Long-Term Pharmacokinetics and Clinical Efficacy of Andromen® Forte 5% Cream for Androgen Replacement Therapy in Hypogonadal Men

Final Study Report

Principal Investigator: Professor DJ Handelsman

Centre Investigators:

Department of Andrology, Concord Hospital
Sydney NSW 2139
S Kelleher, LP Ly, M Jimenez, DJ Handelsman

Prince Henry's Institute of Medical Research,
Monash Medical Centre, Clayton VIC 3168
A Clare, R I Mc Lachlan

Department of Medicine (Endocrinology), St George Hospital
Belgrave Street, Kogarah NSW 2217
A O'Sullivan

Study Sponsor:

Lawley Pharmaceuticals
672 Beaufort Street, Mt Lawley, WA, 6050

Study Administration:

ANZAC Research Institute, Sydney NSW 2139
Phone (02) 9767 0100, FAX (020) 9767 9101
Email: djh@anzac.edu.au

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TABLE OF CONTENTS

Tables

Figures

Summary

Testosterone replacement therapy for men with androgen deficiency results in improved somatic and psychosexual function. The available testosterone preparations in Australia remain limited and a topical cream would be therapeutically useful. We have undertaken a 3 month, open-label, dose-titrated efficacy study of a new testosterone cream (Andromen Forte 5%). The study aimed to determine the pharmacokinetics of this cream, its stability over time, the optimal daily dosage and maintenance of adequate blood testosterone concentrations and clinical efficacy over 3 months. Thirty men with classical androgen deficiency due to hypothalamo-pituitary or testicular disorders (n=15 per group) were randomised to self-administer either 50 or 100mg Andromen Forte 5% cream daily. Based on the week 4 trough blood testosterone concentrations, men underwent individual dose titration. Before and after 4 and 12 weeks of daily use of the testosterone cream, the participants underwent pharmacokinetic study with detailed blood sampling for 24 hrs. Application of the testosterone cream increased blood total testosterone concentrations into the reference range after the 1st dose. Increased blood testosterone concentrations were maintained throughout the study with a dose-response between daily dose and blood testosterone concentrations. Most men (23/30) required up-titration and only 1 man required down-titration. The final titrated dose was <100 mg per day in 5 men, 100 mg per day in 15 men and 150 mg per day in 10 men. There were no serious adverse effects and only one report of a mild skin rash at the application site which did not prevent the participant from completing the study. The clinical efficacy of the cream was evaluated by a variety of clinical and biochemical variables to determine whether the adequate androgen replacement was maintained. Compared with pre-treatment baseline, there were no significant changes in pulse, blood pressure, body composition (lean or fat mass) or muscle strength (hand-grip dynamometry) according to initial or final daily dosage. Blood LH and FSH concentrations were not suppressed in men (n=20) with hypergonadotrophic hypogonadism. Nor were there any significant changes in blood SHBG, PSA, lipids or other routine haematological or biochemical (renal & liver function tests) variables according to daily dosage of cream. Quality of life improvement was shown for vitality (SF-36), reduced tiredness (Lead Symptom Scale) as well as emotional (cheerful, friendly, nervous, irritable) and physical (energy, lethargy, fatigue, tired, active, vigorous) dimensions and increased sexual thoughts and intercourse (Mood, Energy and Sexual Function Scale). Most men (22/30) expressed a wish to continue using the cream after the end of the study. We conclude that Andromen Forte 5% cream increases in a dose-dependent manner and maintains for at least 3 months blood testosterone concentration into the therapeutic range for men with classical androgen deficiency. The optimal dosage following individual titration range from 50 mg to 150 mg per day with the most frequent being 100 mg per day. However some men did not achieve adequate testosterone delivery with the highest dose. The cream was safe and easy to use. Clinical and biochemical efficacy was generally adequate to provide stable testosterone delivery to normalise blood testosterone concentrations for at least 3 months in most men with classical androgen deficiency.

1. Study Design

1.1 Title:

Long-Term Pharmacokinetics and Clinical Efficacy of Andromen Forte 5% Cream for Androgen Replacement Therapy in Hypogonadal Men

1.2 Objective:

Using Andromen Forte (5% testosterone) topical cream over a 3 month study, to determine the

- pharmacokinetics of transdermal testosterone delivery and its stability over time,
- optimal daily dose of cream,
- maintenance of adequate blood testosterone concentrations,
- clinical efficacy of once daily topical cream application for androgen replacement therapy in men with classical androgen deficiency for 3 months.

1.3 Product:

Preparation: Andromen® Forte is manufactured by Lawley Pharmaceuticals. It is a white vanishing cream intended for topical administration. It contains 5% testosterone with dl-α-tocopherol acetate (vitamin E), almond and macadamia oils.

Chemical name of active compound: 19β-hydroxyandrost-4-en-3one.

Molecular weight active compound: 288.4

Molecular formula of active compound: $C_{19}H_{28}0_2$

Intended route of administration: Transdermal (topical application of cream)

Formulation: Andromen® Forte was supplied with a dose applicator calibrated in centimetre graduations. Patients were directed to measure the appropriate dosage using the graduated applicator and then apply the cream to clean, dry skin. Once applied, the cream was massaged into the skin until absorption was complete (typically ~1 minute). Presentation of the drug is in tubes containing 50mg/g testosterone in a 50g tube. Andromen Forte 5% cream contains 50 mg testosterone BP per gram of cream and is packaged so that a 2cm measurement of cream constitutes 1 gm (25 mg testosterone per 1 cm cream).

1.4 Regulatory status:

Andromen Forte (5%) cream is not registered in any country for any indication.

The active ingredient, testosterone, has been marketed in numerous formulations for decades in Australia and most countries for androgen replacement therapy.

The delivery vehicle is a generic base cream whose components are generally regarded as safe ingredients for topical administration to the skin.

1.5 Protocol features

Design:

Prospective, open-label study of clinical efficacy of daily topical application of testosterone dermal cream for androgen replacement therapy with detailed 24 hr pharmacokinetic evaluations performed before and after 4 and 12 weeks of daily topical cream usage.

Participants:

In order to evaluate the utility of the cream for standard androgen replacement therapy, only men with genuine androgen deficiency due to hypothalamic-pituitary or testicular disorders were recruited. Men with age-related testosterone deficiency (also known variously as "andropause", "male menopause", or late-onset hypogonadism) were not eligible.

Run-off from prior androgen replacement therapy:

All participants had well established androgen deficiency and were requiring regular testosterone treatment. All had been previously treated with various forms of testosterone. They were eligible to enter the study once their last standard treatment cycle was completed and androgen deficiency symptoms had returned. Run off from previous treatment was defined as at least 5 months after last testosterone implant, 5 weeks after last testosterone ester injection or 2 weeks after last dose of oral testosterone undecanoate or transdermal testosterone patch.

Dose titration:

Dosage titration (up and down) was allowed based on the trough blood total testosterone concentration achieved after 6 weeks usage for the following 6 weeks.

Titration procedure:

A study target population of 30 androgen deficient men were to be randomized into treatment with either 50 mg or 100 mg Andromen Forte 5% cream applied once daily to abdominal skin.

After 4 weeks of daily use, a dose titration was performed based on a blood total testosterone concentration taken 24 hours after the last dose (trough level).
If trough blood testosterone concentration

- was below the lower limit of the young eugonadal male reference range (<11 nmol/L), the dose for the next 6 weeks was increased from 50 mg to 75 mg or from 100 mg to 150 mg daily
- exceeded the upper limit of the young eugonadal male reference range (>35 nmol/L), the dose was reduced from 50 mg to 25 mg or from 100 mg to 75 mg daily for the second 6 week period
- was within the young eugonadal male reference range (11-35 nmol/L), the dose was not changed

Any change in dose had to be completed by 6^{th} week of the study.

Pharmacokinetic sampling:

Detailed pharmacokinetic sampling was obtained before and after 4 and 12 weeks of daily topical application of Andromen Forte 5% cream.

The detailed pharmacokinetic blood sampling was commenced as close as possible to 24 hours after the last cream dose.

Blood samples (10 mL) were obtained before and at 1, 2, 3, 4, 6, 8, 10, 12, 16 and 24 hours after a single application of the cream to the abdominal skin in the clinic. Plasma was stored for hormone measurement.

Based on the study design, detailed 24 hr pharmacokinetics were available for the 1st dose and the same dose after 4 week for all 30 men, with 15 using each of the 50 mg and 100 mg doses. The 3rd pharmacokinetic sampling (at 12 weeks) evaluated a wider range of doses that included 50mg, 75 mg, 100 mg and 150mg due to dose titration.

Study end-points:

The primary end-points were

(a) pharmacokinetic parameters (AUC, C_{avg}, T_{max}, C_{max}, C_{min}) of blood total testosterone during the 24 hr sampling periods before and after 4 and 12 weeks of using cream at the original randomized dose (weeks 0 & 4) and subsequently at the individually optimized dose (week 12)
(b) optimal dose of testosterone cream based on individual titration according to blood testosterone concentration at week 4 on treatment
(c) maintenance of trough blood total testosterone concentrations at weeks 4 and 12
(d) maintenance of androgen effects (symptoms scores, body composition, muscle strength) at weeks 4 and 12

Secondary end-points included blood LH and FSH concentrations; urinary testosterone, DHT and epitestosterone output; and safety (skin irritation, hematological and biochemical safety parameters) variables.

Sample size:

The sample size was 30 androgen deficient men studied for 3 months. The sample size was based on previous studies of transdermal testosterone and the feasibility of recruitment.

Participants not completing the full study were replaced. Two participants from the Melbourne centre withdrew and were replaced following the first overnight pharmacokinetic sampling visits when inadequate venous access prevented detailed blood sampling.

1.6 Ethical approval

Risks-Benefit Evaluation and Ethical Considerations:

The study aimed to provide participants with the direct benefit of continuation of androgen replacement therapy using a new non-invasive treatment, a transdermal cream containing testosterone.

The study was approved on by the Central Sydney Area Health Service Human Research Ethics Committee (Concord Hospital; approval # CH/62/6/2002-005, 12 March 2002), Southern Health Human Research Ethics Committee B (Monash Medical Centre; approval #02060B, 18 June 2002) and St George Hospital (University of NSW Human Research Ethics Committee #HREC 03072 SEHSS 02/1090, 23 April 2003).

Prior to entering the study, all patients were provided with written information and verbal explanation, following which they signed the study consent form. All study data was maintained in locked filing cabinets with access restricted to the study investigators on a needs basis.

Patients were paid $300 for their participation in this study, as travel reimbursement and compensation for the three weekend sampling visits they were required to attend. Additionally, in return for their full participation in this open-label study the sponsor agreed to provide ongoing supply of the cream at the patient's request without charge until it is commercially available in Australia. Patients were not offered any other inducement or payments to participate.

No serious adverse effects were expected from the study. The men were already using testosterone replacement therapy so no new risks from testosterone were considered likely.

One potential risk of participation was skin irritation or reaction at the administration site was to be specifically monitored.

Minor adverse effects expected included localized bruising and/or infection at the site of cannula insertion for pharmacokinetic study. This risk was minimized by having experienced blood collectors and using sterile, disposable equipment.

