patients with primary hypogonadism show an intact feedback mechanism in that the low serum testosterone concentrations are associated with high FSH and LH concentrations. However, because of testicular or other failures, the high LH concentrations are not effective at stimulating testosterone production.

Secondary hypogonadism involves an idiopathic gonadotropin or LH-releasing hormone deficiency. This type of hypogonadism includes Kallman's Syndrome, Prader-Labhart-Willi's Syndrome, Laurence-Moon-Biedl's Syndrome, pituitary insufficiency/adenomas, Pasqualini's

Syndrome, hemochromatosis, hyperprolactinemia, or pituitary-hypothalamic injury from tumors, trauma, radiation, or obesity. Because patients with secondary hypogonadism do not demonstrate an intact feedback pathway, the lower testosterone concentrations are not associated with increased LH or FSH levels. Thus, these men have low testosterone serum levels but have gonadotropins in the normal to low range.

Third, hypogonadism may be age-related. Men experience a slow but continuous decline in average serum testosterone after approximately age 20 to 30 years. Researchers estimate that the decline is about 1-2% per year. Cross-sectional studies in men have found that the mean testosterone value at age 80 years is approximately 75% of that at age 30 years. Because the serum concentration of SHBG increases as men age, the fall in bioavailable and free testosterone is even greater than the fall in total testosterone. Researchers have estimated that approximately 50% of healthy men between the ages of 50 and 70 have levels of bioavailable testosterone that are below the lower normal limit. Moreover, as men age, the circadian rhythm of testosterone concentration is often muted, dampened, or completely lost. The major problem with aging appears to be within the hypothalamic-pituitary unit. For example, researchers have found that with aging, LH levels do not increase despite the low testosterone levels. Regardless of the cause, these untreated testosterone deficiencies in older men may lead to a variety of physiological changes, including sexual dysfunction, decreased libido, loss of muscle mass, decreased bone density, depressed mood, and decreased cognitive function. The net result is geriatric hypogonadism, or what is commonly referred to as "male menopause."

Today, hypogonadism is the most common hormone deficiency in men, affecting 5 in every 1,000 men. At present, it is estimated that only five percent of the estimated four to five million American men of all ages with hypogonadism currently receive testosterone replacement

therapy. Thus, for years, researchers have investigated methods of delivering testosterone to men. These methods include intramuscular injections (43%), oral replacement (24%), pellet implants (23%), and transdermal patches (10%). A summary of these methods is shown in Table 2.

Table 2: Mode of Application and Dosage of Various Testosterone Preparations

Preparation	Route Of Application	Full Substitution Dose
In Clinical Use		
Testosterone enanthate	Intramuscular injection	200-25.0 g every 2-3 weeks
Testosterone cypionate	Intramuscular injection	200 mg every 2 weeks
Testosterone undecanoate	Oral	2-4 capsules at 40 mg per day
Transdermal testosterone patch	Scrotal skin	1 membrane per day
Transdermal testosterone patch	Non-scrotal skin	1 or 2 systems per day
Testosterone implants	Implantation under the abdominal skin	3-6 implants of 200 mg every 6 months
Under Development		
Testosterone cyclodextrin	Sublingual	2.5-5.0 mg twice daily
Testosterone undecanoate	Intramuscular injection	1000 mg every 8-10 weeks
Testosterone buciclate	Intramuscular injection	1000 mg every 12-16 weeks
Testosterone microspheres	Intramuscular injection	315 mg for 11 weeks
Obsolete		
17α-Methyltestosterone	Oral	25-5.0 g per day
Fluoxymesterone	Sublingual	10-25 mg per day
	Oral	10-20 mg per day

As discussed below, all of the testosterone replacement methods currently employed suffer from one or more drawbacks, such as undesirable pharmacokinetic profiles or skin irritation. Thus, although the need for an effective testosterone replacement methodology has existed for decades, an alternative replacement therapy that overcomes these problems has never been developed. The present invention is directed to a 1% testosterone hydroalcoholic gel that overcomes the problems associated with current testosterone replacement methods.

1. Subdermal Pellet Implants

Subdermal implants have been used as a method of testosterone replacement since the 1940s. The implant is produced by melting crystalline testosterone into a cylindrical form.

Today, pellet implants are manufactured to contain either 100 mg (length 6 mm, surface area 117 mm^2) or 200 mg of testosterone (length 12 mm, surface area 202 mm^2). Patients receive dosages ranging from 100 to 1,200 mg, depending on the individual's requirements. The implants are inserted subcutaneously either by using a trocar and cannula or by open surgery into an area where there is relatively little movement. Frequently, the implant is placed in the lower abdominal wall or the buttock. Insertion is made under local anesthesia, and the wound is closed with an adhesive dressing or a fine suture.

Implants have several major drawbacks. First, implants require a surgical procedure which many hypogonadal men simply do not wish to endure. Second, implant therapy includes a risk of extrusion (8.5%), bleeding (2.3%), or infection (0.6%). Scarring is also a risk. Perhaps most important, the pharmacokinetic profile of testosterone pellet implant therapy fails to provide men with a suitable consistent testosterone level. In general, subdermal testosterone implants produce supra-physiologically high serum testosterone levels which slowly decline so that before the next injection subnormally low levels of testosterone are reached. For example, in one recent pharmacokinetic study, hypogonadal patients who received six implants (1,200 mg testosterone) showed an initial short-lived burst release of testosterone within the first two days after application. A stable plateau was then maintained over then next two months (day 2: 1,015 ng/dL; day 63: 990 ng/dL). Thereafter, the testosterone levels declined to baseline by day 300. DHT serum concentrations also rose significantly above the baseline, peaking at about 63 days after implementation and greatly exceeding the upper limit of the normal range. From day 21 to day 189, the DHT/T ratio was significantly increased. The pharmacokinetic profiles for testosterone, DHT, and DHT/T in this study are shown in FIG. 1. *See* Jockenhovel et al., *Pharmacokinetics and Pharmacodynamics of Subcutaneous Testosterone Implants in*

Hypogonadal Men, 45 CLINICAL ENDOCRINOLOGY 61-71 (1996). Other studies involving implants have reported similar undesirable pharmacokinetic profiles.

2. Injection of Testosterone Esters

Since the 1950s, researchers have experimented with the intermuscular depot injection of testosterone esters (such as enanthate, cypionate) to increase testosterone serum levels in hypogonadal men. More recent studies have involved injection of testosterone buciclate or testosterone undecanoate in an oil-based vehicle. Other researchers have injected testosterone microcapsule formulations.

Testosterone ester injection treatments suffer from many problems. Patients receiving injection therapy often complain that the delivery mechanism is painful and causes local skin reactions. In addition, testosterone microcapsule treatment requires two simultaneous intramuscular injections of a relatively large volume, which may be difficult to administer due to the high viscosity of the solution and the tendency to block the needle. Other men generally find testosterone injection therapy inconvenient because injection usually requires the patient to visit his physician every two to three weeks.

Equally important, injection-based testosterone replacement treatments still create an undesirable pharmacokinetic profile. The profile generally shows a supra-physiologic testosterone concentration during the first 24 to 48 hours followed by a gradual fall – often to sub-physiologic levels – over then next few weeks. These high serum testosterone levels, paralleled by increases in E_2, are also considered the reason for acne and gynecomastia occurring in some patients, and for polycythaemia, occasionally encountered especially in older patients using injectable testosterone esters. In the case of testosterone buciclate injections, the treatment barely provides normal androgen serum levels and the maximal increase of serum testosterone over baseline does not exceed 172 ng/dL (6 nmol/dL) on average. Because libido, potency,

mood, and energy are thought to fluctuate with the serum testosterone level, testosterone injections have largely been unsuccessful in influencing these variables. Thus, testosterone injection remains an undesirable testosterone replacement treatment method.

3. Oral/Sublingual/Buccal Preparations of Androgens

In the 1970s, researchers began using with oral, sublingual, or buccal preparations of androgens (such as fluoxymesterone, 17α-methyl-testosterone or testosterone undecanoate) as a means for testosterone replacement. More recently, researchers have experimented with the sublingual administration of testosterone-hydroxypropyl-beta-cyclodextrin inclusion complexes. Predictably, both fluoxymesterone and methyl testosterone are 17-alkylated and thus associated with liver toxicity. Because these substances must first pass through the liver, they also produce an unfavorable effect on serum lipid profile, increasing LDL and decreasing HDL, and carbohydrate metabolism. While testosterone undecanoate has preferential absorption through the intestinal lymphatics, it has not been approved in the United States.

The pharmacokinetic profiles for oral, sublingual, and buccal delivery mechanisms are also undesirable because patients are subjected to super-physiologic testosterone levels followed by a quick return to the baseline. For example, one recent testing of a buccal preparation showed that patients obtained a peak serum hormone levels within 30 minutes after administration, with a mean serum testosterone concentration of 2,688 +/- 147 ng/dL and a return to baseline in 4 to 6 hours. *See* Dobs et al., *Pharmacokinetic Characteristics, Efficacy and Safety of Buccal Testosterone in Hypogonadal Males: A Pilot Study*, 83 J. CLINICAL ENDOCRINOLOGY & METABOLISM 33-39 (1998). To date, the ability these testosterone delivery mechanisms to alter physiological parameters (such muscle mass, muscle strength, bone resorption, urinary calcium excretion, or bone formation) is inconclusive. Likewise, researchers have postulated that super-

physiologic testosterone levels may not have any extra beneficial impact on mood parameters such anger, nervousness, and irritability.

4. Testosterone Transdermal Patches

The most recent testosterone delivery systems have involved transdermal patches. Currently, there are three patches used in the market: TESTODERM®, TESTODERM® TTS, and ANDRODERM®.

a. TESTODERM®

TESTODERM® (Alza Pharmaceuticals, Mountain View, CA) was the first testosterone-containing patch developed. The TESTODERM® patch is currently available in two sizes (40 or 60 cm^2). The patch contains 10 or 15 mg of testosterone and delivers 4.0 mg or 6.0 mg of testosterone per day. TESTODERM® is placed on shaved scrotal skin, aided by application of heat for a few seconds from a hair dryer.

FIG. 2 shows a typical pharmacokinetic profile testosterone profile for both the 40 cm^2 and 60 cm^2 patch. Studies have also shown that after two to four weeks of continuous daily use, the average plasma concentration of DHT and DHT/T increased four to five times above normal. The high serum DHT levels are presumably caused by the increased metabolism of 5α-reductase in the scrotal skin.

Several problems are associated with the TESTODERM® patch. Not surprisingly, many men simply do not like the unpleasant experience of dry-shaving the scrotal hair for optimal contact. In addition, patients may not be able to wear close-fitting underwear when undergoing treatment. Men frequently experience dislodgment of the patch, usually with exercise or hot weather. In many instances, men experience itching and/or swelling in the scrotal area. Finally, in a number of patients, there is an inability to achieve adequate serum hormone levels.

b. TESTODERM® TTS

The most recently developed non-scrotal patch is TESTODERM® TTS (Alza Pharmaceuticals, Mountain View, CA). It is an occlusive patch applied once daily to the arm, back, or upper buttocks. The system is comprised of a flexible backing of transparent polyester/ethylene-vinyl acetate copolymer film, a drug reservoir of testosterone, and an ethylene-vinyl acetate copolymer membrane coated with a layer of polyisobutylene adhesive formulation. A protective liner of silicone-coated polyester covers the adhesive surface.

Upon application, serum testosterone concentrations rise to a maximum at two to four hours and return toward baseline within two hours after system removal. Many men, however, are unable to obtain and/or sustain testosterone levels within the normal range. The pharmacokinetic parameters for testosterone concentrations are shown as follows:

Table 3: TESTODERM® TTS Testosterone Parameters

Parameters	Day 1	Day 5
C_{max} (ng/dL)	482 ± 149	473 ± 148
T_{max} (h)	3.9	3.0
C_{min} (ng/dL)	164 ± 104	189 ± 86
T_{min} (h)	0	0

The typical 24-hour steady state testosterone concentration achieved with TESTODERM® TTS patch is shown in FIG. 3.

Because of TESTODERM® patch is applied to the scrotal skin while the TESTODERM TTS® patch is applied to non-scrotal skin, the two patches provide different steady-state concentrations of the two major testosterone metabolites, DTH and E_2,:

Table 4: Hormone Levels Using TESTODERM® and TESTODERM® TTS

Hormone	Placebo	TESTODERM®	TESTODERM® TTS
DHT (ng/dL)	11	134	38
E_2 (pg/ml)	3.8	10	21.4

Likewise, in contrast to the scrotal patch, TESTODERM TTS® treatment creates a DHT/T ratio that is not different from that of a placebo treatment. Both systems, however, suffer from similar problems. In clinical studies, TESTODERM® TTS is associated with transient itching in 12% of patients, erythema in 3% of patients, and puritus in 2% of patients. Moreover, in one 14-day study, 42% of patients reported three or more detachments, 33% of which occurred during exercise.

c. ANDRODERM®

ANDRODERM® (Watson Laboratories, Inc., Corona, CA) is a testosterone-containing patch applied to non-scrotal skin. The circular patch has a total surface area of 37 cm.^{2} The patch consists of a liquid reservoir containing 12.2 mg of testosterone and a permeation-enhanced vehicle containing ethanol, water, monoglycerides, fatty acid esters, and gelling agents. The suggested dose of two patches, applied each night in a rotating manner on the back, abdomen, upper arm, or thigh, delivers 4.1 to 6.8 mg of testosterone.

The steady state pharmacokinetic profile of a clinical study involving ANDRODERM® is shown in FIG. 4. In general, upon repeated application of the ANDRODERM® patch, serum testosterone levels increase gradually for eight hours after each application and then remain at this plateau level for about another eight hours before declining.

In clinical trials, ANDRODERM® is associated with skin irritation in about a third of the patients, and 10% to 15% of subjects have been reported to discontinue the treatment because of chronic skin irritation. Preapplication of corticosteroid cream at the site of application of ANDRODERM® has been reported to decrease the incidence and severity of the skin irritation. A recent study, however, found that the incidence of skin reactions sufficiently noxious enough to interrupt therapy was as high as 52%. *See* Parker et al., *Experience with Transdermal*

Testosterone Replacement in Hypogonadal Men, 50 CLINICAL ENDOCRINOLOGY (OXF) 57-62 (1999). The study reported:

> Two-thirds of respondents found the Andropatch unsatisfactory. Patches were variously described as noisy, visually indiscrete, embarrassing, unpleasant to apply and remove, and generally to be socially unacceptable. They fell off in swimming pools and showers, attracted ribald comments from sporting partners, and left bald red marks over trunk and limbs. Dogs, wives, and children were distracted by noise of the patches with body movements. Those with poor mobility or manual dexterity (and several were over 70 years of age) found it difficult to remove packaging an apply patches dorsally.

d. Transdermal Patch Summary

In sum, the transdermal patch generally offers an improved pharmacokinetic profile compared to other currently used testosterone delivery mechanisms. However, as discussed above, the clinical and survey data shows that all of these patches suffer from significant drawbacks, such as buritus, burn-like blisters, and erythema. Moreover, one recent study has concluded that the adverse effects associated with transdermal patch systems are "substantially higher" than reported in clinical trials. *See* Parker, *supra*. Thus, the transdermal patch still remains an inadequate testosterone replacement therapy alternative for most men.

5. DHT Gels

Researchers have recently begun investigating the application of DHT to the skin in a transdermal gel. However, the pharmacokinetics of a DHT-gel is markedly different from that of a testosterone gel. Application of DTH-gel results in decreased serum testosterone, E_2, LH, and FSH levels. Thus, DHT gels are not effective at increasing testosterone levels in hypogonadal men.

Accordingly, there is a definite need for a testosterone formulation that safely and effectively provides an optimal and predictable pharmacokinetic profile.

SUMMARY OF THE INVENTION

The foregoing problems are solved and a technical advance is achieved with the present invention. The present invention generally comprises a testosterone gel. Daily transdermal application of the gel in hypogonadal men results in a unique pharmacokinetic steady-state profile for testosterone. Long-term treatment further results in increased bone mineral density, enhanced libido, enhanced erectile frequency and satisfaction, increased positive mood, increased muscle strength, and improved body composition without significant skin irritation. The present invention is also directed to a unique method of administering the testosterone gel employing a packet having a polyethylene liner compatible with the components of the gel.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a graph of testosterone concentrations, DHT concentrations, and the DHT/T ratio for patients receiving a subdermal testosterone pellet implant over a period of 300 days after implantation.

FIG. 2 is a 24-hour testosterone pharmacokinetic profile for patients receiving the 40 cm^2 or 60 cm^2 TESTODERM® patch.

FIG. 3 is a 24-hour testosterone pharmacokinetic profile for patients receiving the TESTODERM® TTS patch.

FIG. 4 is a 24-hour testosterone pharmacokinetic profile for patients receiving the ANDRODERM® patch.

FIG. 5(a) is a graph showing the 24-hour testosterone pharmacokinetic profile for hypogonadal men prior to receiving 5.0 g/day of AndroGel®, 10.0 g/day of AndroGel®, or the testosterone patch (by initial treatment group).

FIG. 5(b) is a graph showing the 24-hour testosterone pharmacokinetic profile for hypogonadal men on the first day of treatment with either 5.0 g/day of AndroGel®, 10.0 g/day of AndroGel®, or the testosterone patch (by initial treatment group).

FIG. 5(c) is a graph showing the 24-hour testosterone pharmacokinetic profile for hypogonadal men on day 30 of treatment with either 5.0 g/day of AndroGel®, 10.0 g/day of AndroGel, or the testosterone patch (by initial treatment group).

FIG. 5(d) is a graph showing the 24-hour testosterone pharmacokinetic profile for hypogonadal men on day 90 of treatment with either 5.0 g/day of AndroGel®, 10.0 g/day of AndroGel®, or the testosterone patch (by initial treatment group).

FIG. 5(e) is a graph showing the 24-hour testosterone pharmacokinetic profile for hypogonadal men on day 180 of treatment with either 5.0 g/day of AndroGel®, 10.0 g/day of AndroGel®, or the testosterone patch (by final treatment group).