1.7 Subject selection criteria

Inclusion criteria:

- Established androgen deficiency
- Requiring androgen replacement therapy
- Able to understand and willing to comply with the study design
- Sufficient time has elapsed since last testosterone treatment
- On stable medication unlikely to change during course of study

Exclusion criteria:

- Contra-indications to testosterone (notably history of prostate cancer)
- Generalized skin disease which would interfere with topical dermal administration
- Significant systemic illness such as severe, unstable or end-stage chronic renal, hepatic, respiratory or cardiac failure
- History of serious mental illness (requiring ongoing psychotropic medications other than benzodiazepines), including drug or alcohol abuse, making involvement in the study unwise in the investigator's opinion
- Known allergy to components of Andromen Forte 5% cream (testosterone or excipients)
- Concurrent usage of other androgenic compounds

Discontinuation criteria:

- Allergic or severe adverse reaction to cream
- Development of contraindications to testosterone

Participants could discontinue at any time, and for any reason, without affecting ongoing their medical care.

1.8 Procedures

Participants:

Participants were recruited from men under 70 years of age with classical androgen deficiency due to testicular or hypothalamo-pituitary disorders attending the study centre for routine androgen replacement therapy. Men with only age-related low blood total testosterone concentrations (also known as "andropause", "male menopause", "late-onset hypogonadism") were not included.

Eligible men who provided written consent were transferred from their regular androgen replacement therapy to daily administration of Andromen Forte 5% cream. After screening and entry criteria were satisfied, participants were provided with Andromen Forte 5% cream and instructed how to apply the cream daily to truncal skin after the daily shower or bath. They were instructed to avoid showers, baths or swimming for at least 4 hours after daily cream application. Participants were encouraged to rub in the cream for at least one minute. They were advised to avoid major changes in their soap or daily hygiene routines and to avoid using cosmetics on the skin region to be used for Andromen cream application.

Study centres:

There were 3 Australian study centres involved in the trial.

- Department of Andrology, Concord Hospital (21 participants)
- Prince Henry's Institute of medical Research, Monash Medical Centre (6 participants)
- Department of Medicine (Endocrinology), St George Hospital, (3 participants)

Blood and urine sampling:

During the study, blood samples were obtained with special care to avoid contamination with testosterone carried over from the cream onto the venesection equipment or person taking the blood samples. These precautions included asking patients not to apply the cream to their arms below the shoulder during the week prior to any visit. On each occasion when a blood sample was obtained, the patient was asked to confirm he had not applied the cream to the arm to be used for venesection. No one handling the cream, apart from when in sealed unopened tubes, was allowed to take blood samples for the study.

During the study, blood samples (10 mL) were obtained for measurements of blood testosterone and other hormones before and after 4 and 12 weeks of daily use of the cream.

A hematological and biochemical profile, blood PSA and fasting lipids were obtained prior to the start and at the end of the study.

Blood samples were centrifuged and the plasma stored frozen until they were assayed together. Timed urine collections were obtained, the volume measured and 50 mL aliquots stored frozen for subsequent measurement of testosterone, creatinine and other analytes.

Physical Assessment:

The physical assessment included a visual assessment of the cream application site, as well as height, weight pulse and blood pressure measurement.

Muscle strength was measured by using a Jamar handgrip dynamometer (Sammons Preston Inc). Participants performed three grip/release sequences using the dynamometer and the mean of these results was recorded.

Body composition was evaluated by bio-impedance analysis using the SEAC Model Bim 3 bioelectric impedance monitor, recording both the direct bioelectric readings as well as the body composition estimates calculated by the Lukaski algorithm.

All physical assessments were performed at baseline, and at study weeks 4 and 12.

Application site monitoring:

Sites of application of the cream were assessed for possible skin irritation before and throughout the study. Application sites were checked at each blood sampling time point during the overnight sampling visits.

Questionnaires:

Participants completed

- Mood, Energy and Sexual Function (MESF) scale – an in-house scale comprising key components of other validated scales
- Leading Symptom Scale (LSS) an in-house developed self-report linear analog scale identifying (in order of significance) the 3 most obvious symptoms of androgen deficiency and the perceived degree of nuisance that these symptoms posed
- International Prostate Symptom Score (IPSS) for lower urinary tract symptoms
- SF-36 for quality of life.

1.9 Assays

Testosterone assays from detailed pharmacokinetic sampling were performed in one laboratory using an immunofluorimetric (Delfia) assay within a single assay run. Other hormone assays for screening and dose titration were performed by the immunoassays used at the centres. Urinary testosterone, dihydrotestosterone and epitestosterone were measured by urinary gas chromatography–mass spectrometry methods.

Hematology and biochemistry tests were performed by standard autoanalyzer techniques. HDL cholesterol was estimated from the Friedwald equation using cholesterol (total, LDL) and triglycerides measured by standard enzymatic methods. Urinary bone markers were measured by immunoassay.

1.10 Data Analysis

Data were analysed according to the initial doses (50 mg, 100 mg daily) assigned by randomisation for variables at baseline and after 4 weeks of treatment. In addition data were also analysed according to the final doses, which were assigned after individual dose titration based on the trough week 4 blood testosterone concentration. Data were analysed by paired t-test or analysis of variance for repeated measures for continuous variables using SPSS software, which provided exact P values those <0.05 being considered statistically significant. Categorical variables were analysed by exact methods using StatXact software.

Pharmacokinetics of the transdermal testosterone delivery was assessed using the area under the curve (AUC) from 0-24 hr (AUC_{0-24}) generated by the blood samples taken at baseline, weeks 4 and 12. The average testosterone concentration over the 24 hours after that application of the cream (C_{avg}) was calculated as the AUC_{0-24} divided by 24. Additional pharmacokinetic parameters included C_{min}, C_{max} and T_{max} were calculated by standard methods.

2. Results

2.1 Participants

The descriptive data for the 30 men who completed the study is in table 1. Two men withdrew after the first 24-hour sampling period due to inadequate venous access to complete venous blood sampling.

Of the 30 men completing the study, 20 had primary (hypergonadotrophic) hypogonadism and 10 had secondary (hypogonadotrophic) hypogonadism. The causes of primary hypogonadism were cryptorchidism/bilateral orchidectomy (n=7), primary testicular failure (n=7), Klinefelter's syndrome and variants (n=6). The causes of secondary hypogonadism were idiopathic hypogonadotrophic hypogonadism including Kallmann's syndrome (n=5) and pituitary tumour or disease (n=5).

2.2 Doses: Randomisation and Titration

There were 15 men randomised to each of the baseline dosages (50mg/day and 100mg/day). The two groups based on randomisation were balanced apart from a chance difference in age (table 3a & 3b).

After 4 weeks of treatment at the initial randomized dose of 50 mg or 100 mg daily, men underwent individual dose optimisation by titration based on the week 4 trough (obtained 24 hr after the last dose) blood total testosterone concentration.

Doses were increased for 23 men comprising 14 men on the initial 50 mg per day dose and 9 men on the initial 100 mg per day dose, a difference between doses of marginal significance (p=0.08). Of the 14 men having the lower initial dose, 3 were switched to 75 mg, 10 to 100 mg and 1 to 150 mg per day. All 9 men up-titrated from the higher initial dose were switched to 150 mg per day.

Doses were unchanged for 6 men. One remaining on 50mg per day while 5 remained on 100mg per day.

Dose was reduced from 100 mg to 75 mg per day in one man.

The titration was undertaken by the participant's own doctor according to their patients needs. In some cases when the 50 mg dose was considered clinical suboptimal and the blood testosterone concentration required up-titration, the higher doses exceeded the protocol stipulated 75 mg.

The 3 final dose groups (<100 mg (n=5), 100 mg (n=15), 150 mg (n=10)) were well balanced in baseline variables.

2.3 Adverse effects

No major adverse effects were reported.

One minor adverse effect was reported. One man at the Concord centre developed a mild localized rash around the application site. The rash continued but did not prevent the subject from completing the study. This was reported to the Ethics Committee as a minor adverse effect of mild severity and probably related to the cream.

2.4 Continuation of cream usage after study

After completing the study, 22 men requested further supply of the cream and were studied in an open-extension phase. Of the 8 men who did not seek continued post-study cream supply, 1 had a skin rash at the cream application site, and the other 7 reported inadequate efficacy judged by their androgen deficiency symptoms even at the ceiling dosage (150mg/day) and preferred to transfer back to prior form of treatment. Of these 7 men, 3 were castrate; the other 4 had severe testicular failure (2 primary testicular failure, 1 mumps orchitis, 1 Klinefelter's syndrome variant, 46XX).

2.5 Subject compliance

Attendance at scheduled visits was 100%.
Compliance with continued treatment during the study was high as judged by replacement of cream tubes.

2.6 Pharmacokinetics

Baseline and trough blood testosterone concentration:

Pre-treatment baseline blood total testosterone concentration for the whole group was 8.7 ± 0.9 nmol/L (n=30) and were well balanced between the two randomly assigned initial dose groups (table 2). There were slight differences between the screening blood testosterone concentrations, which were slightly lower than the zero time samples for the 24 hr detailed sampling period (table 3a) possibly reflecting regression to the mean.

After 4 weeks of treatment, trough blood total testosterone concentration increased significantly for both doses compared with pre-study. The week 4 blood testosterone concentrations reached higher levels for men treated with 100 mg/day compared with men treated with 50mg/day (27.3 ± 5.8 nmol/L vs 9.7 ± 3.7 nmol/L) (Tables 3a, b & c).

At the end of study following 12 weeks of treatment (the last 8 weeks with individually titrated dosage based on week 4 trough blood testosterone concentration), trough blood testosterone concentrations were 13 nmol/L in the one man remaining on 50 mg per day, 16.5 ± 8.0 nmol/L for men who were up-titrated from 50 mg to 75 mg per day, 13.7 ± 3.1 nmol/L for the men who remained on 100 mg per day and 7.6 ± 1.2 nmol/L for the men who were up-titrated from 100 mg to 150 mg per day (Tables 4a, b & c).

Blood testosterone AUC and other pharmacokinetic variables:

During the first 24 hr detailed sampling period, T_{24} was higher but not significantly so for the 100 mg dose compared with 50 mg dose (table 3a).

After 4 weeks treatment, C_{avg}, C_{max} and C_{min} blood testosterone concentrations during the 24 hr sampling period were higher in men on the higher testosterone dosage with each approximating a 2:1 ratio consistent with the difference in daily dose. The C_{avg} for the higher dose group was near the middle of the young male eugonadal reference range.

After 12 weeks treatment with all men having had 8 weeks of individual dose titration, C_{avg} for all groups was within the young male reference range.

2.7 Blood LH, FSH and SHBG

Among the 20 men with primary (hypergonadotrophic) hypogonadism, there was no consistent pattern of reduced blood LH or FSH after 4 (figure 4) or 12 (figure 5) weeks of treatment (table 3a & 4a). A significant but minor reduction in blood FSH limited to the final low dose group was observed (table 4a).

Blood SHBG was not consistently changed during the study remaining within the eugonadal reference range.

2.8 Hematology and Biochemistry

There were no consistent changes between weeks 0 and 12 in hematology (hemoglobin, hematocrit, white cell, platelet and red call counts), biochemistry (urea, creatinine, bilirubin, ALP, GGT, ALT, AST) and lipids (total, HDL & LDL cholesterol, triglycerides). All remained within the normal adult reference ranges.

There were no significant changes in urinary deoxypyridinoline excretion from pre-treatment baseline according to either initial (week 4) or final (week 12) dose.

2.9 Anthropometric measures

There was no change in pulse, blood pressure or body composition (fat or lean mass by bioimpedance) or muscle strength (hand-grip dynamometry) during the study according to initial or final testosterone cream daily dose.

2.10 Prostate

Blood PSA concentrations remained within adult reference range for all subjects. There was no significant change in PSA after 4 weeks (table 3a) or 12 weeks (table 4a) treatment.

Lower urinary tract symptom scores (IPSS scale) remained low throughout the study (tables 3a & 4a). Statistically significant reductions in IPSS score at 4 weeks in the higher (100mg daily) dose and increases in the low final dose (<100 mg) daily dose groups were observed; however, all IPSS scores remained low.

2.11 Quality of Life Questionnaires

The SF-36 questionnaire (table 5) indicated significant improvements in vitality (VT) at both 4 and 12 weeks of treatment (tables 3c & 4c). In addition, there were inconsistent changes in

social functioning (SF) in low dose (50 mg) group after 4 weeks treatment and the high final dose (150 mg) after 12 weeks treatment, and emotional functioning (RE) at the high final dose (150 mg) group after 12 weeks treatment. Compared with aged-matched Australian norms, the study group as a whole scored significantly poorer in physical role (RP), vitality (VT), social functioning (SF), emotional functioning (RE) and mental health (MH) at baseline with the deficits in RP, VT, SF and MH persisting at week 12. After 12 weeks of treatment, the study participants describe significantly lower levels of bodily pain (BP) than the aged-matched Australians (P=<0.01).

The Leading Symptom Scale showed that tiredness (17/30, 57%), mood disturbances (6/30, 20%), loss of libido (3/30, 10%) and hot flushes (2/30, 7%) were most prominent symptoms at pre-treatment baseline (table 6). After 12 weeks of treatment, the most prevalent symptoms were similar except that tiredness (7/30, 23%) was significantly less frequent (table 6). The prevalence of "no symptoms" increased from 2/30 before treatment to 7/30 at the end of 12 weeks treatment (odds ratio = 4.3, 95% confidence limits 0.70 – 44.9 (2 sided) or 0.88 – 30.3 (1 sided)).

The Mood, Energy and Sexual Function scale showed improvements in both emotional (cheerful, friendly, nervous, irritable) and physical variables (energy, lethargy, fatigue, tired, active, vigorous) together with an increase in sexual thoughts and intercourse, but no change in morning erections, ejaculations or sexual satisfaction (tables 3c & 4c).

3. Discussion

The present study evaluated Andromen Forte, a 5% testosterone cream, over a 3 month period in 30 men with classical androgen deficiency. The study recruited men with classical androgen deficiency whose previous testosterone treatment was withdrawn before entry in a run-off period. During the study clinical, biochemical and hormonal markers of androgen effects were studied with allowance for individual dose-titration to optimise individual dosing.

As this transdermal cream had not previously been clinically evaluated for testosterone replacement therapy, a preliminary dose-finding study concluded that the target dose should best be in range of 50 to 100 mg per day. Based on this, the present study was designed to randomise men initially to either 50 mg or 100 mg per day for the first month and to subsequently allow for individual dose titration. The dose titration was performed according to steady state, trough blood testosterone concentrations after 4 weeks of treatment and the optimised dose was continued for an additional 8 weeks of treatment. On this basis, most men (23/30) required up-titration while only a single man required down-titration. The net effect was however to produce mean blood testosterone concentrations within the eugonadal reference range for all final optimised doses.

The study was designed to recruit men with classical androgen deficiency due to underlying hypothalamo-pituitary or testicular disorders and not men with age-related partial androgen deficiency where such underlying pathology is absent. The aim was to evaluate the product in an appropriate target population, men with classical androgen deficiency, in whom the natural history and clinical practice dictate that testosterone replacement therapy was unequivocally justified. The latter follows from the fact that in routine clinical practice such men are usually treated permanently with testosterone replacement therapy so that a placebo control would not be ethically acceptable. By contrast, the justification for testosterone treatment in the older men with age-related decline in blood testosterone concentrations remains controversial and not justified by available evidence {Gruenewald, 2003 #3051; Liverman, 2004 #3251}. As a consequence it was not feasible to recruit only untreated men with classical androgen deficiency due to the low frequency of new cases. By contrast other studies of new transdermal products

have often recruited men with age-related androgen deficiency, a population in whom the justification for treatment and the risk-benefit analysis are distinctly different but where previously untreated men are more available. While a placebo control is then justifiable, the underlying variability in endogenous testosterone production and the uncertain natural history, complicate interpretation of efficacy, safety and cost-effectiveness.

The necessity to maintain testosterone replacement in this population, which renders a placebo control unacceptable, also limited the degree of testosterone withdrawal feasible in the study population. While the run-off period was long enough to allow blood testosterone concentrations from previous testosterone treatment to decline towards untreated baseline, it is not certain that all tissue androgen effects were fully returned to the equivalent of an untreated state. Hence, the realistic expectation of adequate testosterone replacement in this study was that the topical testosterone cream would maintain androgen effects.

The pharmacokinetics of the testosterone cream demonstrated delayed absorption but ultimately effective increases in blood testosterone for most men into the eugonadal reference range. The delayed absorption is illustrated by the blood testosterone levels that were restored to normal levels only towards the end of the first 24 hr sampling period. The effectiveness of testosterone delivery was attested to by the subsequent detailed sampling studies which showed that, at after 4 and 12 weeks treatment, the pre-treatment baseline and the full 24 hr sampling period demonstrated blood testosterone concentrations were well within the eugonadal blood testosterone reference range. Dosage effects was demonstrated by the higher blood testosterone concentrations (all remaining within the eugonadal reference range) of men randomised to the higher (100 mg per day) compared with the lower (50 mg per day) blood testosterone concentrations both during the first 24 hr sampling period and subsequently after 4 weeks daily testosterone cream treatment. Following individual dose titration, after 12 weeks of treatment mean blood testosterone concentrations were within the eugonadal reference range for all 3 groups of final testosterone doses (<100 mg, 100 mg & 150 mg per day).

The study featured individual dose titration in order to optimise testosterone dosage as this product that had not previously been trialled in men with classical androgen deficiency. A striking feature was that most (23/30, 77%) men required up-titration of dosage whereas down-titration was only required by a single individual. Interestingly even in the 150 mg per day group that were up-titrated from the initial randomised dose of 100 mg per day, blood testosterone concentrations remained low and gonadotrophin suppression minimal suggesting that this subgroup of men had either lower transdermal absorption or more rapid whole body metabolism of testosterone. Similar findings of subgroups requiring individual dose optimisation have been reported with other transdermal products suggesting that the former explanation is more likely. As there were no clinical, demographic, medical history or anthropometric predictors that served to identify this subgroup of men, the present study reinforced the need for individual dose titration in clinical practice. In addition, this highlights the limited knowledge of factors determining variability in transdermal testosterone absorption. An important consequence of these observations is that, in addition to the final testosterone dose, other determinants of clinical efficacy notably degree of testosterone delivery may influence clinical efficacy and that, in particular, men on the same final dose may differ according to whether they were up, down or not titrated to reach that dose.

Pharmacodynamic effects of testosterone delivery in replacement therapy regimens is most sensitively reflected by the degree and consistency of gonadotrophin suppression among the subgroup of men with hypergonadotrophic (primary, or testicular) hypogonadism. In this context, the present study showed minimal suppression of blood LH and FSH concentrations in men with primary hypogonadism. This suggest the delivery of testosterone by this formulation is relatively low compared with net endogenous testosterone production rate as any regimen involving parenteral testosterone delivery can completely suppress blood LH and, to a lesser

extent, FSH in men with primary hypogonadism. On the other hand this indicates the testosterone cream may not suppress whatever residual LH-dependent endogenous testosterone production capacity remains. This is most relevant to men with partial androgen deficiency whether due to milder forms of classical androgen deficiency or to men with chronic diseases or ageing itself. However, the use of any form of testosterone supplementation in the latter conditions remains unproven and unjustified by present knowledge.

The safety of the testosterone cream was evaluated by conventional clinical safety variables as well as for local irritation of skin at the application site. The single skin rash reported at the application site was deemed minor by the patient who chose to continue using the cream to complete the study. The absence of any adverse clinical or biochemical reactions supports the short-term safety of this testosterone cream. The acceptability of the testosterone cream was supported by the relative high level of continuation of cream usage after completion of the study. A relevant factor is that the only transdermal alternative available is the relatively irritating non-scrotal skin patch whereas the more popular testosterone gel was not an available alternative choice. As costs of return to prior testosterone products was minimal due to third-party subsidy, the relative cost differential was minimal and is unlikely to have had major influence on this choice. These findings suggest the testosterone cream is well accepted and has reasonable short-term safety but its popularity relative to a testosterone gel as well as its long-term safety remain to be determined.

4. Conclusion

It is concluded that the Andromen Forte 5% testosterone cream has demonstrated reasonable efficacy, safety and acceptability over a 3 month period. While for most men it maintains adequate testosterone replacement therapy, this requires individual dose titration and there are some men for whom even the maximum practical dosage has suboptimal testosterone delivery as judged by blood testosterone pharmacokinetics and pharmacodynamic effects on gonadotrophin suppression. It is most suitable for men with partial androgen deficiency, notably those retaining some residual endogenous testosterone production such as for those men with milder forms of classical hypogonadism as well as those with chronic diseases and age-related partial androgen deficiency, subject in the latter categories to adequate proof of safety and efficacy.