FIG. 5(f) is a graph showing the 24-hour testosterone pharmacokinetic profile for hypogonadal men on day 0, 1, 30, 90, and 180 of treatment with 5.0 g/day of AndroGel®.

FIG. 5(g) is a graph showing the 24-hour testosterone pharmacokinetic profile for hypogonadal men on day 0, 1, 30, 90, and 180 of treatment with 10.0 g/day of AndroGel®.

FIG. 5(h) is a graph showing the 24-hour testosterone pharmacokinetic profile for hypogonadal men on day 0, 1, 30, 90, and 180 of treatment with the testosterone patch.

FIG. 6(a) is a graph showing the 24-hour free testosterone pharmacokinetic profile for hypogonadal men on day 1 of treatment with either 5.0 g/day of AndroGel®, 10.0 g/day of AndroGel®, or the testosterone patch (by initial treatment group).

FIG. 6(b) is a graph showing the 24-hour free testosterone pharmacokinetic profile for hypogonadal men on day 30 of treatment with either 5.0 g/day of AndroGel®, 10.0 g/day of AndroGel®, or the testosterone patch (by initial treatment group).

FIG. 6(c) is a graph showing the 24-hour free testosterone pharmacokinetic profile for hypogonadal men on day 90 of treatment with either 5.0 g/day of AndroGel®, 10.0 g/day of AndroGel®, or the testosterone patch (by initial treatment group).

FIG. 6(d) is a graph showing the 24-hour free testosterone pharmacokinetic profile for hypogonadal men on day 180 of treatment with either 5.0 g/day of AndroGel®, 10.0 g/day of AndroGel®, or the testosterone patch (by final treatment group).

FIG. 6(e) is a graph showing the 24-hour free testosterone pharmacokinetic profile for hypogonadal men on day 0, 1, 30, 90, and 180 of treatment with 5.0 g/day of AndroGel®.

FIG. 6(f) is a graph showing the 24-hour free testosterone pharmacokinetic profile for hypogonadal men on day 0, 1, 30, 90, and 180 of treatment with 10.0 g/day of AndroGel®.

FIG. 6(g) is a graph showing the 24-hour free testosterone pharmacokinetic profile for hypogonadal men on day 0, 1, 30, 90, and 180 of treatment with the testosterone patch.

FIG. 7 is a graph showing the DHT concentrations on days 0 through 180 for hypogonadal men receiving either 5.0 g/day of AndroGel®, 10.0 g/day of AndroGel®, or the testosterone patch (by initial treatment group).

FIG. 8 is a graph showing the DHT/T ratio on days 0 through 180 for hypogonadal men receiving either 5.0 g/day of AndroGel®, 10.0 g/day of AndroGel®, or the testosterone patch (by initial treatment group).

FIG. 9 is a graph showing the total androgen concentrations (DHT +T) on days 0 through 180 for hypogonadal men receiving either 5.0 g/day of AndroGel®, 10.0 g/day of AndroGel®, or the testosterone patch (by initial treatment group).

FIG. 10 is a graph showing the E_2 concentrations on days 0 through 180 for hypogonadal men receiving either 5.0 g/day of AndroGel®, 10.0 g/day of AndroGel®, or the testosterone patch (by initial treatment group).

FIG. 11 is a graph showing the SHBG concentrations on days 0 through 180 for hypogonadal men receiving either 5.0 g/day of AndroGel®, 10.0 g/day of AndroGel®, or the testosterone patch (by initial treatment group).

FIG. 12(a) is a graph showing the FSH concentrations on days 0 through 180 for men having primary hypogonadism and receiving either 5.0 g/day of AndroGel®, 10.0 g/day of AndroGel®, or the testosterone patch (by initial treatment group).

FIG. 12(b) is a graph showing the FSH concentrations on days 0 through 180 for men having secondary hypogonadism and receiving either 5.0 g/day of AndroGel®, 10.0 g/day of AndroGel®, or the testosterone patch (by initial treatment group).

FIG. 12(c) is a graph showing the FSH concentrations on days 0 through 180 for men having age-associated hypogonadism and receiving either 5.0 g/day of AndroGel®, 10.0 g/day of AndroGel®, or the testosterone patch (by initial treatment group).

FIG. 12(d) is a graph showing the FSH concentrations on days 0 through 180 for men having hypogonadism of an unknown origin and receiving either 5.0 g/day of AndroGel®, 10.0 g/day of AndroGel®, or the testosterone patch (by initial treatment group).

FIG. 13(a) is a graph showing the LH concentrations on days 0 through 180 for men having primary hypogonadism and receiving either 5.0 g/day of AndroGel®, 10.0 g/day of AndroGel®, or the testosterone patch (by initial treatment group).

FIG. 13(b) is a graph showing the LH concentrations on days 0 through 180 for men having secondary hypogonadism and receiving either 5.0 g/day of AndroGel®, 10.0 g/day of AndroGel®, or the testosterone patch (by initial treatment group).

FIG. 13(c) is a graph showing the LH concentrations on days 0 through 180 for men having age-associated hypogonadism and receiving either 5.0 g/day of AndroGel®, 10.0 g/day of AndroGel®, or the testosterone patch (by initial treatment group).

FIG. 13(d) is a graph showing the LH concentrations on days 0 through 180 for men having hypogonadism of an unknown origin and receiving either 5.0 g/day of AndroGel®, 10.0 g/day of AndroGel®, or the testosterone patch (by initial treatment group).

FIG. 14(a) is a bar graph showing the change in hip BMD for hypogonadal men after 180 days of treatment with 5.0 g/day of AndroGel®, 7.5 g/day of AndroGel®, 10.0 g/day of AndroGel®, or the testosterone patch.

FIG. 14(b) is a bar graph showing the change in spine BMD for hypogonadal men after 180 days of treatment with 5.0 g/day of AndroGel®, 7.5 g/day of AndroGel®, 10.0 g/day of AndroGel®, or the testosterone patch

FIG. 15 is a graph showing PTH concentrations on days 0 through 180 for hypogonadal men receiving either 5.0 g/day of AndroGel®, 10.0 g/day of AndroGel®, or the testosterone patch (by initial treatment group).

FIG. 16 is a graph showing SALP concentrations on days 0 through 180 for hypogonadal men receiving either 5.0 g/day of AndroGel®, 10.0 g/day of AndroGel®, or the testosterone patch (by initial treatment group).

FIG. 17 is a graph showing the osteocalcin concentrations on days 0 through 180 for hypogonadal men receiving either 5.0 g/day of AndroGel®, 10.0 g/day of AndroGel®, or the testosterone patch (by initial treatment group).

FIG. 18 is a graph showing the type I procollagen concentrations on days 0 through 180 for hypogonadal men receiving either 5.0 g/day of AndroGel®, 10.0 g/day of AndroGel®, or the testosterone patch (by initial treatment group).

FIG. 19 is a graph showing the N-telopeptide/Cr ratio on days 0 through 180 for hypogonadal men receiving either 5.0 g/day of AndroGel®, 10.0 g/day of AndroGel®, or the testosterone patch (by initial treatment group).

FIG. 20 is a graph showing the Ca/Cr ratio on days 0 through 180 for hypogonadal men receiving either 5.0 g/day of AndroGel®, 10.0 g/day of AndroGel®, or the testosterone patch (by initial treatment group).

FIG. 21(a) is a graph showing sexual motivation scores on days 0 through 180 for hypogonadal men receiving either 5.0 g/day of AndroGel®, 7.5 g/day 10.0 g/day of AndroGel®, or the testosterone patch.

FIG. 21(b) is a graph showing overall sexual desire scores on days 0 through 180 for hypogonadal men receiving either 5.0 g/day of AndroGel®, 7.5 g/day 10.0 g/day of AndroGel®, or the testosterone patch.

FIG. 21(c) is a graph showing sexual enjoyment (with a partner) scores on days 0 through 180 for hypogonadal men receiving either 5.0 g/day of AndroGel®, 7.5 g/day 10.0 g/day of AndroGel®, or the testosterone patch.

FIG. 22(a) is a graph showing sexual performance scores on days 0 through 180 for hypogonadal men receiving either 5.0 g/day of AndroGel®, 7.5 g/day 10.0 g/day of AndroGel®, or the testosterone patch.

FIG. 22(b) is a graph showing erection satisfaction performance scores on days 0 through 180 for hypogonadal men receiving either 5.0 g/day of AndroGel®, 7.5 g/day 10.0 g/day of AndroGel®, or the testosterone patch.

FIG. 22(c) is a graph showing percent erection scores on days 0 through 180 for hypogonadal men receiving either 5.0 g/day of AndroGel®, 7.5 g/day 10.0 g/day of AndroGel®, or the testosterone patch.

FIG. 23(a) is a graph showing positive mood scores on days 0 through 180 for hypogonadal men receiving either 5.0 g/day of AndroGel®, 7.5 g/day 10.0 g/day of AndroGel®, or the testosterone patch.

FIG. 23(b) is a graph showing negative mood scores on days 0 through 180 for hypogonadal men receiving either 5.0 g/day of AndroGel®, 7.5 g/day 10.0 g/day of AndroGel®, or the testosterone patch.

FIG. 24(a) is a bar graph showing the change in leg strength on days 90 and 180 for hypogonadal men receiving either 5.0 g/day of AndroGel®, 7.5 g/day 10.0 g/day of AndroGel®, or the testosterone patch.

FIG. 24(b) is a bar graph showing the change in arm strength on days 90 and 180 for hypogonadal men receiving either 5.0 g/day of AndroGel®, 7.5 g/day 10.0 g/day of AndroGel®, or the testosterone patch.

FIG. 25(a) is a bar graph showing the change in total body mass on days 90 and 180 for hypogonadal men receiving either 5.0 g/day of AndroGel®, 7.5 g/day 10.0 g/day of AndroGel®, or the testosterone patch.

FIG. 25(b) is a bar graph showing the change in lean body mass on days 90 and 180 for hypogonadal men receiving either 5.0 g/day of AndroGel®, 7.5 g/day 10.0 g/day of AndroGel®, or the testosterone patch.

FIG. 25(c) is a bar graph showing the change in fat mass on days 90 and 180 for hypogonadal men receiving either 5.0 g/day of AndroGel®, 7.5 g/day 10.0 g/day of AndroGel®, or the testosterone patch.

FIG. 25(d) is a bar graph showing the change in percent body fat on days 90 and 180 for hypogonadal men receiving either 5.0 g/day of AndroGel®, 7.5 g/day 10.0 g/day of AndroGel®, or the testosterone patch.

DETAILED DESCRIPTION OF THE INVENTION

While the present invention may be embodied in many different forms, several specific embodiments are discussed herein with the understanding that the present disclosure is to be considered only as an exemplification of the principles of the invention, and it is not intended to limit the invention to the embodiments illustrated.

The present invention is directed to a pharmaceutical composition for percutaneous administration comprising at least one active pharmaceutical ingredient (e.g., testosterone) in a hydroalcoholic gel. In a broad aspect of the invention, the active ingredients employed in the composition may include anabolic steroids such as androisoxazole, bolasterone, clostebol, ethylestrenol, formyldienolone, 4-hydroxy-19-nortestosterone, methenolone, methyltrienolone, nandrolone, oxymesterone, quinbolone, stenbolone, trenbolone; androgenic steroids such as boldenone, fluoxymesterone, mestanolone, mesterolone, methandrostenolone, 17-methyltestosterone, 17 α-methyl-testosterone 3-cyclopentyl enol ether, norethandrolone, normethandrone, oxandrolone, oxymetholone, prasterone, stanlolone, stanozolol, dihydrotestosterone, testosterone; and progestogens such as anagestone, chlormadinone acetate, delmadinone acetate, demegestone, dimethisterone, dihydrogesterone, ethinylestrenol, ethisterone, ethynodiol, ethynodiol diacetate, flurogestone acetate, gestodene, gestonorone caproate, haloprogesterone, 17-hydroxy-16-methylene-progesterone, 17 β-hydroxyprogesterone, 17 α-hydroxyprogesterone caproate, medrogestone, medroxyprogesterone, megestrol acetate, melengestrol, norethindrone, norethindrone acetate, norethynodrel, norgesterone, norgestimate, norgestrel, norgestrienone, 19-norprogesterone, norvinisterone, pentagestrone, progesterone, promegestone, quingestrone, and trengestone; and all enantiomers, isomers and derivatives of

these compounds. (Based upon the list provided in The Merck Index, Merck & Co. Rahway, N.J. (1998)).

In addition to the active ingredient, the gel comprises one or more lower alcohols, such as ethanol or isopropanol; a penetration enhancing agent; a thickener; and water. Additionally, the present invention may optionally include salts, emollients, stabilizers, antimicrobials, fragrances, and propellants.

A "penetration enhancer" is an agent known to accelerate the delivery of the drug through the skin. These agents also have been referred to as accelerants, adjuvants, and sorption promoters, and are collectively referred to herein as "enhancers." This class of agents includes those with diverse mechanisms of action including those which have the function of improving the solubility and diffusibility of the drug, and those which improve percutaneous absorption by changing the ability of the stratum corneum to retain moisture, softening the skin, improving the skin's permeability, acting as penetration assistants or hair-follicle openers or changing the state of the skin such as the boundary layer.

The penetration enhancer of the present invention is a functional derivative of a fatty acid, which includes isosteric modifications of fatty acids or non-acidic derivatives of the carboxylic functional group of a fatty acid or isosteric modifications thereof. In one embodiment, the functional derivative of a fatty acid is an unsaturated alkanoic acid in which the —COOH group is substituted with a functional derivative thereof, such as alcohols, polyols, amides and substituted derivatives thereof. The term "fatty acid" means a fatty acid that has four (4) to twenty-four (24) carbon atoms. Non-limiting examples of penetration enhancers include C8-C22 fatty acids such as isostearic acid, octanoic acid, and oleic acid; C8-C22 fatty alcohols such as oleyl alcohol and lauryl alcohol; lower alkyl esters of C8-C22 fatty acids such as ethyl

oleate, isopropyl myristate, butyl stearate, and methyl laurate; di(lower)alkyl esters of C6-C8 diacids such as diisopropyl adipate; monoglycerides of C8-C22 fatty acids such as glyceryl monolaurate; tetrahydrofurfuryl alcohol polyethylene glycol ether; polyethylene glycol, propylene glycol; 2-(2-ethoxyethoxy)ethanol; diethylene glycol monomethyl ether; alkylaryl ethers of polyethylene oxide; polyethylene oxide monomethyl ethers; polyethylene oxide dimethyl ethers; dimethyl sulfoxide; glycerol; ethyl acetate; acetoacetic ester; N-alkylpyrrolidone; and terpenes.

The thickeners used herein may include anionic polymers such as polyacrylic acid (CARBOPOL® by B.F. Goodrich Specialty Polymers and Chemicals Division of Cleveland, Ohio), carboxymethylcellulose and the like. Additional thickeners, enhancers and adjuvants may generally be found in United States Pharmacopeia/National Formulary (2000); Remington's The Science and Practice of Pharmacy, Meade Publishing Co.

The amount of drug to be incorporated in the composition varies depending on the particular drug, the desired therapeutic effect, and the time span for which the gel is to provide a therapeutic effect. The composition is used in a "pharmacologically effective amount." This means that the concentration of the drug is such that in the composition it results in a therapeutic level of drug delivered over the term that the gel is to be used. Such delivery is dependent on a number of variables including the drug, the form of drug, the time period for which the individual dosage unit is to be used, the flux rate of the drug from the gel, surface area of application site, etc. The amount of drug necessary can be experimentally determined based on the flux rate of the drug through the gel, and through the skin when used with and without enhancers.

One such testosterone gel has only recently been made available in the United States under the trademark AndroGel® by Unimed Pharmaceuticals, Inc., Deerfield, Illinois, one of the assignees of this application. In one embodiment, the gel is comprised of the following substances in approximate amounts:

Table 5: Composition of AndroGel®

SUBSTANCE	AMOUNT (w/w) PER 100g OF GEL
Testosterone	1.0 g
Carbopol 980	0.90 g
Isopropyl myristate	0.50 g
0.1 N NaOH	4.72 g
Ethanol (95% w/w)	72.5 g*
Purified water (qsf)	100 g

*Corresponding to 67 g of ethanol.

One skilled in the art will appreciate that the constituents of this formulation may be varied in amounts yet continue to be within the spirit and scope of the present invention. For example, the composition may contain about 0.1 to about 10.0 g of testosterone, about 0.1 to about 5.0 g Carbopol, about 0.1 to about 5.0 g isopropyl myristate, and about 30.0 to about 98.0 g ethanol.

A therapeutically effective amount of the gel is rubbed onto a given area of skin by the user. The combination of the lipophilic testosterone with the hydroalcoholic gel helps drive the testosterone in to the outer layers of the skin where it is absorbed and then slowly released into the blood stream. As demonstrated by the data presented herein, the administration of the gel of the present invention has a sustained effect.

Toxicity and therapeutic efficacy of the active ingredients can be determined by standard pharmaceutical procedures, *e.g.*, for determining LD_{50} (the dose lethal to 50% of the population) and the ED_{50} (the dose therapeutically effective in 50% of the population). The dose ratio

between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD_{50}/ED_{50}. Compounds which exhibit large therapeutic induces are preferred. While compounds that exhibit toxic side effects may be used, care should be taken to design a delivery system that targets such compounds to the site of affected tissue in order to minimize potential damage to uninfected cells and, thereby, reduce side effects.

The term "treatment" as used herein refers to any treatment of a human condition or disease and includes: (1) preventing the disease or condition from occurring in a subject which may be predisposed to the disease but has not yet been diagnosed as having it, (2) inhibiting the disease or condition, *i.e.*, arresting its development, (3) relieving the disease or condition, *i.e.*, causing regression of the condition, or (4) relieving the conditions caused by the disease, *i.e.*, stopping the symptoms of the disease.

Although the examples of the present invention involve the treatment of disorders associated with hypogonadal men, the composition and method of the present invention may be used to treat these disorders in humans and animals of any kind, such as dogs, pigs, sheep, horses, cows, cats, zoo animals, and other commercially bred farm animals.