Table 1 - Characteristics of subjects at baseline

	N	Mean	SEM	SD	Minimum	Median	Maximum
Anthropometry							
Age (yr)	30	48.2	2.2	12.3	25	49	78
Height (cm)	30	177	1.5	8.0	164	175	194
Weight (kg)	30	88.4	2.8	15.6	64.0	86.0	128.3
Body mass index (BMI, kg/m^2)	30	28.2	0.8	4.5	21.1	27.1	40.1
Body surface area (BSA, m^2)	30	2.1	0.0	0.2	1.7	2.1	2.6
Diastolic blood pressure (mm Hg)	30	77	2	9	60	80	90
Systolic blood pressure (mm Hg)	30	122	3	14	101	120	170
Pulse (beats/min)	30	68	2	9	53	65	84
Lean mass (kg)	30	64.8	1.5	8.4	46.2	64.6	82.8
% lean	30	74.1	1.2	6.6	62.3	74.5	87.7
Fat mass (kg)	30	23.4	1.8	9.7	7.1	20.5	45.7
% fat	30	25.6	1.3	6.9	8.8	25.5	37.6
Hand dynamometry (N/m)	24	43.0	2.1	10.0	22.0	41.4	63.7
Hormones							
Testosterone (nmol/L)	27	7.4	0.8	3.9	0.7	6.9	17.4
T at 00 hr	30	8.8	1.0	5.3	0.1	8.3	22.4
T at 24 hr	30	16.7	2.2	11.9	2.7	13.3	55.0
LH (IU/L)	27	10.8	2.3	12.5	0.1	4.9	43.6
FSH (IU/L)	26	23.0	4.5	24.4	0.1	10.8	67.1
SHBG (nmol/L)	26	26.6	3.4	18.8	9.4	20.4	99.0
Free T (pmol/L)	23	112	15.3	73.3	4	93	265
Urinary T	30	16.0	2.3	12.8	0.6	13.3	60.5
Urinary DHT	30	3.3	0.8	4.2	0.0	1.7	19.6
Urinary epiT	30	5.6	1.0	5.3	0.5	4.0	22.2
Urinary deoxypyridinoline (DPD, nmol)	30	61.2	9.8	53.5	6.0	47.2	225.0
Urinary DPD/creatinine ratio	30	5.6	0.6	3.4	1.0	5.6	13.3
PSA (μg/L)	28	0.9	0.2	1.2	0.1	0.6	6.6
IPSS	30	5.8	1.1	6.0	0.0	3.0	23.0
Hematology							
Hemoglobin (g/L)	27	147.6	2.7	13.8	115.0	149.0	170.0
White cell count (10^9/L)	27	5.4	0.3	1.3	3.6	5.1	8.6
Platelets (10^9/L)	27	225	7.2	37.4	140	226	295
Red cell count (10^12/L)	27	4.8	0.1	0.4	3.8	4.7	5.6
Hematocrit (%)	27	43.0	0.8	4.2	33.0	43.0	50.0
Kidney and Liver Function Tests							
Urea (mmol/L)	28	6.0	0.3	1.4	3.7	5.9	9.8
Creatinine (μmol/L)	27	86	2.7	14.3	58	86	121
ALP (U/L)	28	66.3	3.9	20.6	35	65	106
ALT (U/L)	28	36.2	3.9	20.6	10	33	112
AST (U/L)	27	24.4	1.8	9.3	14	23	52
GGT (U/L)	28	33.4	5.3	27.9	12	25	159
Lipids							
Total cholesterol (mmol/L)	28	5.2	0.2	1.0	3.7	5.0	6.9
HDL cholesterol (mmol/L)	26	1.3	0.1	0.3	0.8	1.3	2.1
LDL cholesterol (mmol/L)	26	3.1	0.2	0.9	1.8	2.9	4.9
Triglyceride (mmol/L)	28	1.6	0.2	1.0	0.5	1.4	4.6
Quality of life (SF-36)							
Physical functioning	29	80	4	24	5	90	100
Role limited by physical	30	63	8	43	0	88	100
Bodily pain	30	64	5	29	31	52	100
General health	29	65	4	21	10	67	100
Vitality	30	45	4	24	5	45	85
Social functioning	30	74	5	26	13	75	100
Role limited by emotion	30	61	8	42	0	67	100
Mental health	30	68	3	17	36	68	96

Table 2 - Testosterone Pharmacokinetic Parameters According to Dose and Time

Parameters	50mg	75mg	100mg	150mg
Week 0 visit				
(n=)	(15)		(15)	
C_0 (nmol/L)	8.2 ± 2.4		9.3 ± 1.6	
C_{avg} (nmol/L)	9.9 ± 1.1		15.8 ± 2.6	
C_{max} (nmol/L)	13.9 ± 1.4		19.5 ± 4.1	
C_{min} (nmol/L)	7.3 ± 0.7		11.7 ± 2.4	
T_{max} (hours)	18.6 **±** 2.1		18.4 **±** 2.1	
Week 4 visit				
(n=)	(15)		(15)	
C_0 (nmol/L)	9.7 ± 3.7		27.3 ± 5.8	
C_{avg} (nmol/L)	10.4 ± 0.7		21.5 ± 4.5	
C_{max} (nmol/L)	13.4 ± 1.5		27.3 ± 5.8	
C_{min} (nmol/L)	9.7 ± 0.8		16.5 ± 4.1	
T_{max} (hours)	15.3 **±** 2.4		9.0 **±** 2.7	
Week 12 visit				
(n=)	(1)	(4)	(15)	(10)
C_0 (nmol/L)	13.3	24.1 ± 10.2	21.4 ± 4.4	14.7 ± 2.9
C_{avg} (nmol/L)	14.6	18.0 ± 7.3	20.9 ± 2.5	11.0 ± 1.5
C_{max} (nmol/L)	20.3	25.9 ± 9.3	25.2 ± 5.9	18.0 ± 8.5
C_{min} (nmol/L)	10.7	14.1 ± 3.2	18.1 ± 1.8	8.8 ± 1.1
T_{max} (hours)	6.0	4.2 ± 1.3	12.5 **±** 5.3	7.6 **±** 2.7

Data expressed as mean ± SEM
C_0 is defined as the blood total testosterone level (nmol/L) prior to the application of the cream
C_{avg} is the time-averaged blood testosterone concentration (nmol/L) over the full 24 hr sampling period calculated by dividing the AUC_{0-24} by 24 hr
C_{max} (nmol/L) is defined as the maximum concentration during 24-hour sampling period
C_{min} (nmol/L) is defined as the minimum concentration during 24-hour sampling period
T_{max} is defined as the time at which C_{max} occurred.

Table 3a Anthropometry and Hormones According to Initial Doses

Characteristics	Initial dose 50 mg T (N = 15)				Initial dose 100 mg T (N = 15)				p value (ANOVA)		
	Baseline	Week 4	Change	P value	Baseline	Week 4	Change	P value	Time	Time**x**Dose Interaction	Initial Dose
Anthropometry											
Age (yr)	53.1 ± 3.5				43.3 ± 2.3						0.03
Height (cm)	176.6 ± 2.1				177.0 ± 2.1						0.90
Weight (kg)	87.1 ± 4.0	87.5 ± 4.0	0.4 ± 0.3	0.25	89.6 ± 4.1	90.2 ± 4.2	0.6 ± 0.4	0.22	0.09	0.74	0.65
Body mass index (BMI)	27.9 ± 1.3	28.1 ± 1.3	0.1 ± 0.1	0.21	28.5 ± 1.1	28.7 ± 1.1	0.2 ± 0.2	0.25	0.09	0.79	0.72
Body surface area (BSA)	2.1 ± 0.1	2.2 ± 0.1	0.1 ± 0.1	0.30	2.1 ± 0.1	2.1 ± 0.1	0.0 ± 0.0	0.24	0.25	0.33	0.98
Diastolic blood pressure	78 ± 2	74 ± 2	-4 ± 2	0.04	77 ± 2	76 ± 2	-1 ± 1	0.58	0.04	0.18	0.75
Systolic blood pressure	122 ± 3	121 ± 3	-1 ± 3	0.60	122 ± 4	122 ± 4	0 ± 3	0.96	0.70	0.73	0.90
Pulse per minute	70 ± 2	73 ± 3	2 ± 2	0.16	66 ± 2	69 ± 2	3 ± 2	0.26	0.08	0.89	0.12
Lean mass (kg)	63.8 ± 2.3	64.0 ± 2.2	0.1 ± 0.5	0.78	65.8 ± 2.0	65.7 ± 2.0	-0.1 ± 0.5	0.78	0.98	0.69	0.54
% lean	73.9 ± 1.6	70.8 ± 3.1	-3.1 ± 2.8	0.29	74.3 ± 1.8	73.7 ± 1.7	-0.6 ± 0.3	0.09	0.20	0.39	0.55
Fat mass (kg)	23.2 ± 2.3	23.6 ± 2.2	0.4 ± 0.4	0.32	23.6 ± 2.8	24.6 ± 2.7	1.0 ± 0.4	0.04	0.03	0.41	0.85
% fat	25.9 ± 1.6	26.2 ± 1.4	0.4 ± 0.5	0.43	25.3 ± 2.0	26.4 ± 1.7	1.1 ± 0.5	0.03	0.03	0.25	0.94
Hand dynamometry (N/m)	41.3 ± 3.2	42.1 ± 3.2	0.8 ± 0.9	0.40	44.9 ± 2.5	45.7 ± 2.2	0.8 ± 1.2	0.52	0.30	0.96	0.39
Hormones											
Testosterone (nmol/L)	6.4 ± 0.7	8.7 ± 0.7	2.1 ± 0.9	0.04	8.4 ± 1.3	16.9 ± 2.8	8.2 ± 3.2	0.02	0.00	0.06	0.01
T at 00 hr	8.2 ± 1.0	10.1 ± 0.8	1.5 ± 0.8	0.08	9.4 ± 1.7	27.2 ± 5.8	18.3 ± 5.6	0.01	0.00	0.01	0.02
T at 24 hr	14.0 ± 1.4	13.5 ± 1.5	-0.5 ± 0.8	0.54	19.4 ± 4.1	18.2 ± 4.1	-1.2 ± 2.7	0.65	0.54	0.80	0.22
LH (IU/L)	18.5 ± 5.1	17.7 ± 6.0	-0.8 ± 3.0	0.80	15.8 ± 3.9	14.1 ± 4.3	-1.8 ± 4.3	0.69	0.67	0.87	0.62
FSH (IU/L)	44.3 ± 9.1	40.9 ± 12.2	-2.4 ± 6.1	0.71	32.4 ± 6.5	32.4 ± 9.4	-0.1 ± 5.1	0.99	0.76	0.78	0.45
SHBG (nmol/L)	27.0 ± 5.7	25.8 ± 4.8	-1.2 ± 1.3	0.39	26.2 ± 4.1	26.0 ± 4.0	-0.2 ± 1.4	0.87	0.47	0.63	0.96
Free T (pmol/L)	83 ± 16	143 ± 21	46 ± 20	0.04	134 ± 23	294 ± 68	165 ± 76	0.05	0.02	0.18	0.02
Urinary deoxypyridinoline(nmol)	56.5 ± 1.6	54.6 ± 2.6	-1.9 ± 1.0	0.87	65.9 ± 6.1	62.5 ±10.7	-3.4 ± 0.0	0.74	0.72	0.92	0.61
Urinary DPD/Creatinine ratio	5.7 ± 0.9	4.2 ± 0.7	-1.5 ± 1.0	0.15	5.6 ± 0.9	5.7 ± 0.6	0.1 ± 0.6	0.82	0.24	0.16	0.46
PSA (μg/L)	1.2 ± 0.4	1.1 ± 0.4	-0.1 ± 0.1	0.51	0.7 ± 0.1	0.6 ± 0.1	-0.2 ± 0.1	0.26	0.19	0.67	0.36
IPSS	6.0 ± 1.7	7.3 ± 1.9	1.3 ± 0.7	0.09	5.7 ± 1.4	4.1 ± 1.2	-1.6 ± 0.6	0.02	0.72	0.01	0.43