The present invention is further illustrated by the following examples, which should not be construed as limiting in any way. The contents of all cited references throughout this application are hereby expressly incorporated by reference. The practice of the present invention will employ, unless otherwise indicated, conventional techniques of pharmacology and pharmaceutics, which are within the skill of the art.

EXAMPLES

Example 1: Treatment of Hypogonadism in Male Subjects

One embodiment of the present invention involves the transdermal application of AndroGel® as a method of treating male hypogonadism. As demonstrated below, application of the gel results in a unique pharmacokinetic profile for testosterone, as well as concomitant modulation of several other sex hormones. Application of the testosterone gel to hypogonadal male subjects also results in: (1) increased bone mineral density, (2) enhanced libido, (3) enhanced erectile capability and satisfaction, (4) increased positive mood, (5) increased muscle strength, and (6) better body composition, such increased total body lean mass and decreased total body fat mass. Moreover, the gel is not associated with significant skin irritation.

Methods

In this example, hypogonadal men were recruited and studied in 16 centers in the United States. The patients were between 19 and 68 years and had single morning serum testosterone levels at screening of less than or equal to 300 ng/dL (10.4 nmol/L). A total of 227 patients were enrolled: 73, 78, and 76 were randomized to receive 5.0 g/day of AndroGel® (delivering 50 mg/day of testosterone to the skin of which about 10% or 5 mg is absorbed), 10.0 g/day of AndroGel® (delivering 100 mg/day of testosterone to the skin of which about 10% or 10 mg is absorbed), or the ANDRODERM® testosterone patch ("T patch") (delivering 50 mg/day of testosterone), respectively.

As shown in the following table, there were no significant group-associated differences of the patients' characteristics at baseline.

Table 6. Baseline Characteristics of the Hypogonadal Men

Treatment Group	T patch	AndroGel® (5.0 g/day)	AndroGel® (10.0 g/day)
No of subjects enrolled	76	73	78

Treatment Group	T patch	AndroGel® (5.0 g/day)	AndroGel® (10.0 g/day)
Age (years)	51.1	51.3	51.0
Range (years)	28-67	23-67	19-68
Height (cm)	179.3 ± 0.9	175.8 ± 0.8	178.6 ± 0.8
Weight (kg)	92.7 ± 1.6	90.5 ± 1.8	91.6 ± 1.5
Serum testosterone (nmol/L)	6.40 ± 0.41	6.44 ± 0.39	6.49 ± 0.37
Causes of hypogonadism			
Primary hypogonadism	34	26	34
Klinefelter's Syndrome	9	5	8
Post Orchidectomy/Anorchia	2	1	3
Primary Testicular Failure	23	20	23
Secondary hypogonadism	15	17	12
Kallman's Syndrome	2	2	0
Hypothalimic Pituitary Disorder	6	6	3
Pituitary Tumor	7	9	9
Aging	6	13	6
Not classified	21	17	26
Years diagnosed	5.8 ± 1.1	4.4 ± 0.9	5.7 ± 1.24
Number previously treated with testosterone	50 (65.8%)	38 (52.1%)	46 (59.0%)
Type of Previous Hormonal Treatment			
Intramuscular injections	26	20	28
Transdermal patch	12	7	8
All others	12	11	10
Duration of treatment (years)	5.8 ± 1.0	5.4 ± 0.8	4.6 ± 80.7

Forty-one percent (93/227) of the subjects had not received prior testosterone replacement therapy. Previously treated hypogonadal men were withdrawn from testosterone ester injection for at least six weeks and oral or transdermal androgens for four weeks before the screening visit. Aside from the hypogonadism, the subjects were in good health as evidenced by medical history, physical examination, complete blood count, urinalysis, and serum biochemistry. If the subjects were on lipid-lowering agents or tranquilizers, the doses were stabilized for at least three months prior to enrollment. Less than 5% of the subjects were taking supplemental calcium or vitamin D during the study. The subjects had no history of chronic medical illness, alcohol or drug abuse. They had a normal rectal examination, a PSA level of less than 4 ng/mL, and a urine flow rate of 12 mL/s or greater. Patients were excluded if they

had a generalized skin disease that might affect the testosterone absorption or prior history of skin irritability with ANDRODERM® patch. Subjects weighing less than 80% or over 140% of their ideal body weight were also excluded.

The randomized, multi-center, parallel study compared two doses of AndroGel® with the ANDRODERM® testosterone patch. The study was double-blind with respect to the AndroGel® dose and open-labeled for the testosterone patch group. For the first three months of the study (days 1 to 90), the subjects were randomized to receive 5.0 g/day of AndroGel®, 10.0 g/day of AndroGel®, or two non-scrotal patches. In the following three months (days 91 to 180), the subjects were administered one of the following treatments: 5.0 g/day of AndroGel®, 10.0 g/day of AndroGel®, 7.5 g/day of AndroGel®, or two non-scrotal patches. Patients who were applying AndroGel® had a single, pre-application serum testosterone measured on day 60 and, if the levels were within the normal range of 300 to 1,000 ng/dL (10.4 to 34.7 nmol/L), then they remained on their original dose. Patients with testosterone levels less than 300 ng/dL and who were originally assigned to apply 5.0 g/day of AndroGel® and those with testosterone levels more than 1,000 ng/dL who had received 10.0 g/day of AndroGel® were then reassigned to administer 7.5 g/day of AndroGel® for days 91 to 180.

Accordingly, at 90 days, dose adjustments were made in the AndroGel® groups based on the pre-application serum testosterone levels on day 60. Twenty subjects in the 5.0 g/day AndroGel® group had the dose increased to 7.5 g/day. Twenty patients in the 10.0 g/day AndroGel® group had the AndroGel® dose reduced to 7.5 g/day. There were three patients in the testosterone patch group who were switched to 5.0 g/day AndroGel® because of patch intolerance. One 10.0 g/day AndroGel® subject was adjusted to receive 5.0 g/day and one 5.0 g/day AndroGel® subject had the dose adjusted to 2.5 g/day. The number of subjects

enrolled into day 91 to 180 of the study thus consisted of 51 receiving 5.0 g/day of AndroGel®, 40 receiving 7.5 g/day of AndroGel®, 52 receiving 10.0 g/day of AndroGel®, and 52 continuing on the ANDRODERM® patch. The treatment groups in this example may thus be characterized in two ways, either by "initial" or by the "final" treatment group.

Subjects returned to the study center on days 0, 30, 60, 90, 120, 150, and 180 for a clinical examination, skin irritation and adverse event assessments. Fasting blood samples for calcium, inorganic phosphorus, parathyroid hormone ("PTH"), osteocalcin, type I procollagen, and skeletal specific alkaline phosphatase ("SALP") were collected on days 0, 30, 90, 120, and 180. In addition, a fasting two-hour timed urine collection for urine creatinine, calcium, and type 1 collagen cross-linked N-telopeptides ("N-telopeptide") were collected on days 0, 30, 90, 120, and 180. Other tests performed were as follows:

(1) *Hematology:* hemoglobin, hematocrit, red blood cell count, platelets, white blood cell counts with differential analysis (neutrophils, lymphocytes, monocytes, eosinophils, and basophils);

(2) *Chemistry:* alkaline phosphatase, alanine aminotransferase, serum glutamic pyruvic transaminase ("ALT/SGPT"), asparate aminotransferase/serum glutamin axaloacetic transaminase ("AST/SGOT"), total bilirubin, creatinine, glucose, and elecrolytes (sodium, potassium, choride, bicarbonate, calcium, and inorganic phosphorus);

(3) *Lipids:* total cholesterol, high-density lipoprotein ("HDL"), low-density lipoprotein ("LDL"), and triglycerides;

(4) *Urinalysis:* color, appearance, specific gravity, pH, protein, glucose, ketones, blood, bilirubin, and nitrites; and

(5) *Other:* PSA (screening days 90-180), prolactin (screening), and testosterone (screening) including electrolytes, glucose, renal, and liver function tests and lipid profile, were performed at all clinic visits. Bone mineral density ("BMD") was analyzed at day 0 and day 180.

A. AndroGel® and ANDRODERM® patch

Approximately 250 g of AndroGel® was packaged in multidose glass bottles that delivered 2.25 g of the gel for each actuation of the pump. Patients assigned to apply 5.0 g/day of AndroGel® testosterone were given one bottle of AndroGel® and one bottle of placebo gel (containing vehicle but no testosterone), while those assigned to receive 10.0 g/day of AndroGel® were dispensed two bottles of the active AndroGel®. The patients were then instructed to apply the bottle contents to the right and left upper arms/shoulders and to the right and left sides of the abdomen on an alternate basis. For example, on the first day of the study, patients applied two actuations from one bottle, one each to the left and right upper arm/shoulder, and two actuations from the second bottle, one each to the left and right abdomen. On the following day of treatment, the applications were reversed. Alternate application sites continued throughout the study. After application of the gel to the skin, the gel dried within a few minutes. Patients washed their hands thoroughly with soap and water immediately after gel application.

The 7.5 g/day AndroGel® group received their dose in an open-label fashion. After 90 days, for the subjects titrated to the AndroGel® 7.5 g/day dose, the patients were supplied with three bottles, one containing placebo and the other two AndroGel®. The subjects were instructed to apply one actuation from the placebo bottle and three actuations from a AndroGel® bottle to four different sites of the body as above. The sites were rotated each day taking the same sequence as described above.

ANDRODERM® testosterone patches each delivering 2.5 mg/day of testosterone were provided to about one-third of the patients in the study. These patients were instructed to apply two testosterone patches to a clean, dry area of skin on the back, abdomen, upper arms, or thighs

once per day. Application sites were rotated with approximately seven days interval between applications to the same site.

On study days when the patients were evaluated, the gel/patches were applied following pre-dose evaluations. On the remaining days, the testosterone gel or patches were applied at approximately 8:00 a.m. for 180 days.

B. Study Method and Results

1. Hormone Pharmacokinetics

On days 0, 1, 30, 90, and 180, the patients had multiple blood samples for testosterone and free testosterone measurements at 30, 15 and 0 minutes before and 2, 4, 8, 12, 16, and 24 hours after AndroGel® or patch application. In addition, subjects returned on days 60, 120, and 150 for a single blood sampling prior to application of the gel or patch. Serum DHT, E_2, FSH, LH and SHBG were measured on samples collected before gel application on days 0, 30, 60, 90, 120, 150, and 180. Sera for all hormones were stored frozen at –20 °C until assay. All samples for a patient for each hormone were measured in the same assay whenever possible. The hormone assays were then measured at the Endocrine Research Laboratory of the UCLA-Harbor Medical Center.

The following table summarizes the pharmacokinetic parameters were measured for each patient:

Table 7: Pharmacokinetic Parameters

$AUC_{0\text{-}24}$	area under the curve from 0 to 24 hours, determined using the linear trapezoidal rule.
C_{base} or C_0	Baseline concentration
C_{avg}	time-averaged concentration over the 24-hour dosing interval determined by $AUC_{0\text{-}24}/24$
C_{max}	maximum concentration during the 24-hour dosing interval

C_{min}	minimum concentration during the 24-hour dosing interval
T_{max}	time at which C_{max} occurred
T_{min}	time at which C_{min} occurred
Fluctuation Index	extent of variation in the serum concentration over the course of a single day, calculated as $(C_{max} - C_{min})/C_{avg}$
Accumulation ratio	increase in the daily drug exposure with continued dosing, calculated as the ratio of the AUC at steady on a particular day over the AUC on day 1 (*e.g.*, $AUC_{day\ 30}/AUC_{day\ 1}$)
Net AUC_{0-24}	AUC_{0-24} on days 30, 90, 180 - AUC_{0-24} on day 0

a. **Testosterone Pharmacokinetics**

(1) **Methods**

Serum testosterone levels were measured after extraction with ethylacetate and hexane by a specific radioimmunoassay ("RIA") using reagents from ICN (Costa Mesa, CA). The cross reactivities of the antiserum used in the testosterone RIA were 2.0% for DHT, 2.3% for androstenedione, 0.8% for 3-β-androstanediol, 0.6% for etiocholanolone and less than 0.01% for all other steroids tested. The lower limit of quantitation ("LLQ") for serum testosterone measured by this assay was 25 ng/dL (0.87 nmol/L). The mean accuracy of the testosterone assay, determined by spiking steroid free serum with varying amounts of testosterone (0.9 nmol/L to 52 nmol/L), was 104% and ranged from 92% to 117%. The intra-assay and inter-assay coefficients of the testosterone assay were 7.3 and 11.1%, respectively, at the normal adult male range. In normal adult men, testosterone concentrations range from 298 to 1,043 ng/dL (10.33 to 36.17 nmol/L) as determined at the UCLA-Harbor Medical Center.

(2) **Baseline Concentration**

As shown in Table 8 and FIG. 5(a), at baseline, the average serum testosterone concentrations over 24 hours (C_{avg}) were similar in the groups and below the adult normal range. Moreover the variations of the serum concentration (based on maximum and minimum

concentrations during the 24-hour period, C_{max} and C_{min}, respectively) during the day were also similar in the three groups. FIG. 5(a) shows that the mean testosterone levels had a the maximum level between 8 to 10 a.m. (*i.e.*, at 0 to 2 hours) and the minimum 8 to 12 hours later, demonstrating a mild diurnal variation of serum testosterone. About one-third of the patients in each group had C_{avg} within the lower normal adult male range on day 0 (24/73 for the 5.0 g/day AndroGel® group, 26/78 for the 10.0 g/day AndroGel® group, and 25/76 for testosterone patch group). All except three of the subjects met the enrollment criterion of serum testosterone less than 300 ng/dL (10.4 nmol/L) on admission.

Table 8(a): Baseline Phamacokinetic Parameters by Initial Treatment Group (Mean ± SD)

	5.0 g/day T-Gel	10.0 g/day T-gel	T-patch
N	73	78	76
C_{avg} (ng/dL)	237 ± 130	248 ± 140	237 ± 139
C_{max} (ng/dL)	328 ± 178	333 ± 194	314 ± 179
T_{max}* (hr)	4.0 (0.0-24.5)	7.9 (0.0-24.7)	4.0 (0.0-24.3)
C_{min} (ng/dL)	175 ± 104	188 ± 112	181 ± 112
T_{min}* (hr)	8.01 (0.0-24.1)	8.0 (0.0-24.0)	8.0 (0.0-23.9)
Fluc Index (ratio)	0.627 ± 0.479	0.556 ± 0.384	0.576 ± 0.341

Median (Range)

Table 8(b): Baseline Testosterone Pharmacokinetic Parameters by Final Treatment Group (Mean ± SD)

	Doses Received During Initial => Extended Treatment Phases				
	5.0 g/day T-gel	5.0 => 7.5 g/day T-gel	10.0 => 7.5 g/day T-gel	10.0 g/day T-gel	T-patch
N	53	20	20	58	76
C_{avg} (ng/dL)	247 ± 137	212 ± 109	282 ± 157	236 ± 133	237 ± 140
C_{max} (ng/dL)	333 ± 180	313 ± 174	408 ± 241	307 ± 170	314 ± 179
T_{max}* (hr)	4.0 (0.0-24.5)	4.0 (0.0-24.0)	19.7 (0.0-24.3)	4.0 (0.0-24.7)	4.0 (0.0-24.3)
C_{min} (ng/dL)	185 ± 111	150 ± 80	206 ± 130	182 ± 106	181 ± 112
T_{min}* (hr)	8.0 (0.0-24.1)	11.9 (0.0-24.0)	8.0 (0.0-23.3)	8.0 (0.0-24.0)	8.0 (0.0-23.9)
Fluc Index (ratio)	0.600 ± 0.471	0.699 ± 0.503	0.678 ± 0.580	0.514 ± 0.284	0.576 ± 0.341

*Median (range)

(3) **Day 1**

FIG. 5(b) and Tables 8(c)-(d) show the pharmacokinetic profile for all three initial treatment groups after the first application of transdermal testosterone. In general, treatment

with AndroGel® and the testosterone patch produced increases in testosterone concentrations sufficiently large to bring the patients into the normal range in just a few hours. However, even on day 1, the pharmacokinetic profiles were markedly different in the AndroGel® and patch groups. Serum testosterone rose most rapidly in the testosterone patch group reaching a maximum concentration (C_{max}) at about 12 hours (T_{max}). In contrast, serum testosterone rose steadily to the normal range after AndroGel® application with C_{max} levels achieved by 22 and 16 hours in the 5.0 g/day AndroGel® group and the 10.0 g/day AndroGel® group, respectively.