Table 3b Hematology and Biochemistry According to Initial Doses

Characteristics	Initial dose 50 mg T (N = 15)				Initial dose 100 mg T (N = 15)				p value (ANOVA)		
	Baseline	Week 4	Change	P value	Baseline	Week 4	Change	P value	Time	Interaction	Initial Dose
Hematology											
Hemoglobin (g/L)	143.9 ± 3.2	144.1 ± 3.8	-0.3 ± 1.3	0.82	151.6 ± 4.2	148.5 ± 4.6	-3.7 ± 2.0	0.10	0.10	0.16	0.16
White cell count (10^9/L)	5.1 ± 0.3	5.3 ± 0.3	0.0 ± 0.2	0.97	5.9 ± 0.4	5.4 ± 0.2	-0.3 ± 0.3	0.37	0.41	0.44	0.39
Platelets (10^9/L)	212 ± 10	222 ± 9	9 ± 5	0.10	239 ± 9	230 ± 11	-6 ± 9	0.52	0.74	0.13	0.41
Red cell count (10^12/L)	4.7 ± 0.1	4.7 ± 0.1	0.0 ± 0.0	0.77	4.9 ± 0.1	4.9 ± 0.2	-0.1 ± 0.1	0.26	0.22	0.36	0.13
Hematocrit (%)	41.9 ± 0.9	42.4 ± 1.1	0.3 ± 0.4	0.42	44.2 ± 1.3	43.6 ± 1.3	-1.0 ± 0.7	0.19	0.36	0.09	0.12
Kidney and Liver Function Tests											
Urea (mmol/L)	6.5 ± 0.2	6.5 ± 0.4	0.1 ± 0.4	0.82	5.6 ± 0.4	5.5 ± 0.4	-0.1 ± 0.3	0.61	0.91	0.63	0.12
Creatinine (μmol/L)	87 ± 4	88 ± 4	1 ± 2	0.71	85 ± 4	84 ± 3	1 ± 3	0.67	0.57	0.84	0.59
ALP (U/L)	64 ± 5	62 ± 6	-3 ± 3	0.43	68 ± 6	62 ± 4	-7 ± 3	0.04	0.04	0.37	0.83
ALT (U/L)	33 ± 3	32 ± 4	-3 ± 2	0.14	39 ± 7	45 ± 9	2 ± 3	0.50	0.87	0.17	0.29
AST (U/L)	21 ± 2	22 ± 1	0 ± 1	0.86	28 ± 3	26 ± 2	-2 ± 1	0.11	0.18	0.13	0.07
GGT (U/L)	37 ± 10	34 ± 6	-5 ± 5	0.32	29 ± 3	27 ± 3	-4 ± 2	0.04	0.11	0.83	0.40
Lipids											
Total cholesterol (mmol/L)	5.4 ± 0.3	5.3 ± 0.2	-0.2 ± 0.1	0.14	5.0 ± 0.2	4.9 ± 0.2	-0.1 ± 0.1	0.66	0.17	0.44	0.10
HDL cholesterol (mmol/L)	1.3 ± 0.1	1.3 ± 0.1	0.0 ± 0.0	0.98	1.2 ± 0.1	1.3 ± 0.1	0.0 ± 0.0	0.26	0.42	0.44	0.88
LDL cholesterol (mmol/L)	3.2 ± 0.2	3.1 ± 0.2	-0.1 ± 0.1	0.16	3.1 ± 0.3	3.1 ± 0.3	-0.1 ± 0.2	0.73	0.30	0.67	0.61
Triglyceride (mmol/L)	1.6 ± 0.3	1.7 ± 0.2	0.0 ± 0.1	0.74	1.6 ± 0.3	1.4 ± 0.2	-0.3 ± 0.3	0.35	0.48	0.31	0.78

Table 3c Quality of Life According to Initial Doses

Characteristics	Initial dose 50 mg T (N = 15)				Initial dose 100 mg T (N = 15)				p value (ANOVA)		
	Baseline	Week 4	Change	P value	Baseline	Week 4	Change	P value	Time	Interaction	Dose
Quality of life (SF-36)											
Physical functioning	79 ± 7	85 ± 5	6 ± 5	0.27	82 ± 6	79 ± 6	-5 ± 6	0.46	0.86	0.20	0.78
Role limited by physical	67 ± 11	71 ± 12	5 ± 5	0.34	58 ± 12	60 ± 11	2 ± 11	0.88	0.58	0.77	0.53
Bodily pain	76 ± 7	66 ± 7	-10 ± 7	0.21	52 ± 7	62 ± 7	10 ± 9	0.33	0.99	0.12	0.10
General health	67 ± 6	68 ± 5	4 ± 3	0.31	63 ± 5	63 ± 5	0 ± 3	1.00	0.46	0.46	0.65
Vitality	53 ± 6	66 ± 5	13 ± 4	0.01	38 ± 6	51 ± 4	13 ± 5	0.02	0.00	0.96	0.04
Social functioning	72 ± 6	83 ± 5	11 ± 4	0.03	75 ± 7	78 ± 7	3 ± 6	0.62	0.08	0.35	0.88
Role limited by emotion	60 ± 11	67 ± 12	5 ± 10	0.63	62 ± 11	80 ± 8	18 ± 14	0.23	0.21	0.46	0.58
Mental health	71 ± 4	72 ± 5	1 ± 4	0.73	65 ± 4	71 ± 5	6 ± 4	0.20	0.23	0.45	0.49
Mood and Energy											
Angry	3.0 ± 0.7	2.1 ± 0.7	-0.6 ± 0.5	0.25	1.9 ± 0.7	2.9 ± 0.7	1.1 ± 0.6	0.10	0.55	0.05	0.88
Full of energy	4.7 ± 0.9	6.3 ± 0.8	1.1 ± 0.9	0.24	2.5 ± 0.7	3.9 ± 0.6	1.5 ± 0.6	0.02	0.02	0.74	0.02
Friendly	7.1 ± 0.8	8.0 ± 0.4	0.6 ± 0.6	0.31	6.0 ± 0.8	7.1 ± 0.4	1.1 ± 0.7	0.12	0.06	0.59	0.17
Cheerful	6.4 ± 0.9	7.2 ± 0.6	0.1 ± 0.6	0.90	5.5 ± 0.8	6.5 ± 0.6	0.9 ± 0.5	0.08	0.19	0.27	0.28
Alert	6.2 ± 0.8	7.0 ± 0.6	0.2 ± 0.7	0.75	4.5 ± 0.9	5.9 ± 0.6	1.1 ± 0.7	0.15	0.20	0.40	0.06
Sad	3.9 ± 1.0	1.7 ± 0.5	-1.7 ± 0.9	0.08	4.6 ± 0.9	2.9 ± 0.7	-1.5 ± 1.2	0.23	0.04	0.90	0.19
Nervous	3.5 ± 0.8	2.2 ± 0.5	-1.4 ± 0.9	0.13	3.4 ± 0.9	2.8 ± 0.6	-0.6 ± 0.6	0.36	0.07	0.45	0.85
Fatigued	5.3 ± 1.0	3.7 ± 0.7	-1.6 ± 0.8	0.09	6.5 ± 0.9	5.7 ± 0.6	-0.8 ± 0.8	0.32	0.05	0.50	0.07
Lethargic	4.8 ± 0.9	3.0 ± 0.8	-1.7 ± 0.7	0.02	6.5 ± 0.9	5.9 ± 0.7	-0.8 ± 0.7	0.25	0.01	0.34	0.02
Irritable	4.3 ± 0.8	2.5 ± 0.7	-1.7 ± 0.7	0.03	4.9 ± 0.9	4.5 ± 0.7	-0.4 ± 0.6	0.53	0.03	0.18	0.24
Tired	5.4 ± 0.9	4.9 ± 0.6	-0.5 ± 0.7	0.51	7.1 ± 0.7	6.3 ± 0.6	-1.0 ± 0.5	0.08	0.10	0.54	0.05
Active	5.7 ± 0.8	6.9 ± 0.6	0.8 ± 0.8	0.33	4.4 ± 0.7	4.5 ± 0.5	0.1 ± 0.7	0.92	0.42	0.50	0.01
Well	7.0 ± 0.6	7.0 ± 0.7	0.1 ± 0.5	0.89	5.1 ± 0.9	7.1 ± 0.5	1.9 ± 0.8	0.03	0.05	0.06	0.34
Vigorous	4.5 ± 0.8	6.1 ± 0.7	1.5 ± 0.5	0.02	2.9 ± 0.7	3.7 ± 0.6	0.8 ± 0.6	0.23	0.01	0.44	0.02
Sexual functions											
Many sexual thoughts	4.9 ± 0.7	5.3 ± 0.7	0.4 ± 0.5	0.48	4.4 ± 0.8	4.6 ± 0.8	0.2 ± 0.8	0.81	0.55	0.84	0.53
Strong sexual desires	3.6 ± 0.8	4.1 ± 0.6	0.5 ± 0.8	0.57	3.5 ± 0.8	4.3 ± 0.8	0.9 ± 0.7	0.26	0.23	0.72	0.94
Erections on wakening	3.6 ± 0.9	3.1 ± 0.6	-0.5 ± 0.7	0.54	4.3 ± 0.09	3.6 ± 0.08	-1.0 ± 1.3	0.43	0.31	0.70	0.65
Frequent sexual intercourse	1.6 ± 0.5	2.8 ± 0.7	1.2 ± 0.8	0.16	2.4 ± 0.8	3.4 ± 1.0	0.8 ± 1.0	0.42	0.12	0.78	0.51
Strong sexual satisfaction	3.1 ± 0.8	3.9 ± 0.9	0.9 ± 0.9	0.34	3.3 ± 0.9	3.9 ± 0.9	0.6 ± 1.0	0.54	0.27	0.83	0.95
Having ejaculated	5.0 ± 1.0	6.2 ± 1.0	1.2 ± 1.1	0.30	4.3 ± 1.1	5.2 ± 1.0	0.9 ± 1.1	0.39	0.18	0.89	0.51

Mood and Energy scored on a 10 point Likert scale, Sexual function on a visual linear analogue scale analysed as quasi-continuous variables.