Table 8(c): Testosterone Pharmacokinetic Parameters on Day 1 by Initial Treatment Group (Mean ± SD)

	5.0 g/day T-Gel	10.0 g/day T-gel	T-patch
N	73	76	74
C_{avg} (ng/dL)	398 ± 156	514 ± 227	482 ± 204
C_{max} (ng/dL)	560 ± 269	748 ± 349	645 ± 280
T_{max}* (hr)	22.1 (0.0-25.3)	16.0 (0.0-24.3)	11.8 (1.8-24.0)
C_{min} (ng/dL)	228 ± 122	250 ± 143	232 ± 132
T_{min}* (hr)	1.9 (0.0-24.0)	0.0 (0.0-24.2)	1.5 (0.0-24.0)

*Median (Range)

Table 8(d): Testosterone Phamacokinetic Parameters on Day 1 by Final Treatment Group (Mean ± SD)

	Doses Received During Initial => Extended Treatment Phases				
	5.0 g/day T-gel	5.0 => 7.5 g/day T-gel	10.0 => 7.5 g/day T-gel	10.0 g/day T-gel	T-patch
N	53	20	19	57	74
C_{avg} (ng/dL)	411 ± 160	363 ± 143	554 ± 243	500 ± 223	482 ± 204
C_{max} (ng/dL)	573 ± 285	525 ± 223	819 ± 359	724 ± 346	645 ± 280
T_{max}* (hr)	22.1 (0.0-25.3)	19.5 (1.8-24.3)	15.7 (3.9-24.0)	23.0 (0.0-24.3)	11.8 (1.8-24.0)
C_{min} (ng/dL)	237 ± 125	204 ± 112	265 ± 154	245 ± 140	232 ± 132
T_{min}* (hr)	1.8 (0.0-24.0)	3.5 (0.0-24.0)	1.9 (0.0-24.2)	0.0 (0.0-23.8)	1.5 (0.0-24.0)
Fluc Index (ratio)	0.600 ± 0.471	0.699 ± 0.503	0.678 ± 0.580	0.514 ± 0.284	0.576 ± 0.341

*Median (range)

(4) **Days 30, 90, and 180**

FIGS. 5(c) and 5(d) show the unique 24-hour pharmacokinetic profile of AndroGel®-treated patients on days 30 and 90. In the AndroGel® groups, serum testosterone levels showed small and variable increases shortly after dosing. The levels then returned to a relatively constant level. In contrast, in the testosterone patch group, patients exhibited a rise over the first

8 to 12 hours, a plateau for another 8 hours, and then a decline to the baseline of the prior day. Further, after gel application on both days 30 and 90, the C_{avg} in the 10.0 g/day AndroGel® group was 1.4 fold higher than in the 5.0 g/day AndroGel® group and 1.9 fold higher than the testosterone patch group. The testosterone patch group also had a C_{min} substantially below the lower limit of the normal range. On day 30, the accumulation ratio was 0.94 for testosterone patch group, showing no accumulation. The accumulation ratios at 1.54 and 1.9 were significantly higher in the 5.0 g/day AndroGel® group and 10.0 g/day AndroGel® group, respectively. The differences in accumulation ratio among the groups persisted on day 90. This data indicates that the AndroGel® preparations had a longer effective half-life than testosterone patch.

FIG. 5(e) shows the 24-hour pharmacokinetic profile for the treatment groups on day 180. In general, as Table 8(e) shows, the serum testosterone concentrations achieved and the pharmacokinetic parameters were similar to those on days 30 and 90 in those patients who continued on their initial randomized treatment groups. Table 8(f) shows that the patients titrated to the 7.5 g/day AndroGel® group were not homogeneous. The patients that were previously in the 10.0 g/day group tended to have higher serum testosterone levels than those previously receiving 5.0 g/day. On day 180, the C_{avg} in the patients in the 10.0 g/day group who converted to 7.5 g/day on day 90 was 744 ng/dL, which was 1.7 fold higher than the. C_{avg} of 450 ng/dL in the patients titrated to 7.5 g/day from 5.0 g/day. Despite adjusting the dose up by 2.5 g/day in the 5.0 to 7.5 g/day group, the C_{avg} remained lower than those remaining in the 5.0 g/day group. In the 10.0 to 7.5 g/day group, the C_{avg} became similar to those achieved by patients remaining in the 10.0 g/day group without dose titration. These results suggest that many of the under-responders may actually be poorly compliant patients. For example, if a

patient does not apply AndroGel® properly (*e.g.*, preferentially from the placebo container or shortly before bathing), then increasing the dose will not provide any added benefit.

FIGS. 5(f)-(h) compare the pharmacokinetic profiles for the 5.0 g/day AndroGel® group, the 10.0 AndroGel® g/day group, and the testosterone patch group at days 0, 1, 30, 90, and 180, respectively. In general, the mean serum testosterone levels in the testosterone patch group remained at the lower limit of the normal range throughout the treatment period. In contrast, the mean serum testosterone levels remained at about 490-570 ng/dL for the 5.0 g/day AndroGel® group and about 630-860 ng/dL AndroGel® for the 10.0 g/day group.

Table 8(e): Testosterone Phamacokinetic Parameters on Day 1 by Initial Treatment Group (Mean ± SD)

	5.0 g/day T-Gel	10.0 g/day T-gel	T-patch
Day 30	N = 66	N = 74	N = 70
C_{avg} (ng/dL)	566 ± 262	792 ± 294	419 ± 163
C_{max} (ng/dL)	876 ± 466	1200 ± 482	576 ± 223
T_{max}*(hr)	7.9 (0.0-24.0)	7.8 (0.0-24.3)	11.3 (0.0-24.0)
C_{min} (ng/dL)	361 ± 149	505 ± 233	235 ± 122
T_{min}* (hr)	8.0 (0.0-24.1)	8.0 (0.0-25.8)	2.0 (0.0-24.2)
Fluc Index (ratio)	0.857 ± 0.331	0.895 ± 0.434	0.823 ± 0.289
Accum Ratio (ratio)	1.529 ± 0.726	1.911 ± 1.588	0.937 ± 0.354
Day 90	N = 65	N = 73	N = 64
C_{avg} (ng/dL)	553 ± 247	792 ± 276	417 ± 157
C_{max} (ng/dL)	846 ± 444	1204 ± 570	597 ± 242
T_{max}*(hr)	4.0 (0.0-24.1)	7.9 (0.0-25.2)	8.1 (0.0-25.0)
C_{min} (ng/dL)	354 ± 147	501 ± 193	213 ± 105
T_{min}* (hr)	4.0 (0.0-25.3)	8.0 (0.0-24.8)	2.0 (0.0-24.0)
Fluc Index (ratio)	0.851 ± 0.402	0.859 ± 0.399	0.937 ± 0.442
Accum Ratio (ratio)	1.615 ± 0.859	1.927 ± 1.310	0.971 ± 0.453
Day 180	N = 63	N = 68	N = 45
C_{avg} (ng/dL)	520 ± 227	722 ± 242	403 ± 163
C_{max} (ng/dL)	779 ± 359	1091 ± 437	580 ± 240
T_{max}*(hr)	4.0 (0.0-24.0)	7.9 (0.0-24.0)	10.0 (0.0-24.0)
C_{min} (ng/dL)	348 ± 164	485 ± 184	223 ± 114
T_{min}* (hr)	11.9 (0.0-24.0)	11.8 (0.0-27.4)	2.0 (0.0-25.7)
Fluc Index (ratio)	0.845 ± 0.379	0.829 ± 0.392	0.891 ± 0.319
Accum Ratio (ratio)	1.523 ± 1.024	1.897 ± 2.123	0.954 ± 0.4105

*Median (Range)

Table 8(f): Testosterone Pharmacokinetic Parameters on Days 30, 90, 180 by Final Treatment Group (Mean ± SD)

	Doses Received During Initial => Extended Treatment Phases				
	5.0 g/day T-gel	5.0 => 7.5 g/day T-gel	10.0 => 7.5 g/day T-gel	10.0 g/day T-gel	T-patch
Day 30	N = 47	N = 19	N = 19	N = 55	N = 70
C_{avg} (ng/dL)	604 ± 288	472 ± 148	946 ± 399	739 ± 230	419 ± 163
C_{max} (ng/dL)	941 ± 509	716 ± 294	1409 ± 556	1128 ± 436	576 ± 223
T_{max}* (hr)	7.9 (0.0-24.0)	8.0 (0.0-24.0)	8.0 (0.0-24.3)	7.8 (0.0-24.3)	11.3 (0.0-24.0)
C_{min} (ng/dL)	387 ± 159	296 ± 97	600 ± 339	471 ± 175	235 ± 122
T_{min}* (hr)	8.1 (0.0-24.1)	1.7 (0.0-24.1)	11.4 (0.0-24.1)	8.0 (0.0-25.8)	2.0 (0.0-24.2)
Fluc Index (ratio)	0.861 ± 0.341	0.846 ± 0.315	0.927 ± 0.409	0.884 ± 0.445	0.823 ± 0.289
Accum Ratio (ratio)	1.543 ± 0.747	1.494 ± 0.691	2.053 ± 1.393	1.864 ± 1.657	0.937 ± 0.354
Day 90	N = 45	N = 20	N = 18	N = 55	N = 64
C_{avg} (ng/dL)	596 ± 266	455 ± 164	859 ± 298	771 ± 268	417 ± 157
C_{max} (ng/dL)	931 ± 455	654 ± 359	1398 ± 733	1141 ± 498	597 ± 242
T_{max}* (hr)	3.8 (0.0-24.1)	7.7 (0.0-24.0)	7.9 (0.0-24.0)	7.9 (0.0-25.2)	8.1 (0.0-25.0)
C_{min} (ng/dL)	384 ± 147	286 ± 125	532 ± 181	492 ± 197	213 ± 105
T_{min}* (hr)	7.9 (0.0-25.3)	0.0 (0.0-24.0)	12.0 (0.0-24.1)	4.0 (0.0-24.8)	2.0 (0.0-24.0)
Fluc Index (ratio)	0.886 ± 0.391	0.771 ± 0.425	0.959 ± 0.490	0.826 ± 0.363	0.937 ± 0.442
Accum Ratio (ratio)	1.593 ± 0.813	1.737 ± 1.145	1.752 ± 0.700	1.952 ± 1.380	0.971 ± 0.453
Day 180	N = 44	N = 18	N = 19	N = 48	N = 41
C_{avg} (ng/dL)	555 ± 225	450 ± 219	744 ± 320	713 ± 209	408 ± 165
C_{max} (ng/dL)	803 ± 347	680 ± 369	1110 ± 468	1083 ± 434	578 ± 245
T_{max}* (hr)	5.8 (0.0-24.0)	2.0 (0.0-24.0)	7.8 (0.0-24.0)	7.7 (0.0-24.0)	10.6 (0.0-24.0)
C_{min} (ng/dL)	371 ± 165	302 ± 150	505 ± 233	485 ± 156	222 ± 116
T_{min}* (hr)	11.9 (0.0-24.0)	9.9 (0.0-24.0)	12.0 (0.0-24.0)	8.0 (0.0-27.4)	2.0 (0.0-25.7)
Fluc Index (ratio)	0.853 ± 0.402	0.833 ± 0.335	0.824 ± 0.298	0.818 ± 0.421	0.866 ± 0.311
Accum Ratio (ratio)	1.541 ± 0.917	NA	NA	2.061 ± 2.445	0.969 ± 0.415

*Median (range)

(5) Dose Proportionality for AndroGel®

Table 8(g) shows the increase in AUC_{0-24} on days 30, 90, and 180 from the pretreatment baseline (net AUC_{0-24}). In order to assess dose-proportionality, the bioequivalence assessment was performed on the log-transformed AUCs using "treatment" as the only factor. The AUCs were compared after subtracting away the AUC contribution from the endogenous secretion of testosterone (the AUC on day 0) and adjusting for the two-fold difference in applied doses. The AUC ratio on day 30 was 0.95 (90% C.I.: 0.75-1.19) and on day 90 was 0.92 (90% C.I.: 0.73-1.17). When the day 30 and day 90 data was combined, the AUC ratio was 0.93 (90% C.I.: 0.79-1.10).

The data shows dose proportionality for AndroGel® treatment. The geometric mean for the increase in AUC_{0-24} from day 0 to day 30 or day 90 was twice as great for the 10.0 g/day

group as for the 5.0 g/day group. A 125 ng/dL mean increase in serum testosterone C_{avg} level was produced by each 2.5 g/day of AndroGel®. In other words, the data shows that 0.1 g/day of AndroGel® produced, on the average, a 5 ng/dL increase in serum testosterone concentration. This dose proportionality aids dosing adjustment by the physician. Because AndroGel® is provided in 2.5 g packets (containing 25 mg of testosterone), each 2.5 g packet will produce, on average, a 125 ng/dL increase in the C_{avg} for serum total testosterone.

Table 8(g): Net AUC_{0-24} (nmol*h/L) on Days 30, 90, and 180 after Transdermal Testosterone Application

	T Patch	T gel 5.0 g/day	T gel 10.0 g/day
Day 30	154 ± 18	268 ± 28	446 ± 30
Day 90	157 ± 20	263 ± 29	461 ± 28
Day 180	160 ± 25	250 ± 32	401 ± 27

The increase in AUC_{0-24} from pretreatment baseline achieved by the 10.0 g/day and the 5.0 g/day groups were approximately 2.7 and 1.7 fold higher than that resulting from application of the testosterone patch.

b. Pharmacokinetics of Serum Free Testosterone Concentration

(1) Methods

Serum free testosterone was measured by RIA of the dialysate, after an overnight equilibrium dialysis, using the same RIA reagents as the testosterone assay. The LLQ of serum free testosterone, using the equilibrium dialysis method, was estimated to be 22 pmol/L. When steroid free serum was spiked with increasing doses of testosterone in the adult male range, increasing amounts of free testosterone were recovered with a coefficient of variation that ranged from 11.0-18.5%. The intra- and interassay coefficients of free testosterone were 15% and 16.8% for adult normal male values, respectively. As estimated by the UCLA-Harbor Medical

Center, free testosterone concentrations range from 3.48-17.9 ng/dL (121-620 pmol/L) in normal adult men.

(2) **Pharmacokinetic Results**

In general, as shown in Table 9, the pharmacokinetic parameters of serum free testosterone mirrored that of serum total testosterone as described above. At baseline (day 0), the mean serum free testosterone concentrations (C_{avg}) were similar in all three groups which were at the lower limit of the adult male range. The maximum serum free testosterone concentration occurred between 8 and 10 a.m., and the minimum about 8 to 16 hours later. This data is consistent with the mild diurnal variation of serum testosterone.

FIG. 6(a) shows the 24-hour pharmacokinetic profiles for the three treatment groups on day 1. After application of the testosterone patch, the serum free testosterone levels peaked at 12 hours about 4 hours earlier than those achieved by the AndroGel® groups The serum free testosterone levels then declined in the testosterone patch group whereas in the AndroGel® groups, the serum free testosterone levels continued to rise.

FIGS. 6(b) and 6(c) show the pharmacokinetic profiles of free testosterone in the AndroGel®-treated groups resembled the unique testosterone profiles on days 30 and 90. After AndroGel® application, the mean serum free testosterone levels in the three groups were within normal range. Similar to the total testosterone results, the free testosterone C_{avg} achieved by the 10.0 g/day group was 1.4 fold higher than the 5.0 g/day group and 1.7 fold higher than the testosterone patch group. Moreover, the accumulation ratio for the testosterone patch was significantly less than that of the 5.0 g/day AndroGel® group and the 10.0 g/day AndroGel® group.

FIG. 6(d) shows the free testosterone concentrations by final treatment groups on day 180. In general, the free testosterone concentrations exhibited a similar pattern as serum testosterone. The 24-hour pharmacokinetic parameters were similar to those on days 30 and 90 in those subjects who remained in the three original randomized groups. Again, in the subjects

titrated to receive 7.5 g/day of AndroGel®, the group was not homogenous. The free testosterone C_{avg} in the patients with doses adjusted upwards from 5.0 to 7.5 g/day remained 29% lower than those of subjects remaining in the 5.0 g/day group. The free testosterone C_{avg} in the patients whose doses were decreased from 10.0 to 7.5 g/day was 11% higher than those in remaining in the 10.0 g/day group.

FIGS. 6(e)-(g) show the free testosterone concentrations in the three groups of subjects throughout the 180-day treatment period. Again, the free testosterone levels followed that of testosterone. The mean free testosterone levels in all three groups were within the normal range with the 10.0 g/day group maintaining higher free testosterone levels than both the 5.0 g/day and the testosterone patch groups.

Table 9: Free Testosterone Pharmacokinetic Parameters by Final Treatment (Mean ± SD)

	Doses Received During Initial =>Extended Treatment Phases				
	5.0 g/day T-gel	5.0 => 7.5 g/day T-gel	10.0 => 7.5 g/day T-gel	10/0 g/day T gel	T-patch
Day 0	N = 53	N = 20	N = 20	N = 58	N = 76
Cavg (ng/dL)	4.52 ± 3.35	4.27 ± 3.45	4.64 ± 3.10	4.20 ± 3.33	4.82 ± 3.64
Cmax (ng/dL)	5.98 ± 4.25	6.06 ± 5.05	6.91 ± 4.66	5.84 ± 4.36	6.57 ± 4.90
Tmax* (hr)	4.0 (0.0-24.5)	2.0 (0.0-24.0)	13.5 (0.0-24.2)	2.1 (0.0-24.1)	3.8 (0.0-24.0)
Cmin (ng/dL)	3.23 ± 2.74	3.10 ± 2.62	3.14 ± 2.14	3.12 ± 2.68	3.56 ± 2.88
Tmin* (hr)	8.0 (0.0-24.2)	9.9 (0.0-16.0)	4.0 (0.0-23.3)	8.0 (0.0-24.0)	7.9 (0.0-24.0)
Fluc Index (ratio)	0.604 ± 0.342	0.674 ± 0.512	0.756 ± 0.597	0.634 ± 0.420	0.614 ± 0.362
Day 1	N = 53	N = 20	N = 19	N = 57	N = 74
Cavg (ng/dL)	7.50 ± 4.83	6.80 ± 4.82	9.94 ± 5.04	8.93 ± 6.09	9.04 ± 4.81
Cmax (ng/dL)	10.86 ± 7.45	10.10 ± 7.79	15.36 ± 7.31	13.20 ± 8.61	12.02 ± 6.14
Tmax* (hr)	16.0 (0.0-25.3)	13.9 (0.0-24.3)	15.7 (2.0-24.0)	23.5 (1.8-24.3)	12.0 (1.8-24.0)
Cmin (ng/dL)	4.30 ± 3.33	3.69 ± 3.24	3.88 ± 2.73	4.40 ± 3.94	4.67 ± 3.52
Tmin* (hr)	0.0 (0.0-24.1)	1.8 (0.0-24.0)	0.0 (0.0-24.2)	0.0 (0.0-23.9)	0.0 (0.0-24.0)
Day 30	N = 47	N = 19	N = 19	N = 55	N = 70
Cavg (ng/dL)	11.12 ± 6.22	7.81 ± 3.94	16.18 ± 8.18	13.37 ± 7.13	8.12 ± 4.15
Cmax (ng/dL)	16.93 ± 10.47	11.62 ± 6.34	25.14 ± 10.80	19.36 ± 9.75	11.48 ± 5.78
Tmax* (hr)	8.0 (0.0-27.8)	8.0 (0.0-26.3)	8.0 (0.0-24.3)	8.0 (0.0-24.3)	8.0 (0.0-24.0)
Cmin (ng/dL)	6.99 ± 3.82	4.78 ± 3.10	9.99 ± 7.19	8.25 ± 5.22	4.31 ± 3.20
Tmin* (hr)	4.0 (0.0-24.1)	3.5 (0.0-24.1)	11.4 (0.0-24.1)	7.8 (0.0-25.8)	2.0 (0.0-24.8)
Fluc Index (ratio)	0.853 ± 0.331	0.872 ± 0.510	1.051 ± 0.449	0.861 ± 0.412	0.929 ± 0.311
Accum Ratio (ratio)	1.635 ± 0.820	1.479 ± 0.925	2.065 ± 1.523	1.953 ± 1.626	0.980 ± 0.387

	Doses Received During Initial =>Extended Treatment Phases				
	5.0 g/day T-gel	5.0 => 7.5 g/day T-gel	10.0 => 7.5 g/day T-gel	10/0 g/day T gel	T-patch
Day 90	N = 45	N = 20	N = 18	N = 55	N = 64
Cavg (ng/dL)	12.12 ± 7.78	8.06 ± 3.78	17.65 ± 8.62	13.11 ± 5.97	8.50 ± 5.04
Cmax (ng/dL)	18.75 ± 12.90	10.76 ± 4.48	25.29 ± 12.42	18.61 ± 8.20	12.04 ± 6.81
Tmax* (hr)	4.0 (0.0-24.0)	9.7 (0.0-24.0)	8.0 (0.0-24.0)	8.0 (0.0-25.2)	11.6 (0.0-25.0)
Cmin (ng/dL)	7.65 ± 4.74	4.75 ± 2.86	10.56 ± 6.07	8.40 ± 4.57	4.38 ± 3.70
Tmin* (hr)	8.0 (0.0-24.0)	1.9 (0.0-24.0)	5.9 (0.0-24.1)	4.0 (0.0-24.8)	2.0 (0.0-24.1)
Fluc Index (ratio)	0.913 ± 0.492	0.815 ± 0.292	0.870 ± 0.401	0.812 ± 0.335	0.968 ± 0.402
Accum Ratio (ratio)	1.755 ± 0.983	1.916 ± 1.816	1.843 ± 0.742	2.075 ± 1.866	1.054 ± 0.498
Day 180	N = 44	N = 18	N = 19	N = 48	N = 41
Cavg (ng/dL)	11.01 ± 5.24	7.80 ± 4.63	14.14 ± 7.73	12.77 ± 5.70	7.25 ± 4.90
Cmax (ng/dL)	16.21 ± 7.32	11.36 ± 6.36	22.56 ± 12.62	18.58 ± 9.31	10.17 ± 5.90
Tmax* (hr)	7.9 (0.0-24.0)	2.0 (0.0-23.9)	7.8 (0.0-24.0)	8.0 (0.0-24.0)	11.1 (0.0-24.0)
Cmin (ng/dL)	7.18 ± 3.96	5.32 ± 4.06	9.54 ± 6.45	8.23 ± 4.01	3.90 ± 4.20
Tmin* (hr)	9.9 (0.0-24.2)	7.9 (0.0-24.0)	8.0 (0.0-23.2)	11.8 (0.0-27.4)	2.5 (0.0-25.7)
Fluc Index (ratio)	0.897 ± 0.502	0.838 ± 0.378	0.950 ± 0.501	0.815 ± 0.397	0.967 ± 0.370
Accum Ratio (ratio)	1.712 ± 1.071	NA	NA	2.134 ± 1.989	1.001 ± 0.580

*Median (Range)

c. Serum DHT Concentrations

Serum DHT was measured by RIA after potassium permanganate treatment of the sample followed by extraction. The methods and reagents of the DHT assay were provided by DSL (Webster, TX). The cross reactivities of the antiserum used in the RIA for DHT were 6.5% for 3-β-androstanediol, 1.2% for 3-α-androstanediol, 0.4% for 3-α-androstanediol glucuronide, and 0.4% for testosterone (after potassium permanganate treatment and extraction), and less than 0.01% for other steroids tested. This low cross-reactivity against testosterone was further confirmed by spiking steroid free serum with 35 nmol/L (1,000 pg/dL) of testosterone and taking the samples through the DHT assay. The results even on spiking with over 35 nmol/L of testosterone was measured as less than 0.1 nmol/L of DHT. The LLQ of serum DHT in the assay was 0.43 nmol/L. The mean accuracy (recovery) of the DHT assay determined by spiking steroid free serum with varying amounts of DHT from 0.43 nmol/L to 9 nmol/L was 101% and ranged from 83 to 114%. The intra-assay and inter-assay coefficients of variation for the DHT

assay were 7.8 and 16.6%, respectively, for the normal adult male range. The normal adult male range of DHT was 30.7-193.2 ng/dL (1.06 to 6.66 nmol/L) as determined by the UCLA-Harbor Medical Center.

As shown in Table 10, the pretreatment mean serum DHT concentrations were between 36 and 42 ng/dL, which were near the lower limit of the normal range in all three initial treatment groups. None of the patients had DHT concentrations above the upper limit of the normal range on the pretreatment day, although almost half (103 patients) had concentrations less than the lower limit.

FIG. 7 shows that after treatment, the differences between the mean DHT concentrations associated with the different treatment groups were statistically significant, with patients receiving AndroGel® having a higher mean DHT concentration than the patients using the patch and showing dose-dependence in the mean serum DHT concentrations. Specifically, after testosterone patch application mean serum DHT levels rose to about 1.3 fold above the baseline. In contrast, serum DHT increased to 3.6 and 4.8 fold above baseline after application of 5.0 g/day and 10.0 g/day of AndroGel®, respectively.

Table 10: DHT Concentrations (ng/dL) on Each of the Observation Days By Initial Treatment (Mean ± SD)

	Day 0	Day 30	Day 60	Day 90	Day 120	Day 150	Day 180
5.0 g/day T-gel	N = 73 36.0 ± 19.9	N = 69 117.6 ± 74.9	N = 70 122.4 ± 99.4	N = 67 130.1 ± 99.2	N = 65 121.8 ± 89.2	N = 63 144.7 ± 110.5	N = 65 143.7 ± 105.9
10.0 g/day T-gel	N = 78 42.0 ± 29.4	N = 78 200.4 ± 127.8	N = 74 222.0 ± 126.6	N = 75 207.7 ± 111.0	N = 68 187.3 ± 97.3	N = 67 189.1 ± 102.4	N = 71 206.1 ± 105.9
T-Patch	N = 76 37.4 ± 21.4	N = 73 50.8 ± 34.6	N = 68 49.3 ± 27.2	N = 66 43.6 ± 26.9	N = 49 53.0 ± 52.8	N = 46 54.0 ± 42.5	N = 49 52.1 ± 34.3
Across RX	0.6041	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001

The increase in DHT concentrations are likely attributed to the concentration and location of 5α-reductase in the skin. For example, the large amounts of 5α-reductase in the scrotal skin presumably causes an increase in DHT concentrations in the TESTODERM® patch. In contrast,

the ANDRODERM® and TESTODERM TTS® patches create little change in DTH levels because the surface area of the patch is small and little 5α-reductase is located in nonscrotal skin. AndroGel® presumably causes an increase in DHT levels because the gel is applied to a relatively large skin area and thus exposes testosterone to greater amounts of the enzyme.

To date, elevated DHT levels have not been reported to have any adverse clinical effects. Moreover, there is some evidence to suggest that increased DHT levels may inhibit prostate cancer.

d. DHT/T Ratio

The UCLA-Harbor Medical Center reports a DHT/T ratio of 0.052-0.328 for normal adult men. In this example, the mean ratios for all three treatments were within the normal range on day 0. As shown in FIG. 8 and Table 11, there were treatment and concentration-dependent increases observed over the 180-day period. Specifically, the AndroGel® treatment groups showed the largest increase in DHT/T ratio. However, the mean ratios for all of the treatment groups remained within the normal range on all observation days.

Table 11: DHT/T Ratio on Each of the Observation Days By Initial Treatment (Mean ± SD)

	Day 0	Day 30	Day 60	Day 90	Day 120	Day 150	Day 180
5.0 g/day T-gel	N = 73 0.198 ± 0.137	N = 68 0.230 ± 0.104	N = 70 0.256 ± 0.132	N = 67 0.248 ± 0.121	N = 65 0.266 ± 0.119	N = 62 0.290 ± 0.145	N = 64 0.273 ± 0.160
10.0 g/day T-gel	N = 78 0.206 ± 0.163	N = 77 0.266 ± 0.124	N = 74 0.313 ± 0.160	N = 74 0.300 ± 0.131	N = 68 0.308 ± 0.145	N = 67 0.325 ± 0.142	N = 71 0.291 ± 0.124
T-Patch	N = 76 0.204 ± 0.135	N = 73 0.192 ± 0.182	N = 68 0.175 ± 0.102	N = 65 0.175 ± 0.092	N = 49 0.186 ± 0.134	N = 46 0.223 ± 0.147	N = 46 0.212 ± 0.160
Across RX	0.7922	0.0001	0.0001	0.0001	0.0001	0.0001	0.0002

e. Total Androgen (DHT + T)

The UCLA-Harbor Medical Center has determined that the normal total androgen concentration is 372 to 1,350 ng/dL. As shown in FIG. 9 and Table 12, the mean pre-dose total androgen concentrations for all three treatments were below the lower limit of the normal range

on pretreatment day 0. The total androgen concentrations for both AndroGel® groups were within the normal range on all treatment observation days. In contrast, the mean concentrations for patients receiving the testosterone patch was barely within the normal range on day 60 and 120, but were below the lower normal limit on days 30, 90, 150, and 180.

Table 12: Total Androgen (DHT +T) (ng/dL) on Each of the Observation Days By Initial Treatment (Mean ± SD)

	Day 0	Day 30	Day 60	Day 90	Day 120	Day 150	Day 180
5.0 g/day T-gel	N = 73 281 ± 150	N = 68 659 ± 398	N = 70 617 ± 429	N = 67 690 ± 431	N = 65 574 ± 331	N = 62 631 ± 384	N = 64 694 ± 412
10.0 g/day T-gel	N = 78 307 ± 180	N = 77 974 ± 532	N = 74 1052 ± 806	N = 74 921 ± 420	N = 68 827 ± 361	N = 67 805 ± 383	N = 71 944 ± 432
T-Patch	N = 76 282 ± 159	N = 73 369 ± 206	N = 68 392 ± 229	N = 65 330 ± 173	N = 49 378 ± 250	N = 46 364 ± 220	N = 46 355 ± 202
Across RX	0.7395	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001

f. E_2 Concentrations

Serum E_2 levels were measured by a direct assay without extraction with reagents from ICN (Costa Mesa, CA). The intra-assay and inter-assay coefficients of variation of E_2 were 6.5 and 7.1% respectively. The UCLA-Harbor Medical Center reported an average E_2 concentration ranging from 7.1 to 46.1 pg/mL (63 to 169 pmol/L) for normal adult male range. The LLQ of the E_2 was 18 pmol/L. The cross reactivities of the E_2 antibody were 6.9% for estrone, 0.4% for equilenin, and less than 0.01% for all other steroids tested. The accuracy of the E_2 assay was assessed by spiking steroid free serum with increasing amount of E_2 (18 to 275 pmol/L). The mean recovery of E_2 compared to the amount added was 99.1% and ranged from 95 to 101%.

FIG. 10 depicts the E_2 concentrations throughout the 180-day study. The pretreatment mean E_2 concentrations for all three treatment groups were 23-24 pg/mL. During the study, the E_2 levels increased by an average 9.2% in the testosterone patch during the treatment period, 30.9% in the 5.0 g/day AndroGel® group, and 45.5% in the 10.0 g/day AndroGel® group. All of the mean concentrations fell within the normal range.

**Table 13: Estradiol Concentration (pg/mL)
on Each of the Observation Days
By Initial Treatment (Mean ± SD)**

	Day 0	Day 30	Day 60	Day 90	Day 120	Day 150	Day 180
5.0 g/day T-gel	N = 73 23.0 ± 9.2	N = 69 29.2 ± 11.0	N = 68 28.1 ± 10.0	N = 67 31.4 ± 11.9	N = 64 28.8 ± 9.9	N = 65 30.8 ± 12.5	N = 65 32.3 ± 13.8
10.0 g/day T-gel	N = 78 24.5 ± 9.5	N = 78 33.7 ± 11.5	N = 74 36.5 ± 13.5	N = 75 37.8 ± 13.3	N = 71 34.6 ± 10.4	N = 66 35.0 ± 11.1	N = 71 36.3 ± 13.9
T-Patch	N = 76 23.8 ± 8.2	N = 72 25.8 ± 9.8	N = 68 24.8 ± 8.0	N = 66 25.7 ± 9.8	N = 50 25.7 ± 9.4	N = 49 27.0 ± 9.2	N = 49 26.9 ± 9.5
Across RX	0.6259	0.0001	0.0001	0.0001	0.0001	0.0009	0.0006

E_2 is believed to be important for the maintenance of normal bone. In addition, E_2 has a positive effect on serum lipid profiles.

g. Serum SHBG Concentrations

Serum SHBG levels were measured with a fluoroimmunometric assay ("FIA") obtained from Delfia (Wallac, Gaithersberg, MD). The intra- and interassay coefficients were 5% and 12% respectively. The LLQ was 0.5 nmol/L. The UCLA-Harbor Medical Center determined that the adult normal male range for the SHBG assay is 0.8 to 46.6 nmol/L.

As shown in FIG. 11 and Table 11, the serum SHBG levels were similar and within the normal adult male range in the three treatment groups at baseline. None of the treatment groups showed major changes from these the baseline on any of the treatment visit days. After testosterone replacement serum SHBG levels showed a small decrease in all three groups. The most marked change occurred in the 10.0 g/day AndroGel® group.

**Table 14: SHBG Concentration (nmol/L)
on Each of the Observation Days
By Initial Treatment (Mean ± SD)**

	Day 0	Day 30	Day 60	Day 90	Day 120	Day 150	Day 180
5.0 g/day T-gel	N = 73 26.2 ± 14.9	N = 69 24.9 ± 14.0	N = 69 25.9 ± 14.4	N = 67 25.5 ± 14.7	N = 66 25.2 ± 14.1	N = 65 24.9 ± 12.9	N = 65 24.2 ± 13.6
10.0 g/day T-gel	N = 78 26.6 ± 17.8	N = 78 24.8 ± 14.5	N = 75 25.2 ± 15.5	N = 75 23.6 ± 14.7	N = 72 25.5 ± 16.5	N = 68 23.8 ± 12.5	N = 71 24.0 ± 14.5
T-Patch	N = 76 30.2 ± 22.6	N = 72 28.4 ± 21.3	N = 68 28.2 ± 23.8	N = 66 28.0 ± 23.6	N = 50 26.7 ± 16.0	N = 49 26.7 ± 16.4	N = 49 25.8 ± 15.1
Across RX	0.3565	0.3434	0.5933	0.3459	0.8578	0.5280	0.7668

b. Gonadotropins

Serum FSH and LH were measured by highly sensitive and specific solid-phase FIA assays with reagents provided by Delfia (Wallac, Gaithersburg, MD). The intra-assay coefficient of variations for LH and FSH fluroimmunometric assays were 4.3 and 5.2%, respectively; and the interassay variations for LH and FSH were 11.0% and 12.0%, respectively. For both LH and FSH assays, the LLQ was determined to be 0.2 IU/L. All samples obtained from the same subject were measured in the same assay. The UCLA-Harbor Medical Center reports that the adult normal male range for LH is 1.0-8.1 U/L and for FSH is 1.0-6.9U/L.

(1) FSH

Table 15(a)-(d) shows the concentrations of FSH throughout the 180-day treatment depending on the cause of hypogonadism: (1) primary, (2) secondary, (3) age-associated, or (4) unknown.

As discussed above, patients with primary hypogonadism have an intact feedback inhibition pathway, but the testes do not secrete testosterone. As a result, increasing serum testosterone levels should lead to a decrease in the serum FSH concentrations. In this example, a total of 94 patients were identified as having primary hypogonadism. For these patients, the mean FSH concentrations in the three treatment groups on day 0 were 21-26 mIU/mL, above the upper limit of the normal range. As shown in FIG. 12(a) and Table 15(a), the mean FSH concentrations decreased during treatment in all three treatment regimens. However, only the 10.0 g/day AndroGel® group reduced the mean concentrations to within the normal range during the first 90 days of treatment. Treatment with the 10.0 g/day AndroGel® group required approximately 120 days to reach steady state. The mean FSH concentration in patients applying 5.0 g/day of AndroGel® showed an initial decline that was completed by day 30 and another declining phase at day 120 and continuing until the end of treatment. Mean FSH concentrations

in the patients receiving the testosterone patch appeared to reached steady state after 30 days but were significantly higher than the normal range.

Table 15(a): FSH Concentrations (mIU/mL) on Each of the Observation Days by Initial Treatment Group for Patients Having Primary Hypogonadism (Mean ± SD)

	N	5 g/day	N	10 g/day	N	T-patch
Day 0	26	21.6 ± 21.0	33	20.9 ± 15.9	34	25.5 ± 25.5
Day 30	23	10.6 ± 15.0	34	10.6 ± 14.1	31	21.4 ± 24.6
Day 60	24	10.8 ± 16.9	32	7.2 ± 12.6	31	21.7 ± 23.4
Day 90	24	10.4 ± 19.7	31	5.7 ± 10.1	30	19.5 ± 20.0
Day 120	24	8.1 ± 15.2	28	4.6 ± 10.2	21	25.3 ± 28.4
Day 150	22	6.7 ± 15.0	29	5.3 ± 11.0	21	18.6 ± 24.0
Day 180	24	6.2 ± 11.3	28	5.3 ± 11.2	22	24.5 ± 27.4

Patients with secondary hypogonadism have a deficient testosterone negative feedback system. As shown in FIG. 12(b), of 44 patients identified as having secondary hypogonadism, the mean FSH concentrations decreased during treatment, although the decrease over time was not statistically significant for the testosterone patch. The patients in the 5.0 g/day AndroGel® group showed a decrease in the mean FSH concentration by about 35% by day 30, with no further decrease evident by day 60. Beyond day 90, the mean FSH concentration in the patients appeared to slowly return toward the pretreatment value. By day 30, all of the 10.0 g/day AndroGel® group had FSH concentrations less than the lower limit.