Table 4a Anthropometry and Hormones According to Final Doses

Characteristics	Final dose < 100 mg (N = 5)			Final dose 100 mg (N = 15)			Final dose 150 mg (N = 10)			p (ANOVA)		
	Baseline	Week 12	Change (p)	Baseline	Week 12	Change (p)	Baseline	Week 12	Change (p)	Time	Interaction	Final Dose
Anthropometry												
Age (yr)	53.8 ± 2.9			50.5 ± 3.2			42.0 ± 3.9					0.13
Height (cm)	172 ± 3			176 ± 2			181 ± 3					0.09
Weight (kg)	81.0 ± 5.4	81.3 ± 5.8	0.3 ± 0.7 (0.74)	87.1 ± 3.9	87.8 ± 3.9	0.7 ± 0.4 (0.13)	94.0 ± 5.4	95.2 ± 5.2	1.2 ± 0.6 (0.05)	0.04	0.55	0.27
Body mass index (BMI)	24.8 ± 1.0	24.9 ± 1.1	0.1 ± 0.2 (0.75)	27.8 ± 1.0	28.1 ± 1.0	0.2 ± 0.1 (0.11)	30.5 ± 1.7	31.0 ± 1.6	0.4 ± 0.2 (0.06)	0.04	0.51	0.05
Body surface area (BSA)	2.0 ± 0.1	2.0 ± 0.1	0.0 ± 0.0 (0.77)	2.1 ± 0.1	2.1 ± 0.1	0.0 ± 0.0 (0.13)	2.2 ± 0.1	2.2 ± 0.1	0.0 ± 0.0 (0.05)	0.05	0.55	0.46
Diastolic blood pressure	76 ± 5	72 ± 2	-4 ± 7 (0.59)	77 ± 3	74 ± 2	-3 ± 2 (0.11)	78 ± 2	75 ± 3	-3 ± 3 (0.34)	0.09	0.97	0.85
Systolic blood pressure	134 ± 11	122 ± 7	-12 ± 7 (0.17)	119 ± 3	118 ± 3	-1 ± 4 (0.77)	121 ± 3	116 ± 3	-5 ± 2 (0.10)	0.03	0.26	0.24
Pulse per minute	68 ± 4	72 ± 5	4 ± 4 (0.35)	67 ± 2	71 ± 2	4 ± 2 (0.04)	70 ±3	69 ± 3	-1 ± 2 (0.53)	0.12	0.16	0.96
Lean mass (kg)	60.4 ± 2.8	61.0 ± 2.7	0.5 ± 0.9 (0.59)	65.0 ± 2.3	66.3 ± 3.4	1.3 ± 2.0 (0.53)	66.7 ± 2.6	67.0 ± 2.3	0.3 ± 0.9 (0.75)	0.55	0.91	0.46
% lean	75.3 ± 3.5	75.7 ± 3.3	0.5 ± 1.3 (0.74)	75.3 ± 1.6	75.5 ± 1.7	0.2 ± 1.8 (0.90)	71.9 ± 2.1	71.2 ± 2.0	-0.6 ± 0.6 (0.34)	0.99	0.90	0.26
Fat mass (kg)	20.4 ± 4.3	20.2 ± 4.3	-0.1 ± 1.0 (0.91)	21.9 ± 2.3	21.5 ± 1.8	-0.4 ± 1.9 (0.85)	27.2 ± 3.4	28.0 ± 3.2	0.8 ± 0.6 (0.20)	0.93	0.87	0.18
% fat	24.4 ± 3.6	24.3 ± 3.4	-0.1 ± 1.1 (0.92)	24.4 ± 1.7	24.4 ± 1.7	0.0 ± 1.8 (1.00)	28.0 ± 2.1	28.7 ± 2.0	0.7 ± 0.7 (0.34)	0.86	0.94	0.28
Hand dynamometry (N/m)	42.9 ± 3.9	47.7 ± 4.4	4.8 ± 0.5 (0.07)	40.8 ± 2.9	41.8 ± 3.1	1.0 ± 0.9 (0.28)	46.7 ± 3.2	46.8 ± 3.7	0.1 ± 2.0 (0.97)	0.11	0.38	0.50
Hormones												
Testosterone (nmol/L)	6.8 ± 2.1	15.8 ± 6.2	9.1 ± 4.4 (0.11)	7.4 ± 1.2	13.2 ± 3.2	6.3 ± 2.7 (0.04)	7.7 ± 1.1	7.6 ± 1.2	0.0 ± 1.8 (0.99)	0.01	0.13	0.52
T at 00 hr	7.2 ± 2.5	22.0 ± 8.2	14.8 ± 7.0 (0.10)	9.4 ± 1.6	21.4 ± 4.5	12.1 ± 4.1(0.01)	8.8 ± 1.3	14.3 ± 2.9	5.5 ± 3.1 (0.11)	0.00	0.40	0.58
T at 24 hr	12.4 ± 3.5	14.9 ± 2.6	2.4 ± 3.3 (0.50)	20.7 ± 3.8	19.3 ± 2.4	-1.3 ± 5.4 (0.81)	12.9 ± 1.8	11.1 ± 1.5	-1.8 ± 1.7 (0.31)	0.94	0.87	0.01
LH (IU/L)	22.2 ± 6.3	18.6 ± 7.8	-3.7 ± 2.8 (0.28)	16.7 ± 6.9	4.2 ± 3.1	-12.5 ± 7.3 (0.15)	14.4 ± 3.6	21.0 ± 6.0	6.6 ± 4.1 (0.16)	0.35	0.05	0.39
FSH (IU/L)	47.5 ± 11.1	38.8 ± 11.8	-8.7 ± 1.4 (0.01)	26.8 ± 6.7	9.4 ± 6.7	-17.4 ± 7.6 (0.07)	38.7 ± 9.3	48.8 ± 12.2	10.1 ± 6.5 (0.17)	0.21	0.02	0.18
SHBG (nmol/L)	46.9 ± 15.6	41.4 ± 10.9	-5.5 ± 5.6 (0.38)	22.3 ± 2.8	21.3 ± 3.1	-1.0 ± 1.7 (0.56)	22.9 ± 3.8	25.1 ± 5.5	2.2 ± 2.0 (0.30)	0.40	0.20	0.04
Free T (pmol/L)	86 ± 39	271 ± 120	201 ± 115 (0.18)	108 ± 21	274 ± 66	165 ± 70 (0.04)	127 ± 27	133 ± 41	5 ± 41 (0.90)	0.01	0.13	0.61
Urinary T	24.0 ± 9.9	26.4 ± 8.4	2.4 ± 14.7 (0.88)	15.7 ± 2.5	21.4 ± 3.4	5.8 ± 2.7 (0.05)	12.4 ± 3.4	22.0 ± 4.6	8.2 ± 2.0 (0.00)	0.09	0.79	0.46
Urinary DHT	8.8 ± 3.3	6.8 ± 2.3	-2.0 ± 4.9 (0.71)	2.3 ± 0.6	2.4 ± 0.5	0.1 ± 0.8 (0.89)	2.1 ± 0.5	5.0 ± 0.9	3.0 ± 0.8 (0.01)	0.72	0.19	0.00
Urinary epiT	6.7 ± 4.0	7.4 ± 2.7	0.8 ± 2.4 (0.77)	5.4 ± 1.1	7.4 ± 1.0	2.0 ± 1.0 (0.05)	5.3 ± 1.5	7.2 ± 1.9	1.3 ± 1.3 (0.35)	0.12	0.82	0.96
Urinary deoxypyridinoline(nmol/L)	75.0 ± 12.2	86.8 ± 37.8	11.8 ±35.1(0.75)	53.1 ± 15.7	76.0 ± 17.8	22.9 ± 14.3(0.13)	66.3 ± 16.9	63.9 ± 12.8	-2.4 ± 10.(0.83)	0.33	0.52	0.83
Urinary DPD/creatining ratio	7.2 ± 1.3	6.0 ± 0.9	-1.2 ± 1.5 (0.49)	5.4 ± 1.0	5.9 ± 1.0	0.6 ± 0.7 (0.42)	5.3 ± 0.9	4.8 ± 0.8	-0.5 ± 0.7 (0.55)	0.51	0.39	0.65
PSA (μg/L)	0.2 ± 0.1	0.8 ± 0.5	0.1 ± 0.0 (0.18)	1.2 ± 0.4	1.2 ± 0.4	0.0 ± 0.1 (0.63)	0.8 ± 0.2	0.7 ± 0.1	-0.2 ± 0.2 (0.44)	0.54	0.65	0.33
IPSS	9.2 ± 2.9	15.6 ± 3.6	6.4 ± 2.0 (0.03)	5.4 ± 1.7	6.4 ± 1.7	1.0 ± 0.6 (0.12)	4.8 ± 1.4	3.0 ± 0.9	-1.8 ± 0.9 (0.07)	0.00	0.00	0.04

Table 4b Hematology and Biochemistry According to Final Doses

Characteristics	Final dose < 100 mg (N = 5)			Final dose 100 mg (N = 15)			Final dose 150 mg (N = 10)			p (ANOVA)		
	Baseline	Week 12	Change (p)	Baseline	Week 12	Change (p)	Baseline	Week 12	Change (p)	Time	Interaction	Dose
Hematology												
Hemoglobin (g/L)	143.5 ± 5.6	144.8 ± 6.4	1.3 ± 3.4 (0.74)	144.8 ± 4.1	148.3 ± 2.7	3.0 ± 2.4 (0.23)	153.8 ± 3.7	145.9 ± 2.6	-6.6 ± 2.4 (0.04)	0.67	0.05	0.79
White cell count (10^9/L)	5.6 ± 0.5	5.3 ± 0.6	-0.3 ± 0.1 (0.09)	5.3 ± 0.4	5.5 ± 0.2	0.1 ± 0.3 (0.63)	5.6 ± 0.5	5.3 ± 0.5	-0.3 ± 0.3 (0.27)	0.43	0.46	0.82
Platelets (10^9/L)	204 ± 19	218 ± 12	14 ± 14 (0.39)	219 ± 10	243 ± 9	28 ± 8 (0.00)	245 ± 11	256 ± 15	0 ± 9 (0.96)	0.04	0.11	0.24
Red cell count (10^12/L)	4.6 ± 0.1	4.7 ± 0.1	0.1 ± 0.1 (0.23)	4.7 ± 0.1	4.7 ± 0.1	0.0 ± 0.1 (0.76)	5.1 ± 0.1	4.8 ± 0.1	-0.2 ± 0.1 (0.03)	0.68	0.05	0.31
Hematocrit (%)	41.8 ± 1.6	43.0 ± 1.7	1.3 ± 0.8 (0.19)	42.2 ± 1.2	42.7 ± 0.8	0.4 ± 0.6 (0.57)	44.7 ± 1.2	42.3 ± 0.7	-2.3 ± 0.8 (0.03)	0.65	0.02	0.85
Kidney and Liver Function Tests												
Urea (mmol/L)	5.7 ± 0.7	5.7 ± 0.6	-0.3 ± 0.4 (0.49)	6.1 ± 0.3	5.7 ± 0.4	-0.4 ± 0.4 (0.39)	6.1 ± 0.6	5.9 ± 0.4	-0.5 ± 0.2 (0.10)	0.19	0.98	0.68
Creatinine (μmol/L)	74 ± 3	84 ± 7	3 ± 4 (0.53)	91 ± 4	83 ± 3	-7 ± 2 (0.01)	83 ± 5	87 ± 5	1 ± 2 (0.74)	0.62	0.05	0.22
ALP (U/L)	70 ± 14	57 ± 14	-9 ± 4 (0.15)	63 ± 5	60 ± 4	0 ± 3 (0.95)	70 ± 6	71 ± 6	0 ± 2 (0.95)	0.14	0.16	0.45
ALT (U/L)	32 ± 4	31 ± 3	-4 ± 5 (0.46)	32 ± 4	30 ± 3	-2 ± 2 (0.32)	45 ± 10	50 ± 9	0 ± 3 (0.89)	0.28	0.63	0.11
AST (U/L)	23 ± 4	20 ± 1	-3 ± 3 (0.41)	23 ± 2	23 ± 1	0 ± 2 (0.81)	27 ± 4	26 ± 2	-2 ± 2 (0.29)	0.22	0.74	0.17
GGT (U/L)	22 ± 2	23 ± 3	-1 ± 1 (0.22)	36 ± 9	28 ± 5	-6 ± 6 (0.32)	34 ± 5	33 ± 4	-3 ± 4 (0.44)	0.38	0.84	0.62
Lipids												
Total cholesterol (mmol/L)	5.1 ± 0.3	5.1 ± 0.1	0.0 ± 0.3 (0.87)	5.0 ± 0.3	5.1 ± 0.3	0.0 ± 0.2 (0.81)	5.4 ± 0.3	5.5 ± 0.2	0.3 ± 0.2 (0.30)	0.56	0.66	0.73
HDL cholesterol (mmol/L)	1.3 ± 0.2	1.3 ± 0.1	0.0 ± 0.1 (0.70)	1.3 ± 0.1	1.3 ± 0.1	0.0 ± 0.0 (0.70)	1.3 ± 0.1	1.4 ± 0.2	0.2 ± 0.1 (0.10)	0.25	0.21	0.90
LDL cholesterol (mmol/L)	3.0 ± 0.2	3.1 ± 0.1	0.1 ± 0.2 (0.56)	3.0 ± 0.2	3.0 ± 0.3	0.0 ± 0.1 (0.74)	3.4 ± 0.4	3.3 ± 0.3	-0.1 ± 0.2 (0.52)	0.88	0.70	0.94
Triglyceride (mmol/L)	1.8 ± 0.9	1.6 ± 0.5	-0.3 ± 0.3 (0.37)	1.4 ± 0.2	1.8 ± 0.3	0.4 ± 0.2 (0.11)	1.8 ± 0.4	1.9 ± 0.5	0.3 ± 0.2 (0.25)	0.46	0.32	0.85