Table 15(b): FSH Concentrations (mIU/mL) on Each of the Observation Days by Initial Treatment Group for Patients Having Secondary Hypogonadism (Mean ± SD)

	N	5 g/day	N	10 g/day	N.	T-patch
Day 0	17	4.2 ± 6.6	12	2.1 ± 1.9	15	5.1 ± 9.0
Day 30	16	2.8 ± 5.9	12	0.2 ± 0.1	14	4.2 ± 8.0
Day 60	17	2.8 ± 6.1	12	0.2 ± 0.1	13	4.2 ± 7.4
Day 90	15	2.9 ± 5.6	12	0.2 ± 0.1	14	4.9 ± 9.0
Day 120	14	3.0 ± 6.1	12	0.1 ± 0.1	12	6.1 ± 10.7
Day 150	14	3.5 ± 7.5	12	0.2 ± 0.2	11	4.6 ± 6.5
Day 180	14	3.7 ± 8.6	12	0.1 ± 0.1	12	4.9 ± 7.4

Twenty-five patients were diagnosed with age-associated hypogonadism. As shown in FIG. 12(c), the 5.0 g/day AndroGel® group had a mean pretreatment FSH concentration above the normal range. The mean concentration for this group was within the normal range by day 30 and had decreased more than 50% on days 90 and 180. The decrease in FSH mean concentration in the 10.0 g/day AndroGel® group showed a more rapid response. The concentrations in all six patients decreased to below the lower normal limit by day 30 and remained there for the duration of the study. The six patients who received the testosterone patch exhibited no consistent pattern in the mean FSH level; however, there was an overall trend towards lower FHS levels with continued treatment.

Table 15(c): FSH Concentrations (mIU/mL) on Each of the Observation Days by Initial Treatment Group for Patients Having Age-Related Hypogonadism (Mean ± SD)

	N	5 g/day	N	10 g/day	N	T-patch
Day 0	13	8.0 ± 9.1	6	5.2 ± 1.9	6	4.7 ± 1.7
Day 30	12	4.6 ± 7.4	6	0.4 ± 0.3	6	3.7 ± 2.0
Day 60	12	3.9 ± 6.6	6	0.3 ± 0.3	4	4.3 ± 3.3
Day 90	11	3.8 ± 7.0	6	0.4 ± 0.7	4	3.5 ± 1.9
Day 120	11	4.2 ± 8.3	6	0.4 ± 0.7	4	4.2 ± 3.3
Day 150	11	4.3 ± 8.1	5	0.2 ± 0.2	4	3.4 ± 2.7
Day 180	11	4.0 ± 7.2	6	0.2 ± 0.2	4	2.7 ± 2.1

Sixty-four patients in the study suffered from unclassified hypogonadism. As shown in FIG. 12(d), the patients showed a marked and comparatively rapid FSH concentration decrease in all three groups, with the greatest decrease being in the 10.0 g/day AndroGel® group. The 10.0 g/day AndroGel® group produced nearly a 90% decrease in the mean FSH concentration by day 30 and maintained the effect to day 180. The 5.0 g/day AndroGel® group produced about a 75% drop in mean FSH concentration by day 30 and stayed at that level for the remainder of treatment. The 21 patients receiving the testosterone patch had a 50% decrease in the mean FSH

concentration by day 30, a trend that continued to day 90 when the concentration was about one-third of its pretreatment value.

Table 15(d): Concentrations (mIU/mL) for FSH on Each of the Observation Days by Initial Treatment Group for Patients Having Unknown-Related Hypogonadism (Mean ± SD)

	N	5 g/day	N	10 g/day	N	T-patch
Day 0	17	4.0 ± 1.8	26	4.1 ± 1.6	21	3.7 ± 1.4
Day 30	17	1.1 ± 1.0	26	0.5 ± 0.5	21	1.8 ± 0.8
Day 60	16	1.1 ± 1.1	26	0.3 ± 0.3	18	1.6 ± 1.0
Day 90	17	1.1 ± 1.1	25	0.4 ± 0.7	18	1.2 ± 0.9
Day 120	16	1.2 ± 1.4	26	0.4 ± 0.6	12	1.4 ± 1.0
Day 150	17	1.4 ± 1.4	23	0.3 ± 0.5	13	1.4 ± 1.2
Day 180	16	1.0 ± 0.9	24	0.4 ± 0.4	11	1.3 ± 0.9

This data shows that feedback inhibition of FSH secretion functioned to some extent in all four subpopulations. The primary hypogonadal population showed a dose-dependency in both the extent and rate of the decline in FSH levels. The sensitivity of the feedback process appeared to be reduced in the secondary and age-associated groups in that only the highest testosterone doses had a significant and prolonged impact on FSH secretion. In contrast, the feedback inhibition pathway in the patients in the unclassified group was quite responsive at even the lowest dose of exogenous testosterone.

(2) LH

The response of LH to testosterone was also examined separately for the same four subpopulations. Tables 16(a)-(d) shows the LH concentrations throughout the treatment period.

As shown in FIG. 13(a) and Table 16(a), the LH concentrations prior to treatment were about 175% of the upper limit of the normal range in primary hypogonadal patients. The mean LH concentrations decreased during treatment in all groups. However, only the AndroGel® groups decreased the mean LH concentrations enough to fall within the normal range. As with

FSH, the primary hypogonadal men receiving AndroGel® showed dose-dependence in both the rate and extent of the LH response.

Table 16(a): Concentrations for LH (mIU/mL) on Each of the Observation Days for Patients Having Primary Hypogonadism (Summary of Mean ± SD)

	N	5 g/day	N	10 g/day	N	T-patch
Day 0	26	12.2 ± 12.1	33	13.9 ± 14.9	33	13.3 ± 14.3
Day 30	23	5.6 ± 7.6	34	5.9 ± 8.1	31	10.9 ± 12.9
Day 60	24	6.8 ± 9.0	32	4.8 ± 10.0	31	10.8 ± 11.8
Day 90	24	5.9 ± 9.5	31	4.2 ± 11.0	30	10.0 ± 11.7
Day 120	24	6.4 ± 11.9	28	3.8 ± 10.4	21	11.5 ± 11.5
Day 150	22	4.4 ± 8.5	29	4.0 ± 11.3	21	7.4 ± 6.0
Day 180	24	4.8 ± 6.8	28	4.0 ± 11.9	22	11.2 ± 10.5

The secondary hypogonadal men were less sensitive to exogenous testosterone. For the 44 patients identified as having secondary hypogonadism, the pretreatment mean concentrations were all within the lower limit normal range. The mean LH concentrations decreased during treatment with all three regimens as shown in FIG. 13(b) and Table 16(b).

Table 16(b): Concentrations for LH (mIU/mL) on Each of the Observation Days for Patients Having Secondary Hypogonadism (Summary of Mean ± SD)

	N	5 g/day	N	10 g/day	N	T-patch
Day 0	17	1.8 ± 2.6	12	1.4 ± 1.8	15	1.6 ± 3.1
Day 30	16	1.1 ± 2.2	12	0.2 ± 0.2	14	0.4 ± 0.4
Day 60	17	1.4 ± 3.8	12	0.2 ± 0.2	13	0.6 ± 0.5
Day 90	15	1.2 ± 2.4	12	0.2 ± 0.2	14	0.7 ± 1.0
Day 120	14	1.6 ± 4.0	12	0.2 ± 0.2	12	0.8 ± 0.8
Day 150	14	1.6 ± 3.5	12	0.2 ± 0.2	11	1.2 ± 2.0
Day 180	14	1.5 ± 3.7	12	0.2 ± 0.2	12	1.4 ± 2.1

None of the 25 patients suffering from age-associated hypogonadism had pretreatment LH concentrations outside of the normal range as shown in FIG. 13(c) and Table 16(c). The overall time and treatment effects were significant for the AndroGel® patients but not those patients using the testosterone patch.

Table 16(c): Concentrations for LH (mIU/mL) on Each of the Observation Days for Patients Having Age-Related Hypogonadism (Summary of Mean ± SD)

	N	5 g/day	N	10 g/day	N	T-patch
Day 0	13	3.2 ± 1.1	6	2.4 ± 1.8	6	2.9 ± 0.6
Day 30	12	1.1 ± 1.0	6	0.1 ± 0.0	6	1.8 ± 1.1
Day 60	12	0.8 ± 0.7	6	0.2 ± 0.3	5	3.4 ± 2.8
Day 90	11	0.9 ± 1.2	6	0.1 ± 0.0	4	2.3 ± 1.4
Day 120	11	1.0 ± 1.4	6	0.1 ± 0.0	4	2.2 ± 1.4
Day 150	11	1.3 ± 1.5	5	0.1 ± 0.0	4	1.9 ± 1.2
Day 180	11	1.8 ± 2.1	6	0.1 ± 0.0	4	1.4 ± 1.0

Of the 64 patients suffering from an unclassified hypogonadism, none of the patients had a pretreatment LH concentration above the upper limit. Fifteen percent, however, had pretreatment concentrations below the normal limit. The unclassified patients showed comparatively rapid LH concentration decreases in all treatment groups as shown in FIG. 13(d) and Table 16(d).

Table 16(d): Concentrations for LH (mIU/mL) on Each of the Observation Days for Patients Having Unknown-Related Hypogonadism (Summary of Mean ± SD)

	N	5 g/day	N	10 g/day	N	T-patch
Day 0	17	1.8 ± 1.2	26	2.5 ± 1.5	21	2.5 ± 1.5
Day 30	17	0.3 ± 0.3	26	0.3 ± 0.3	21	1.3 ± 1.3
Day 60	17	0.4 ± 0.5	26	0.3 ± 0.3	18	1.2 ± 1.4
Day 90	17	0.5 ± 0.5	26	0.3 ± 0.4	18	1.0 ± 1.4
Day 120	17	0.4 ± 0.4	26	0.4 ± 0.5	12	1.2 ± 1.1
Day 150	17	0.8 ± 1.1	23	0.3 ± 0.4	13	1.1 ± 1.1
Day 180	15	0.3 ± 0.4	25	0.4 ± 0.4	11	1.5 ± 1.3

(3) Summary: LH and FSH

Patients receiving AndroGel® or the testosterone patch achieve "hormonal steady state" only after long-term treatment. Specifically, data involving FSH and LH show that these hormones do not achieve steady-state until many weeks after treatment. Because testosterone concentrations are negatively inhibited by FSH and LG, testosterone levels do not achieve true steady state until these other hormones also achieve steady state. However, because these

hormones regulate only endogenous testosterone (which is small to begin with in hypogonadal men) in an intact feedback mechanism (which may not be present depending on the cause of hypogonadism), the level of FSH and/or LH may have little effect on the actual testosterone levels achieved. The net result is that the patients do not achieve a "hormonal steady state" for testosterone even though the C_{avg}, C_{min}, and C_{max} for testosterone remains relative constant after a few days of treatment.

2. Bone Mineral Density ("BMD") and Similar Markers

a. BMD

BMD was assessed by dual energy X-ray absorptiometry ("DEXA") using Hologic QDR 2000 or 4500 A (Hologic, Waltham, MA) on days 0 and 180 in the lumbar spine and left hip regions. BMD of spine was calculated as the average of BMD at L1 to L4. BMD of the left hip, which included Ward's triangle, was calculated by the average of BMD from neck, trochanter, and intertrochanter regions. The scans were centrally analyzed and processed at Hologic. BMD assessments were performed at 13 out of the 16 centers (206 out of 227 subjects) because of the lack of the specific DEXA equipment at certain sites.

Table 17 and FIGS. 14(a)-14(b) show that before treatment, the BMD of the hip or the spine was not different among the three treatment groups. Significant increases in BMD occurred only in subjects in the AndroGel® 10.0 g/day group and those who switched from AndroGel® 10.0 to 7.5 g/day groups. The increases in BMD were about 1% in the hip and 2% in the spine during the six-month period. Average increases in BMD of 0.6% and 1% in the hip and spine were seen in those receiving 5.0 g/day of AndroGel® but no increase was observed in the testosterone patch group.

Table 17: BMD Concentrations on Day 0 and Day 180 by Final Treatment Group Mean (± SD)

Final Treatment Group	N	Day 0	N	Day 180	N	% Change from Day 0 to Day 180
Hip						
5.0 g/day T-gel	50	1.026 ± 0.145	41	1.022 ± 0.145	41	0.7 ± 2.1
5.0 to 7.5 g/day T-gel	16	1.007 ± 0.233	15	1.011 ± 0.226	15	1.0 ± 4.9
10.0 to 7.5 g/day T-gel	20	1.002 ± 0.135	19	1.026 ± 0.131	19	1.3 ± 2.4
10.0 g/day T-gel	53	0.991 ± 0.115	44	0.995 ± 0.130	44	1.1 ± 1.9
T-Patch	67	0.982 ± 0.166	37	0.992 ± 0.149	37	-0.2 ± 2.9
Spine						
5.0 g/day T-gel	50	1.066 ± 0.203	41	1.072 ± 0.212	41	1.0 ± 2.9
5.0 to 7.5 g/day T-gel	16	1.060 ± 0.229	15	1.077 ± 0.217	15	0.4 ±5.5
10.0 to 7.5 g/day T-gel	19	1.049 ± 0.175	19	1.067 ± 0.175	18	1.4 ± 3.2
10.0 g/day T-gel	53	1.037 ± 0.126	44	1.044 ± 0.124	44	2.2 ± 3.1
T-Patch	67	1.058 ± 0.199	36	1.064 ± 0.205	36	-0.2 ± 3.4

Note: Day 0 and Day 180 are arithmetic means, while percent change is a geometric mean.

The baseline hip and spine BMD and the change in BMD on day 180 were not significantly correlated with the average serum testosterone concentration on day 0. The changes in BMD in the hip or spine after testosterone replacement were not significantly different in subjects with hypogonadism due to primary, secondary, aging, or unclassified causes; nor were they different between naive and previously testosterone replaced subjects. The change in BMD in the spine was negatively correlated with baseline BMD values, indicating that the greatest increase in BMD occurred in subjects with the lowest initial BMD. The increase in BMD in the hip (but not in the spine) after testosterone treatment was correlated with the change in serum testosterone levels.

b. Bone Osteoblastic Activity Markers

The results described above are supported by measurements of a number of serum and urine markers of bone formation. Specifically, the mean concentrations of the serum markers (PTH, SALP, osteocalcin, type I procollagen) generally increase during treatment in all treatment groups. In addition, the ratios of two urine markers of bone formation (N-telopeptide /creatinine ratio and calcium/creatinine ratio) suggests a decrease in bone resorption.

(1) PTH (Parathyroid or Calciotropic Hormone)

Serum intact PTH was measured by two site immunoradiometric assay ("IRMA") kits from Nichol's Institute (San Juan Capistrano, CA). The LLC for the PTH assay was 12.5 ng/L. The intra- and inter-assay coefficients of variation were 6.9 and 9.6%, respectively. The UCLA-Harbor Medical Center has reported previously that the normal male adult range of PTH is 6.8 to 66.4 ng/L.

Table 18 provides the PTH concentrations over the 180-day study. FIG. 15 shows that the mean serum PTH levels were within the normal male range in all treatment groups at baseline. Statistically significant increases in serum PTH were observed in all subjects as a group at day 90 without inter-group differences. These increases in serum PTH were maintained at day 180 in all three groups.

Table 18: PTH Concentrations on Each of the Observation Days by Final Treatment Group (Mean ± SD)

	N	5 g/day T-gel	N	5 ⇨ 7.5 g/day T-gel	N	10 ⇨ 7.5 g/day T-gel	N	10 g/day T-gel	N	T-Patch
Day 0	53	16.31 ± 8.81	20	17.70 ± 9.66	20	18.02 ± 8.18	58	14.99 ± 6.11	75	15.60 ± 6.57
Day 30	49	17.91 ± 10.36	20	18.33 ± 8.02	20	17.45 ± 5.67	58	18.04 ± 8.95	72	18.33 ± 10.92
Day 90	47	21.32 ± 11.47	20	21.25 ± 10.96	19	17.10 ± 6.04	54	20.01 ± 9.77	66	21.45 ± 13.71
Day 120	46	21.19 ± 11.42	19	21.42 ± 13.20	20	19.62 ± 9..96	50	22.93 ± 12.57	46	21.07 ± 11.44
Day 180	46	22.85 ± 12.89	19	21.34 ± 11.08	19	21.02 ± 10.66	51	25.57 ± 15.59	46	25.45 ± 16.54

(2) SALP

SALP was quantitated by IRMA using reagents supplied by Hybritech (San Diego, CA). The LLQ for the SALP assay was 3.8 µg/L.; and the intra- and inter-assay precision coefficients were 2.9 and 6.5%, respectively. The UCLA-Harbor Medical Center reported that the adult normal male concentration of SALP ranges from 2.4 to 16.6 µg/L.

The pretreatment SALP concentrations were within the normal range. FIG. 16 and Table 19 show that SALP levels increased with testosterone treatment in the first 90 days and

reached statistical difference in the testosterone patch group. Thereafter serum SALP plateaued in all treatment groups.

Table 19: SALP Concentrations on Each of the Observation Days by Final Treatment Group (Mean ± SD)

	N	5 g/day T-gel	N	5 => 7.5 g/day T-gel	N	10 => 7.5 g/day T-gel	N	10 g/day T-gel	N	T-Patch
Day 0	53	9.96 ± 5.61	20	12.36 ± 4.62	20	10.48 ± 3.68	58	9.80 ± 3.57	76	10.44 ± 3.77
Day 30	49	10.20 ± 6.77	20	11.38 ± 4.09	20	11.83 ± 4.32	58	9.93 ± 3.88	71	10.86 ± 3.75
Day 90	47	11.64 ± 7.98	20	11.97 ± 5.03	20	10.97 ± 3.18	55	9.56 ± 3.12	65	11.99 ± 9.36
Day 120	46	11.71 ± 7.85	19	12.12 ± 5.25	20	11.61 ± 2.58	48	9.63 ± 3.58	45	11.63 ± 4.72
Day 180	45	11.12 ± 7.58	19	11.67 ± 5.35	19	11.22 ± 3.44	51	9.19 ± 2.42	46	11.47 ± 3.77

(3) Osteocalcin

Serum osteocalcin was measured by an IRMA from Immutopics (San Clemente, CA). The LLQ was 0.45 µg/L. The intra- and inter- assay coefficients were 5.6 and 4.4%, respectively. The UCLA-Harbor Medical Center reports that the normal male adult range for the osteocalcin assay ranges from 2.9 to 12.7 µg/L.

As shown in FIG. 17 and Table 20, the baseline mean serum osteocalcin levels were within the normal range in all treatment groups. During the first 90-day treatment, mean serum osteocalcin increased with testosterone replacement in all subjects as a group without significant differences between the groups. With continued treatment serum osteocalcin either plateaued or showed a decrease by day 180.

Table 20: Osteocalcin Concentrations on Each of the Observation Days by Final Treatment Group (Mean ± SD)

	N	5 g/day T-gel	N	5 => 7.5 g/day T-gel	N	10 => 7.5 g/day T-gel	N	10 g/day T-gel	N	T-Patch
Day 0	53	4.62 ± 1.55	20	5.01 ± 2.03	20	4.30 ± 1.28	58	4.58 ± 1.92	76	4.53 ± 1.54
Day 30	49	4.63 ± 1.65	20	5.35 ± 2.06	20	4.48 ± 1.72	58	4.91 ± 2.08	72	5.17 ± 1.61
Day 90	47	4.91 ± 2.15	20	5.29 ± 1.87	19	4.76 ± 1.50	55	4.83 ± 2.13	66	5.18 ± 1.53
Day 120	46	4.95 ± 1.97	18	4.97 ± 1.60	20	4.71 ± 1.39	49	4.61 ± 2.01	47	4.98 ± 1.87
Day 180	45	4.79 ± 1.82	19	4.89 ± 1.54	19	4.47 ± 1.49	51	3.76 ± 1.60	46	5.15 ± 2.18

(4) Type I Procollagen

Serum type I procollagen was measured using a RIA kit from Incstar Corp (Stillwater, MN). The LLQ of the procollagen assay was 5 µg/L, and the intra- and inter-assay precisions

were 6.6 and 3.6%, respectively. The UCLA-Harbor Medical Center reports that the normal adult male concentration of type I procollagen ranges from 56 to 310 µg/L.

FIG. 18 and Table 21 show shat serum procollagen generally followed the same pattern as serum osteocalcin. At baseline the mean levels were similar and within the normal range in all treatment groups. With transdermal treatment, serum procollagen increased significantly in all subjects as a group without treatment group differences. The increase in procollagen was highest on day 30 and then plateaued until day 120. By day 180, the serum procollagen levels returned to baseline levels.

Table 21: Procollagen Concentrations on Each of the Observation Days by Final Treatment Group (Mean ± SD)

	N	5 g/day T-gel	N	5 => 7.5 g/day T-gel	N	10 => 7.5 g/day T-gel	N	10 g/day T-gel	N	T-Patch
Day 0	53	115.94 ± 43.68	20	109.27 ± 32.70	20	120.93 ± 28.16	58	125.33 ± 57.57	76	122.08 ± 51.74
Day 30	49	141.09 ± 64.02	20	141.41 ± 77.35	20	147.25 ± 49.85	58	149.37 ± 60.61	71	139.26 ± 59.12
Day 90	47	137.68 ± 68.51	20	129.02 ± 60.20	29	144.60 ± 58.20	55	135.59 ± 51.54	66	130.87 ± 49.91
Day 120	46	140.07 ± 81.48	19	133.61 ± 54.09	20	139.00 ± 64.96	50	128.48 ± 45.56	46	130.39 ± 42.22
Day 180	45	119.78 ± 49.02	19	108.78 ± 35.29	19	123.51 ± 39.30	51	108.52 ± 38.98	45	120.74 ± 56.10

c. Urine Bone Turnover Markers: N-telopeptide/Cr and Ca/Cr Ratios

Urine calcium and creatinine were estimated using standard clinical chemistry procedures by an autoanalyzer operated by the UCLA-Harbor Pathology Laboratory. The procedures were performed using the COBAS MIRA automated chemistry analyzer system manufactured by Roche Diagnostics Systems. The sensitivity of the assay for creatinine was 8.9 mg/dL and the LLQ was 8.9 mg/dL. According to the UCLA-Harbor Medical Center, creatinine levels in normal adult men range from 2.1 mM to 45.1 mM. The sensitivity of the assay for calcium was 0.7 mg/dL and the LLQ was 0.7 mg/dL. The normal range for urine calcium is 0.21 mM to 7.91 mM.

N-telopeptides were measured by an enzyme-linked immunosorbant assay ("ELISA") from Ostex (Seattle, WA). The LLQ for the N-telopeptide assay was 5 nM bone collagen

equivalent ("BCE"). The intra- and inter-assay had a precision of 4.6 and 8.9%, respectively. The normal range for the N-telopeptide assay was 48-2529 nM BCE. Samples containing low or high serum/urine bone marker levels were reassayed after adjusting sample volume or dilution to ensure all samples would be assayed within acceptable precision and accuracy.

The normal adult male range for the N-telopeptide/Cr ratio is 13 to 119 nM BCE/nM Cr. As shown in FIG. 19 and Table 22, urinary N-telopeptide/Cr ratios were similar in all three treatment groups at baseline but decreased significantly in the AndroGel® 10.0 g/day group but not in the AndroGel® 5.0 g/day or testosterone patch group during the first 90 days of treatment. The decrease was maintained such that urinary N-telopeptide/Cr ratio remained lower than baseline in AndroGel® 10.0 g/day and in those subjects adjusted to 7.5 g/day from 10.0 g/day group at day 180. This ratio also decreased in the testosterone patch treatment group by day 180.

Table 22: N-Telopeptide/Cr Ratio on Each of the Observation Days by Initial Treatment Group (Mean ± SD)

Initial Treatment Group	N	5.0 g/day T-gel	N	10.0 g/day T-gel	N	T-Patch	Across-group p-value
Day 0	71	90.3 ± 170.3	75	98.0 ± 128.2	75	78.5 ± 82.5	0.6986
Day 30	65	74.6 ± 79.3	73	58.4 ± 66.4	66	91.6 ± 183.6	0.3273
Day 90	62	70.4 ± 92.6	73	55.2 ± 49.1	63	75.0 ± 113.5	0.5348
Day 120	35	78.8 ± 88.2	36	46.6 ± 36.4	21	71.2 ± 108.8	0.2866
Day 180	64	68.2 ± 81.1	70	46.9 ± 43.1	47	49.4 ± 40.8	0.2285

The normal range for Ca/Cr ratio is 0.022 to 0.745 mM/mM. FIG. 20 shows no significant difference in baseline urinary Ca/Cr ratios in the three groups. With transdermal testosterone replacement therapy, urinary Ca/Cr ratios did not show a significant decrease in any treatment group at day 90. With continued testosterone replacement to day 180, urinary Ca/Cr showed marked variation without significant changes in any treatment groups.

Table 23: Ca/Cr Ratio on Each of the Observation Days by Initial Treatment Group (Mean ± SD)

Initial Treatment Group	N	5.0 g/day T-gel	N	10.0 g/day T-gel	N	T-Patch	Across-group p-value
Day 0	71	0.150 ± 0.113	75	0.174 ± 0.222	75	0.158 ± 0.137	0.6925
Day 30	65	0.153 ± 0.182	73	0.128 ± 0.104	66	0.152 ± 0.098	0.3384
Day 90	63	0.136 ± 0.122	73	0.113 ± 0.075	63	0.146 ± 0.099	0.2531

Initial Treatment Group	N	5.0 g/day T-gel	N	10.0 g/day T-gel	N	T-Patch	Across-group p-value
Day 120	36	0.108 ± 0.073	36	0.117 ± 0.090	21	0.220 ± 0.194	0.0518
Day 180	64	0.114 ± 0.088	70	0.144 ± 0.113	47	0.173 ± 0.108	0.0398

Interestingly, the change in Ca/Cr ratio from baseline at day 90 was inversely related to the baseline Ca/Cr ratios. Similarly, the change in urine N-telopeptide/Cr ratio was also inversely proportional to the baseline N-telopeptide/Cr ratio ($r=-0.80$, $p=0.0001$). Thus subjects with the highest bone resorption markers at baseline showed the largest decreases of these markers during transdermal testosterone replacement. The decreases in urinary bone resorption markers were most prominent in subjects who had highest baseline values, suggesting that hypogonadal subjects with the most severe metabolic bone disease responded most to testosterone replacement therapy.

d. Serum Calcium

Serum calcium showed no significant inter-group differences at baseline, nor significant changes after testosterone replacement. Serum calcium levels showed insignificant changes during testosterone replacement.

3. Libido, Sexual Performance, and Mood

Sexual function and mood were assessed by questionnaires the patients answered daily for seven consecutive days before clinic visits on day 0 and on days 30, 60, 90, 120, 150, and 180 days during gel and patch application. The subjects recorded whether they had sexual day dreams, anticipation of sex, flirting, sexual interaction (*e.g.*, sexual motivation parameters) and orgasm, erection, masturbation, ejaculation, intercourse (*e.g.*, sexual performance parameters) on each of the seven days. The value was recorded as 0 (none) or 1 (any) for analyses and the number of days the subjects noted a parameter was summed for the seven-day period. The average of the four sexual motivation parameters was taken as the sexual motivation score and that of the five sexual motivation parameters as the sexual motivation mean score (0 to 7). The

subjects also assessed their level of sexual desire, sexual enjoyment, and satisfaction of erection using a seven-point Likert-type scale (0 to 7) and the percent of full erection from 0 to 100%. The subjects rated their mood using a 0 to 7 score. The parameters assessed included positive mood responses: alert, friendly, full of energy, well/good feelings and negative mood responses: angry, irritable, sad, tired, nervous. Weekly average scores were calculated. The details of this questionnaire had been described previously and are fully incorporated by reference. *See* Wang et al., *Testosterone Replacement Therapy Improves Mood in Hypogonadal Men – A Clinical Research Center Study*, 81 J. CLINICAL ENDOCRINOLOGY & METABOLISM 3578-3583 (1996).

a. Libido

As shown in FIG. 21(a), at baseline, sexual motivation was the same in all treatment groups. After transdermal testosterone treatment, overall sexual motivation showed significant improvement. The change in the summary score from baseline, however, was not different among the three treatment groups.

Libido was assessed from responses on a linear scale of: (1) overall sexual desire, (2) enjoyment of sexual activity without a partner, and (3) enjoyment of sexual activity with a partner. As shown in FIG. 21(b) and Table 24, as a group, overall sexual desire increased after transdermal testosterone treatment without inter-group difference. Sexual enjoyment with and without a partner (FIG. 21(c) and Tables 25 and 26) also increased as a group.

Similarly the sexual performance score improved significantly in all subjects as a groups. The improvement in sexual performance from baseline values was not different between transdermal preparations.

Table 24: Overall Sexual Desire Changes From Day 0 to Day 180 by Initial Treatment Group (Mean ± SD)

Initial Treatment Group	N	Day 0	N	Day 180	N	Change From Day 0 to Day 180	Within-Group p-value
5.0 g/day T-gel	69	2.1 ± 1.6	63	3.5 ± 1.6	60	1.4 ± 1.9	0.0001
10.0 g/day T-gel	77	2.0 ± 1.4	68	3.6 ± 1.6	67	1.5 ± 1.9	0.0001
T-Patch	72	2.0 ± 1.6	47	3.1 ± 1.9	45	1.6 ± 2.1	0.0001
Across-Groups p-value		0.8955		0.2247		0.8579	

Table 25: Level of Sexual Enjoyment Without a Partner Changes From Day 0 to Day 180 by Initial Treatment Group (Mean ± SD)

Initial Treatment Group	N	Day 0	N	Day 180	N	Change From Day 0 to Day 180	Within-Group p-value
5.0 g/day T-gel	60	1.5 ± 1.9	51	1.9 ± 1.9	44	0.8 ± 1.4	0.0051
10.0 g/day T-gel	63	1.2 ± 1.4	53	2.2 ± 1.9	48	1.1 ± 1.6	0.0001
T-Patch	66	1.4 ± 1.8	44	2.2 ± 2.3	40	1.0 ± 1.9	0.0026
Across-Groups p-value		0.6506		0.7461		0.6126	

Table 26: Level of Sexual Enjoyment With a Partner Change from Day 0 to Day 180 by Initial Treatment Group (Mean ± SD)

Initial Treatment Group	N	Day 0	N	Day 180	N	Change From Day 0 to Day 180	Within-Group p-value
5.0 g/day T-gel	64	2.1 ± 2.1	55	2.6 ± 2.2	48	0.4 ± 2.2	0.0148
10.0 g/day T-gel	66	1.8 ± 1.7	58	3.0 ± 2.2	52	1.0 ± 2.3	0.0053
T-Patch	61	1.5 ± 1.7	40	2.2 ± 2.4	35	0.7 ± 2.3	0.1170
Across-Groups p-value		0.2914		0.1738		0.3911	

b. Sexual Performance

FIG. 22(a) shows that while all treatment groups had the same baseline sexual performance rating, the rating improved with transdermal testosterone treatment in all groups. In addition, as a group, the subjects' self-assessment of satisfaction of erection (FIG. 22(b) and Table 27) and percent full erection (FIG. 22(c) and Table 28) were also increased with testosterone replacement without significant differences between groups.

The improvement in sexual function was not related to the dose or the delivery method of testosterone. Nor was the improvement related to the serum testosterone levels achieved by the various testosterone preparations. The data suggest that once a threshold (serum testosterone level probably at the low normal range) is achieved, normalization of sexual function occurs. Increasing serum testosterone levels higher to the upper normal range does not further improve sexual motivation or performance.

Table 27: Satisfaction with Duration of Erection
Change from Day 0 to Day 180
by Initial Treatment Group (Mean ± SD)

Initial Treatment Group	N	Day 0	N	Day 180	N	Change From Day 0 to Day 180	Within-Group p-value
5.0 g/day T-gel	55	2.5 ± 2.1	57	4.3 ± 1.8	44	1.9 ± 2.0	0.0001
10/0 g/day T-gel	64	2.9 ± 1.9	58	4.5 ± 1.7	53	1.5 ± 2.0	0.0001
T-Patch	45	3.4 ± 2.1	34	4.5 ± 2.0	20	1.3 ± 2.1	0.0524
Across-Groups p-value		0.1117		0.7093		0.5090	

Table 28: Percentage of Full Erection
Change from Day 0 to Day 180
by Initial Treatment Group (Mean ± SD)

Initial Treatment Group	N	Day 0	N	Day 180	N	Change From Day 0 to Day 180	Within-Group p-value
5.0 g/day T-gel	53	53.1 ± 24.1	57	67.4 ± 22.5	43	18.7 ± 22.1	0.0001
10.0 g/day T-gel	62	59.6 ± 22.1	59	72.0 ± 20.2	52	10.4 ± 23.4	0.0001
T-Patch	47	56.5 ± 24.7	33	66.7 ± 26.7	19	12.7 ± 20.3	0.0064
Across-Groups p-value		0.3360		0.4360		0.1947	

c. Mood

The positive and negative mood summary responses to testosterone replacement therapy are shown in FIGS. 23(a) and 23(b). All three treatment groups had similar scores at baseline and all subjects as a group showed improvement in positive mood. Similarly, the negative mood summary scores were similar in the three groups at baseline and as a group the responses to transdermal testosterone applications showed significant decreases without showing between group differences. Specifically, positive mood parameters, such as sense of well being and

energy level, improved and negative mood parameters, such as sadness and irritability, decreased. The improvement in mood was observed at day 30 and was maintained with continued treatment. The improvement in mood parameters was not dependent on the magnitude of increase in the serum testosterone levels. Once the serum testosterone increased into the low normal range, maximal improvement in mood parameters occurred. Thus, the responsiveness in sexual function and mood in hypogonadal men in response to testosterone therapy appeared to be dependent on reaching a threshold of serum testosterone at the low normal range.

4. Muscle Strength

Muscle strength was assessed on days 0, 90, and 180. The one-repetitive maximum ("1-RM") technique was used to measure muscle mass in bench press and seated leg press exercises. The muscle groups tested included those in the hips, legs, shoulders, arms, and chest. The 1-RM technique assesses the maximal force generating capacity of the muscles used to perform the test. After a 5-10 minute walking and stretching period, the test began with a weight believe likely to represent the patient's maximum strength. The test was repeated using increments of about 2-10 pounds until the patient was unable to lift additional weight with acceptable form Muscle strength was assessed in 167 out of the 227 patients. Four out of 16 centers did not participate in the muscle strength testing because of lack of the required equipment.

The responses of muscle strength testing by the arm/chest and leg press tests are shown in FIG. 24(a) and 24(b) and Table 29. There were no statistical significant differences in arm/chest or leg muscle strength among the three groups at baseline. In general, muscle strength improved in both the arms and legs in all three treatment groups without inter-group differences at both day 90 and 180. The results showed an improvement in muscle strength at 90 and 180 days, more in the legs than the arms, which was not different across treatment groups nor on the different days

of assessment. Adjustment of the dose at day 90 did not significantly affect the muscle strength responses to transdermal testosterone preparations.