Table 4c Quality of Life According to Final Doses

Characteristics	Final dose < 100 mg (N = 5)			Final dose 100 mg (N = 15)			Final dose 150 mg (N = 10)			p (ANOVA)		
	Baseline	Week 12	Change (p)	Baseline	Week 12	Change (p)	Baseline	Week 12	Change (p)	Time	TxD	Dose
Quality of life (SF-36)												
Physical functioning	81 ± 7	77 ± 7	-4 ± 6 (0.55)	84 ± 7	79 ± 7	-5 ± 4 (0.22)	74 ± 8	84 ± 7	8 ± 6 (0.22)	0.95	0.15	0.94
Role limited by physical	50 ± 22	45 ± 23	-5 ± 12 (0.70)	63 ± 12	73 ± 10	10 ± 10 (0.33)	67 ± 13	87 ± 8	20 ± 15 (0.21)	0.31	0.53	0.33
Bodily pain	91 ± 9	62 ± 10	-29 ± 13 (0.09)	63 ± 7	67 ± 7	4 ± 4 (0.33)	53 ± 8	72 ± 10	19 ± 10 (0.08)	0.71	0.00	0.57
General health	76 ± 4	77 ± 3	1 ± 4 (0.79)	66 ± 6	68 ± 6	2 ± 5 (0.68)	59 ± 7	68 ± 6	9 ± 4 (0.05)	0.22	0.47	0.54
Vitality	61 ± 8	67 ± 6	6 ± 4 (0.21)	45 ± 7	61 ± 4	16 ± 6 (0.01)	38 ± 7	53 ± 7	15 ± 6 (0.03)	0.00	0.61	0.21
Social functioning	75 ± 8	81 ± 8	3 ± 3 (0.39)	72 ± 6	75 ± 6	3 ± 4 (0.41)	76 ± 10	92 ± 3	16 ± 8 (0.08)	0.07	0.23	0.46
Role limited by emotion	47 ± 20	73 ± 16	27 ± 34 (0.48)	60 ± 11	71 ± 9	11 ± 8 (0.17)	70 ± 13	100 ± 0	30 ± 13 (0.04)	0.02	0.53	0.20
Mental health	74 ± 6	70 ± 11	-4 ± 12 (0.75)	66 ± 4	72 ± 5	6 ± 5 (0.21)	68 ± 6	79 ± 4	10 ± 6 (0.10)	0.30	0.42	0.73
Mood and Energy												
Angry	1.3 ± 0.8	2.4 ± 1.9	1.8 ± 2. (0.57)	2.5 ± 0.7	1.3 ± 0.5	-1.3 ± 0.6 (0.05)	2.7 ± 0.9	3.4 ± 0.7	0.7 ± 1.0 (0.51)	0.56	0.15	0.36
Full of energy	4.0 ± 1.1	6.2 ± 1.2	3.0 ± 2.1 (0.25)	4.4 ± 0.9	5.3 ± 0.7	0.9 ± 0.8 (0.30)	2.0 ± 0.9	4.5 ± 1.1	2.5 ± 0.9 (0.02)	0.01	0.34	0.19
Friendly	8.4 ± 0.9	8.2 ± 0.9	-0.2 ± 0.5 (0.70)	6.7 ± 0.7	7.8 ± 0.6	1.1 ± 0.6 (0.09)	5.2 ± 1.1	7.5 ± 0.6	2.3 ± 1.1 (0.06)	0.05	0.19	0.31
Cheerful	8.0 ± 1.1	7.4 ± 0.9	0.0 ± 0.8 (1.00)	6.0 ± 0.8	7.1 ± 0.7	1.1 ± 0.6 (0.10)	5.0 ± 1.1	7.3 ± 0.7	2.3 ± 1.1 (0.08)	0.06	0.32	0.44
Alert	6.5 ± 2.0	8.0 ± 0.9	1.8 ± 2.1 (0.47)	5.8 ± 0.8	7.4 ± 0.5	1.6 ± 0.6 (0.01)	4.1 ± 1.0	6.2 ± 0.8	1.9 ± 1.1 (0.12)	0.01	0.97	0.16
Sad	4.3 ± 2.2	0.6 ± 0.4	-3.5 ± 2.3 (0.22)	4.2 ± 0.9	2.9 ± 0.7	-1.3 ± 0.9 (0.17)	4.3 ± 1.2	2.1 ± 0.6	-2.0 ± 1.3 (0.16)	0.01	0.56	0.74
Nervous	4.4 ± 1.8	3.8 ± 1.7	-0.6 ± 2.9 (0.84)	3.7 ± 0.9	2.1 ± 0.6	-1.7 ± 0.8 (0.06)	2.6 ± 0.9	2.1 ± 0.8	-0.5 ± 0.5 (0.36)	0.20	0.68	0.41
Fatigued	4.3 ± 1.8	2.6 ± 0.9	-1.0 ± 1.4 (0.53)	5.6 ± 1.0	3.8 ± 0.8	-1.8 ± 1.0 (0.08)	7.0 ± 1.0	5.6 ± 0.8	-1.4 ± 1.3 (0.31)	0.10	0.91	0.19
Lethargic	3.5 ± 1.3	2.0 ± 1.3	-1.0 ± 2.1 (0.67)	5.1 ± 0.9	3.1 ± 0.8	-2.1 ± 0.6 (0.01)	7.4 ± 0.9	5.6 ± 0.7	-1.9 ± 1.2 (0.15)	0.02	0.83	0.06
Irritable	2.8 ± 1.5	3.2 ± 1.9	1.3 ± 0.8 (0.19)	5.5 ± 0.8	4.1 ± 0.8	-1.4 ± 0.8 (0.09)	4.0 ± 1.0	3.5 ± 0.7	-0.5 ± 1.0 (0.62)	0.72	0.27	0.51
Tired	5.0 ± 1.2	4.0 ± 0.8	-1.3 ± 1.1 (0.34)	5.8 ± 0.8	5.1 ± 0.6	-0.7 ± 0.9 (0.47)	7.7 ± 0.8	5.8 ± 0.7	-2.0 ± 0.8 (0.05)	0.06	0.59	0.20
Active	4.8 ± 1.0	7.8 ± 0.7	3.3 ± 1.9 (0.18)	6.1 ± 0.6	6.3 ± 0.7	0.1 ± 0.4 (0.73)	3.4 ± 0.9	5.3 ± 0.9	1.9 ± 1.0 (0.09)	0.00	0.05	0.16
Well	8.2 ± 0.6	7.6 ± 0.9	-0.6 ± 0.4 (0.21)	5.7 ± 0.8	6.5 ± 0.7	0.8 ± 1.2 (0.51)	5.5 ± 1.1	6.8 ± 0.7	1.3 ± 0.9 (0.17)	0.51	0.64	0.23
Vigorous	5.8 ± 0.3	5.0 ± 0.9	-0.8 ± 1.3 (0.59)	4.0 ± 0.8	5.5 ± 0.7	1.5 ± 0.6 (0.02)	2.4 ± 0.7	5.9 ± 0.7	3.5 ± 0.8 (0.00)	0.01	0.01	0.65
Sexual Functions												
Many sexual thoughts	4.7 ± 1.4	5.4 ± 1.5	0.7 ± 2.0 (0.75)	4.3 ± 0.7	5.8 ± 0.8	1.5 ± 0.8 (0.08)	5.0 ± 1.1	6.7 ± 0.9	1.7 ± 1.3 (0.21)	0.09	0.87	0.75
Strong sexual desires	3.3 ± 1.1	5.0 ± 1.4	1.7 ± 2.1 (0.46)	3.2 ± 0.7	5.3 ± 0.7	2.1 ± 1.0 (0.06)	4.2 ± 1.0	5.3 ± 1.0	1.0 ± 1.4 (0.46)	0.06	0.83	0.81
Erections on wakening	4.1 ± 1.9	3.9 ± 1.8	-0.2 ± 3.0 (0.96)	3.4 ± 0.8	4.3 ± 0.8	0.9 ± 0.9 (0.35)	4.5 ± 1.2	4.3 ± 0.9	0.1 ± 1.9 (0.96)	0.79	0.89	0.74
Frequent intercourse	1.4 ± 0.9	3.4 ± 2.3	4.5 ± 5.5 (0.56)	1.4 ± 0.5	3.6 ± 0.9	2.2 ± 0.6 (0.00)	3.0 ± 1.0	3.6 ± 1.1	0.4 ± 1.5 (0.77)	0.02	0.29	0.80
Sexual satisfaction	2.2 ± 0.9	5.3 ± 2.0	3.0 ± 2.2 (0.23)	2.8 ± 0.8	4.3 ± 0.8	1.5 ± 0.8 (0.10)	4.1 ± 1.2	5.2 ± 0.8	1.1 ± 1.5 (0.51)	0.03	0.66	0.58
Having ejaculated	3.6 ± 1.5	4.2 ± 2.0	0.6 ± 2.7 (0.84)	4.9 ± 1.0	5.8 ± 1.0	0.9 ± 1.3 (0.49)	4.8 ± 1.3	4.9 ± 1.1	0.1 ± 1.5 (0.97)	0.62	0.92	0.64

Mood and Energy scored on a 10 point Likert scale, Sexual function on a visual linear analogue scale analysed as quasi-continuous variables.