Table 29: Muscle Strength – Days 0, 90, and 180 Levels and Change (lbs.) from Day 0 to Day 90 and from Day 0 to Day 180 by Final Treatment Group

Final Treatment Group	Study Day	Seated Leg Press		Arm/Chest (Bench Press)	
		N	Mean ± SD (lbs.)	N	Mean ± SD (lbs.)
5.0 g/day T-gel	0	37	356.8 ± 170.0	37	100.5 ± 37.4
	90	30	396.4 ± 194.3	31	101.2 ± 30.7
	□ 0-90	30	25.8 ± 49.2	31	4.0 ± 10.0
	180	31	393.4 ± 196.6	31	99.7 ± 31.4
	□ 0-180	31	19.9 ± 62.4	31	1.3 ± 13.0
7.5 g/day T-gel (from 5.0 g/day)	0	16	302.8 ± 206.5	16	102.8 ± 48.9
	90	15	299.8 ± 193.9	15	109.5 ± 47.6
	□ 0-90	15	17.0 ± 88.4	15	5.0 ± 21.3
	180	14	300.6 ± 203.0	14	108.5 ± 49.3
	□ 0-180	14	-0.1 ± 110.2	14	5.6 ± 30.4
7.5 g/day T-gel (From 10.0 g/day)	0	14	363.4 ± 173.8	14	123.3 ± 54.7
	90	14	401.6 ± 176.6	14	134.6 ± 57.5
	□ 0-90	14	38.2 ± 42.9	14	11.3 ± 10.5
	180	12	409.9 ± 180.2	14	132.3 ± 61.5
	□ 0-180	12	33.9 ± 67.3	14	9.0 ± 18.7
10.0 g/day T-gel	0	45	345.9 ± 186.9	43	114.7 ± 55.1
	90	43	373.5 ± 194.8	41	119.8 ± 54.2
	□ 0-90	43	27.6 ± 45.1	41	4.6 ± 12.8
	180	36	364.4 ± 189.1	34	112.0 ± 45.5
	□ 0-180	36	32.2 ± 72.3	34	1.9 ± 14.8
T-Patch	0	55	310.4 ± 169.7	54	99.2 ± 43.1
	90	46	344.9 ± 183.9	46	106.2 ± 44.0
	□ 0-90	46	25.4 ± 37.0	46	3.2 ± 12.0
	180	36	324.8 ± 199.0	35	104.8 ± 44.8
	□ 0-180	36	15.2 ± 54.7	35	2.3 ± 15.7

5. Body Composition

Body composition was measured by DEXA with Hologic 2000 or 4500A series on days 0, 90, and 180. These assessments were done in 168 out of 227 subjects because the Hologic DEXA equipment was not available at 3 out of 16 study centers. All body composition measurements were centrally analyzed and processed by Hologic (Waltham, MA).

At baseline, there were no significant differences in total body mass ("TBM"), total body lean mass ("TLN"), percent fat ("PFT"), and total body fat mass ("TFT") in the three treatment groups. As shown in FIGS. 25(a) and Table 30, all treatment groups incurred an overall increase in TBM. The increase in TBM was mainly due to the increases in TLN. FIG. 25(b) and Table 30 show that after 90 days of testosterone replacement the increase in TLN was significantly higher in the 10.0 g/day AndroGel® group than in the other two groups. At day 180, the increases in TLN were further enhanced or maintained in all AndroGel® treated groups, as well as in the testosterone patch group.

FIGS. 25(c) and (d) show that the TFT and the PFT decreased in all transdermal AndroGel® treatment groups. At 90 days of treatment, TFT was significantly reduced by in the 5.0 g/day and 10.0 g/day AndroGel® groups, but was not changed in the testosterone patch group. This decrease was maintained at day 180. Correspondingly, at the 90 and 180, the decrease in PFT remained significantly lower in all AndroGel® treated groups but not significantly reduced in the testosterone patch group.

The increase in TLN and the decrease in TFT associated with testosterone replacement therapy showed significant correlations with the serum testosterone level attained by the testosterone patch and the different doses of AndroGel®. Testosterone gel administered at 10.0 g/day increased lean mass more than the testosterone patch and the 5.0 g/day AndroGel® groups. The changes were apparent on day 90 after treatment and were maintained or enhanced at day 180. Such changes in body composition was significant even though the subjects were withdrawn from prior testosterone therapy for six weeks. The decrease in TFT and PFT was also related to the serum testosterone achieved and were different across the treatment groups. The testosterone patch group did not show a decrease in PFT or TFT after 180 days of treatment.

Treatment with AndroGel® (50 to 10.0 g/day) for 90 days reduced PFT and TFT. This decrease was maintained in the 5.0 and 7.5 g/day groups at 180 days but were further lowered with continued treatment with the higher dose of the AndroGel®.

Table 30: Mean Change in Body Composition Parameters (DEXA) From Baseline to Day 90 and Baseline to Day 180 By Final Treatment Groups

Final Treatment Group	Mean Change from Day 0 – Day 90				
	N	TFT (g)	TLN (g)	TBM (g)	PFT
5.0 g/day T-gel	43	-782 ± 2105	1218 ± 2114	447 ± 1971	-1.0 ± 2.2
7.5 g/day (from 5.0 g/day)	12	-1342 ± 3212	1562 ± 3321	241 ± 3545	-1.0 ± 3.1
7.5 g/day (from 10.0 g/day)	16	-1183 ± 1323	3359 ± 2425	2176 ± 2213	-2.0 ± 1.5
10.0 g/day T-gel	45	-999 ± 1849	2517 ± 2042	1519 ± 2320	-1.7 ± 1.8
T-Patch	52	11 ± 1769	1205 ± 1913	1222 ± 2290	-0.4 ± 1.6

Final Treatment Group	Mean Change from Day 0 – Day 180				
	N	TFT (g)	TLN (g)	TBM (g)	PFT
5.0 g/day T-gel	38	-972 ± 3191	1670 ± 2469	725 ± 2357	-1.3 ± 3.1
7.5 g/day (from 5.0 g/day)	13	-1467 ± 3851	2761 ± 3513	1303 ± 3202	-1.5 ± 3.9
7.5 g/day (from 10.0 g/day)	16	-1333 ± 1954	3503 ± 1726	2167 ± 1997	-2.2 ± 1.7
10.0 g/day T-gel	42	-2293 ± 2509	3048 ± 2284	771 ± 3141	-2.9 ± 2.1
T-Patch	34	293 ± 2695	997 ± 2224	1294 ± 2764	-0.3 ± 2.2

6. Lipid profile and blood chemistry

The serum total, HDL, and LDL cholesterol levels at baseline were not significantly different in all treatment groups. With transdermal testosterone replacement, there were no overall treatment effects nor inter-group differences in serum concentrations of total, HDL- and LDL-cholesterol (FIG. 5(d)) and triglycerides (data not shown). There was a significant change of serum total cholesterol concentrations as a group with time (p=0.0001), the concentrations on day 30, 90, and 180 were significantly lower than day 0.

Approximately 70 to 95% of the subjects had no significant change in their serum lipid profile during testosterone replacement therapy. Total cholesterol levels which were initially

high were lowered into the normal range (of each center's laboratory) at day 180 in 17.2, 20.4, and 12.2% of subjects on testosterone patch, AndroGel® 5.0 g/day and AndroGel® 10.0 g/day, respectively. Serum HDL-cholesterol levels (initially normal) were reduced to below the normal range (of each center's laboratory) in 9.8, 4.0, 9.1, and 12.5% of subjects at day 180 in the testosterone patch, AndroGel® 5.0, 7.5, and 10.0 g/day groups, respectively. There was no clinically significant changes in renal or liver function tests in any treatment group.

7. **Skin Irritations**

Skin irritation assessments were performed at every clinic visit using the following scale: 0 = no erythema; 1 = minimal erythema; 2 = moderate erythema with sharply defined borders; 3 = intense erythema edema; and 4 = intense erythema with edema and blistering/erosion.

Tolerability of the daily application of AndroGel® at the tested dosages was much better than with the permeation-enhanced testosterone patch. Minimal skin irritation (erythema) at the application site was noted in three patients in the AndroGel® 5.0 g/day group (5.7%) and another three in the AndroGel® 10.0 g/day group (5.3%). Skin irritation varying in intensity from minimal to severe (mild erythema to intense edema with blisters) occurred in 65.8% of patients in the patch group. Because of the skin irritation with the testosterone patch, 16 subjects discontinued the study; 14 of these had moderate to severe skin reactions at the medication sites. No patients who received AndroGel® discontinued the study because of adverse skin reactions. The open system and the lower concentration of alcohol in the AndroGel® formulation markedly reduced skin irritation resulting in better tolerability and continuation rate on testosterone replacement therapy.

Moreover, based on the difference in the weight of the dispensed and returned AndroGel® bottles, the mean compliance was 93.1% and 96.0% for the 5.0 g/day and 10.0 g/day AndroGel® groups during days 1-90, respectively. Compliance remained at over 93% for the three

AndroGel® groups from days 91-180. In contrast, based on counting the patches returned by the subjects, the testosterone patch compliance was 65% during days 1-90 and 74% during days 91-180. The lower compliance in the testosterone patch group was mostly due to skin reactions from the subjects' records.

Table 31: Incidence of Skin-Associated Adverse Events: Day 1 to Day 180 in Patients Who Remained on Initial Treatment

	5.0 g/day T-gel N = 53	10.0 g/day T-gel N = 57	T-Patch N = 73
Total	16 (30.2%)	18 (31.6%)	50 (68.5%)
Application Site Reaction	3 (5.7%	3 (5.3%)	48 (65.8%)
Acne	1 (1.9%)	7 (12.3%)	3 (4.1%)
Rash	4 (7.5%)	4 (7.0%)	2 (2.7%)
Skin Disorder	2 (3.8%)	1 (1.8%)	1 (1.4%)
Skin Dry	2 (3.8)	0 (0.0%)	1 (1.4%)
Sweat	0 (0.0%)	2 (3.5%)	0 (0.0%)
Reaction Unevaluable	2 (3.6%)	1 (1.7%)	0 (0.0%)
Cyst	0 (0.0%)	0 (0.0%)	2 (2.7%)

Example 2: Gel Delivery Dosage Forms and Devices

The present invention is also directed to a method for dispensing and packaging the gel. In one embodiment, the invention comprises a hand-held pump capable of delivering about 2.5 g of testosterone gel with each actuation. In another embodiment, the gel is packaged in foil packets comprising a polyethylene liner. Each packet holds about 2.5 g of testosterone gel. The patient simply tears the packet along a perforated edge to remove the gel. However, because isopropyl myristate binds to the polyethylene liner, additional isopropyl myristate is added to the gel in order to obtain a pharmaceutically effective gel when using this delivery embodiment. Specifically, when dispensing the gel via the foil packet, about 41% more isopropyl myristate is used in the gel composition (*i.e.*, about 0.705 g instead of about 0.5 g in Table 5), to compensate for this phenomenon.

The composition can also be dispensed from a rigid multi-dose container (e.g., with a hand pump) having a larger foil packet of the composition inside the container. Such larger packets also comprise a polyethylene liner as above.

Both embodiments permit a patient to deliver accurate but incremental amounts of gel (e.g., either 2.5 g, 5.0 g, 7.5 g, etc.) to the body. These delivery mechanisms thus permit the gel to be administered in unit dose form depending on the particular needs and characteristics of the patient.

Although the invention has been described with respect to specific embodiments and examples, it should be appreciated that other embodiments utilizing the concept of the present invention are possible without departing from the scope of the invention. Th present invention is defined by the claimed elements, and any and all modifications, variations, or equivalents that fall within the true spirit and scope of the underlying principles.

CLAIMS

1. A pharmaceutical composition, consisting essentially of:
   a) about 0.5% to about 10% testosterone;
   b) about 30% to about 98% alcohol selected from the group consisting of ethanol and isopropanol;
   c) about 0.1% to about 5% isopropyl myristate;
   d) about 1% to about 5% 0.1 N sodium hydroxide; and
   e) about 0.1% to about 5% of a gelling agent,
   wherein the percentages of components are weight to weight of the composition.

2. The composition as recited in claim 1, wherein the testosterone is present in a concentration selected from the group consisting of about 0.5%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, and 10% weight of the composition.

3. The composition as recited in claim 1, wherein the composition is contained in a packet selected from the group consisting of a unit dose packet or multiple dose packet.

4. The composition as recited in claim 1, wherein the isopropyl myristate is present in a concentration selected from the group consisting of about 0.5%, 1%, 2%, 3%, 4%, and 5% weight to weight of the composition.

5. The composition as recited in claim 1, wherein the isopropyl myristate is present in a concentration of about 0.5% weight to weight of the composition.

6. The composition as recited in claim 1, wherein the gelling agent is selected from the group consisting of polyacrylic acid and carboxymethylcellulose present in a concentration of about 0.1% to about 5% weight to weight of the composition.

7. The composition as recited in claim 1, wherein the composition is the form of a gel.

8. The composition as recited in claim 1, wherein the gelling agent is polyacrylic acid present in a concentration of about 1% weight to weight of the composition.

9. A hydroalcoholic gel formulation, consisting essentially of:
   a) about 1% to about 2% testosterone;
   b) about 50% to about 75% ethanol;
   c) about 0.5% to about 2% isopropyl myristate;
   d) about 1% to about 3% 0.1 N sodium hydroxide;
   e) about 0.5% to about 2% polyacrylic acid; and
   f) water in an amount sufficient to make the formulation 100%;

   wherein the percentages of components are weight to weight of the formulation.

10. A unit dose packet comprising inner and outer surfaces, and a pharmaceutical composition inside the packet, the composition consisting essentially of:
   a) about 0.5% to about 5% testosterone;
   b) about 30% to about 98% ethanol;
   c) about 0.1% to about 5% isopropyl myristate;
   d) about 1% to about 5% 0.1 N sodium hydroxide; and
   e) about 0.1% to about 5% of a gelling agent;

   wherein the percentages of components are weight to weight of the composition.

11. The packet as recited in claim 10, wherein the composition weighs about 1.0 gram to about 10.0 grams.

12. The packet as recited in claim 10, wherein the composition weighs about 2.5 grams to about 5.0 grams.

13. The packet as recited in claim 10, wherein the composition is in a form of a gel.

14. The packet as recited in claim 10, wherein the testosterone is present in a concentration selected from the group consisting of about 0.5%, 1%, 2%, 3%, 4%, and 5% weight to weight of the composition.

15. The packet recited claim 10, wherein the isopropyl myristate is present in a concentration of about 0.5% weight to weight of the composition.

16. The packet as recited in claim 10, wherein the gelling agent is selected from the group consisting of polyacrylic acid and carboxymethylcellulose.

17. The packet as recited in claim 10, wherein the gelling agent is about 1% polyacrylic acid weight to weight of the composition.

18. Use of a pharmaceutical composition consisting essentially of:
(i) about 0.5% to about 5% testosterone;
(ii) about 0.1% to about 5% of a gelling agent;
(iii) about 0.1% to about 5% isopropyl myristate;
(iv) about 1% to about 5% 0.1 N sodium hydroxide; and
(v) about 30% to about 98% alcohol selected form the group consisting of ethanol and isopropanol;
wherein the percentages are weight to weight of the composition;
in the manufacture of a medicament for treating hypogonadism in male subjects.

19. The use as recited in claim 18, wherein the testosterone is present in a concentration of about 1% weight to weight of the composition.

20. The use as recited in claim 18, wherein the testosterone is present in a concentration of about 2% weight to weight of the composition.

21. The use as recited in claim 18, wherein the isopropyl myristate is present in a concentration of about 0.5% weight to weight of the composition.

22. The use as recited in claim 18, wherein the alcohol is ethanol present in a concentration of about 72.5% weight to weight of the composition.

23. The use as recited in claim 18, wherein the gelling agent is selected from the group consisting of polyacrylic acid and carboxymethylcellulose present in a concentration of about 0.1% to about 5% weight to weight of the composition.

24. The use as recited in claim 18, wherein the composition is the form of a gel.

25. The use as recited in claim 18, wherein the gelling agent is polyacrylic acid present in a concentration of about 1% weight to weight of the composition.

26. The use as recited in claim 18, wherein the medicament is for increasing the average DHT concentration.

27. The use as recited in claim 18, wherein the medicament is for increasing the bone mineral density, especially in the spine and/or hip.

28. The use as recited in claim 18, wherein the medicament is for increasing libido, or improving sexual performance, especially by increasing the percentage of full erection.

29. The use as recited in claim 18, wherein the medicament is for improving mood.

30. The use as recited in claim 18, wherein the medicament is for increasing muscle strength, especially in the legs, or improving body composition, especially by decreasing the fat percentage.

31. Use of a pharmaceutical composition consisting essentially of:
(i) about 0.5% to about 5% testosterone;
(ii) about 0.1% to about 5% isopropyl myristate;
(iii)about 30% to about 98% of an alcohol selected from the group consisting of ethanol and isopropanol; and
(iv)about 0.1% to about 5% of a gelling agent;
wherein the percentages are weight to weight of the composition;
in the manufacture of a medicament for treating hypogonadism in male subjects.

32. The use as recited in claim 31, wherein the testosterone is present in a concentration of about 1% weight to weight of the composition.

33. The use as recited in claim 31, wherein the testosterone is present in a concentration of about 2% weight to weight of the composition.

34. The use as recited in claim 31, wherein the isopropyl myristate is present in a concentration of about 0.5% weight to weight of the composition.

35. The use as recited in claim 31, wherein the alcohol is present in a concentration of about 72.5% weight to weight of the composition.

36. The use as recited in claim 31, wherein the gelling agent is selected from the group consisting of polyacrylic acid and carboxymethylcellulose.

37. The method as recited in claim 31, wherein the composition is in the form of a gel.

38. The method as recited in claim 36, wherein the gelling agent is polyacrylic acid present in a concentration of about 1% weight to weight of the composition.

39. The use as recited in claim 31, wherein the medicament is for increasing the average DHT concentration.

40. The use as recited in claim 31, wherein the medicament is for increasing the bone mineral density, especially in the spine and/or hip.

41. The use as recited in claim 31, wherein the medicament is for increasing libido, or improving sexual performance, especially by increasing the percentage of full erection.

42. The use as recited in claim 31, wherein the medicament is for improving mood.

43. The use as recited in claim 31, wherein the medicament is for increasing muscle strength, especially in the legs, or improving body composition, especially by decreasing the fat percentage.