Table 5 - Quality Of Life (SF-36) Of Androgen Deficient Men Before And At End Of Study Compared With Age-Matched Healthy Australian Norms

	Pre-study (n=30)	+12 weeks (n=30)	Age-matched healthy Australians (n=1538)	P
Physical functioning (PF)	80.3	81.1	84.6	0.45
Role physical (RP)	63.3	74.3#	82.7#+	<0.01
Bodily pain (BP)	76.5	84.3#	77.6+	<0.01
General Health (GH)	64.5	68.7	70.8#	<0.05
Vitality (VT)	44.2	58.7#	67.0#+	<0.01
Social Functioning (SF)	73.5	80.8#	87.2#+	<0.01
Role-Emotional (RE)	62	82#	85.3#	<0.01
Mental Health (MH)	68.7	67.8	76.8#+	<0.01

#= different from baseline Andromen values

+= different from week 12 values

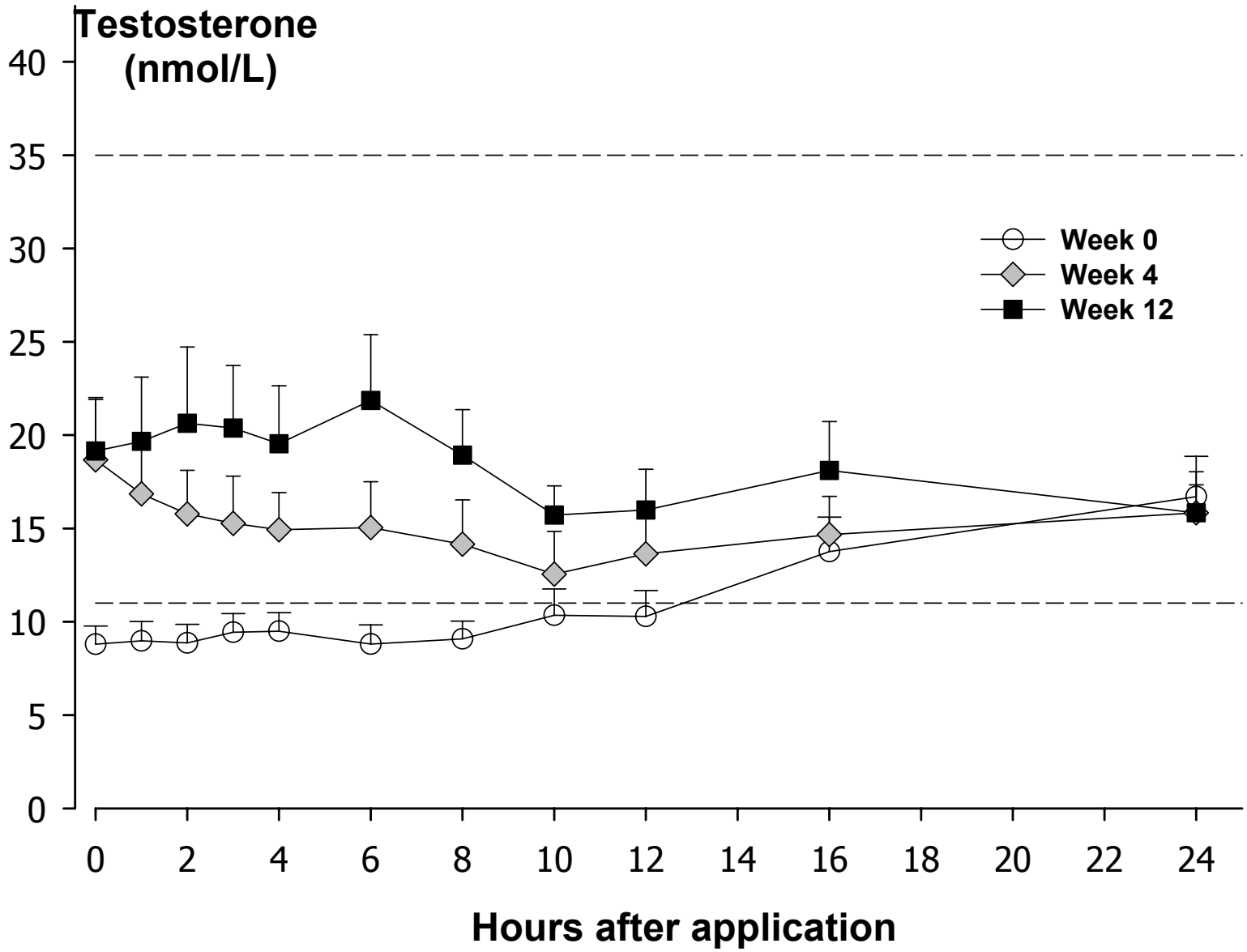
$ = different form all others

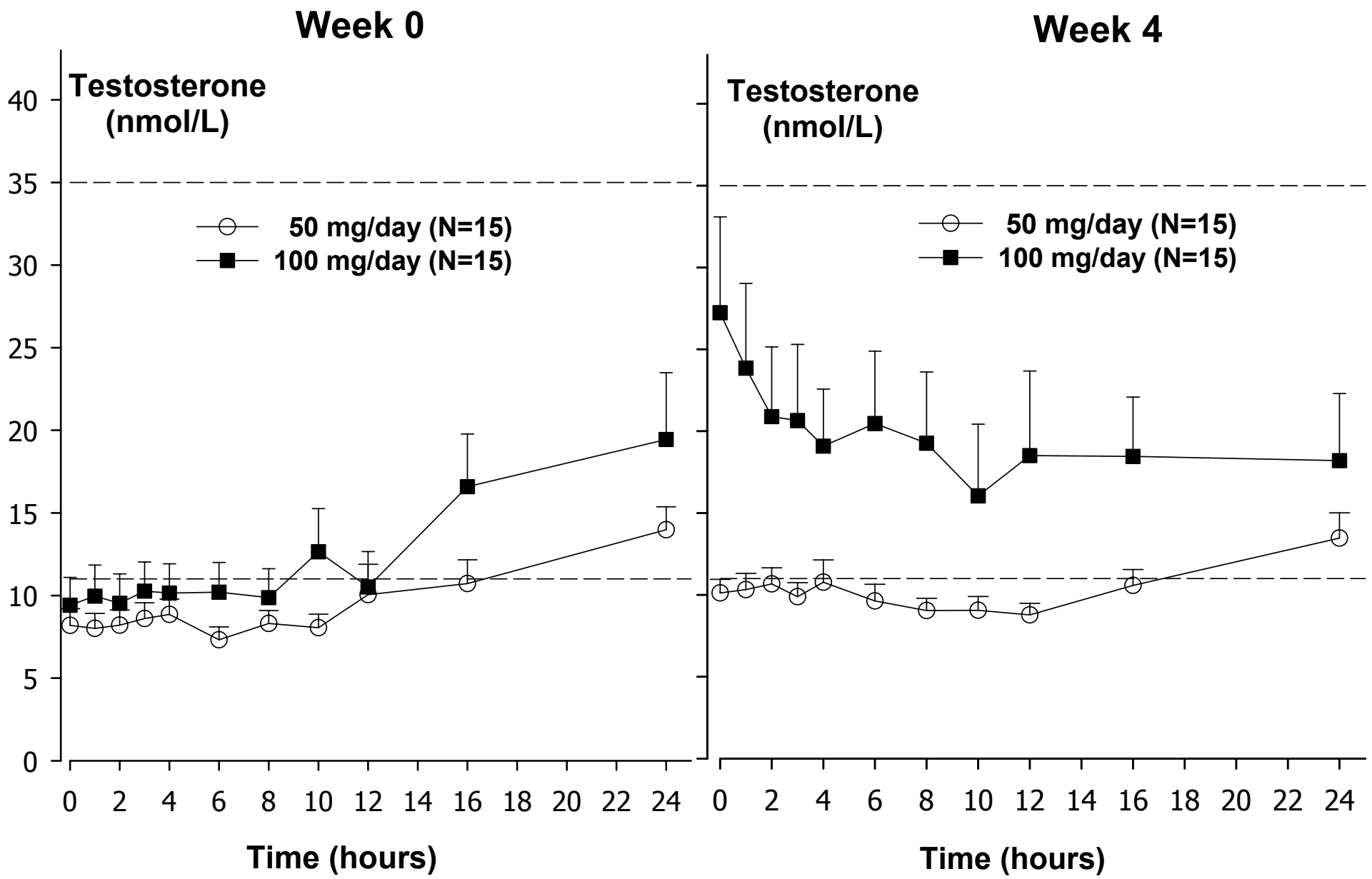
The right two columns are Australian reference populations for comparison.
The 3rd column is Australian normative data in age group 45–54 years.

Table 6 - Leading Symptoms of Androgen Deficiency

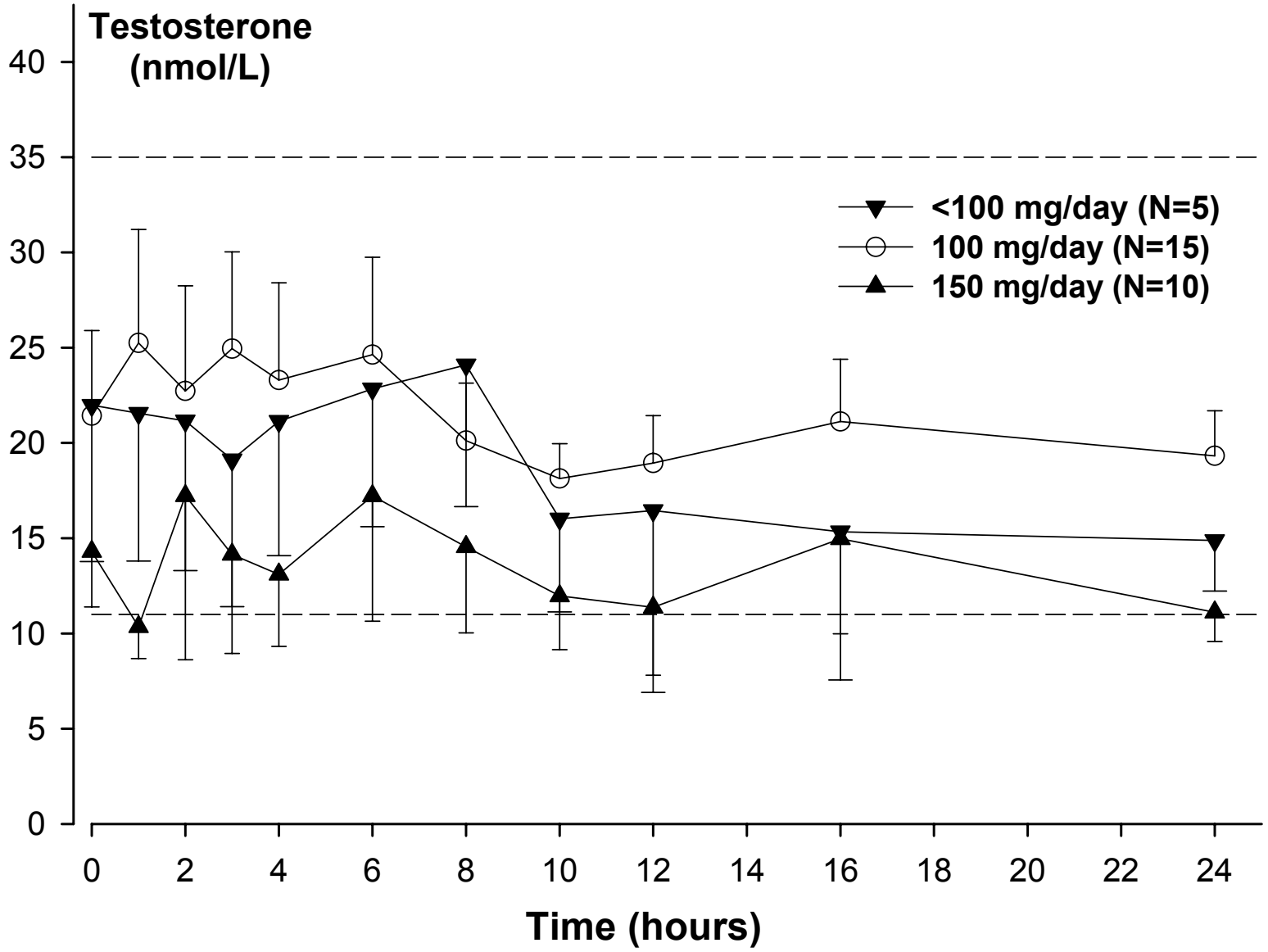
Symptom	Baseline	Week 12	P *
Tiredness	17/30	7/30	0.017
Mood disturbance	6/30	8/30	0.76
Loss of libido	3/30	6/30	0.47
Hot flushes	2/30	0/30	0.49
No appetite	1/30	0/30	1.0
Muscular pain	1/30	0/30	1.0
No symptoms	2/30	7/30	0.15

* Fishers exact test

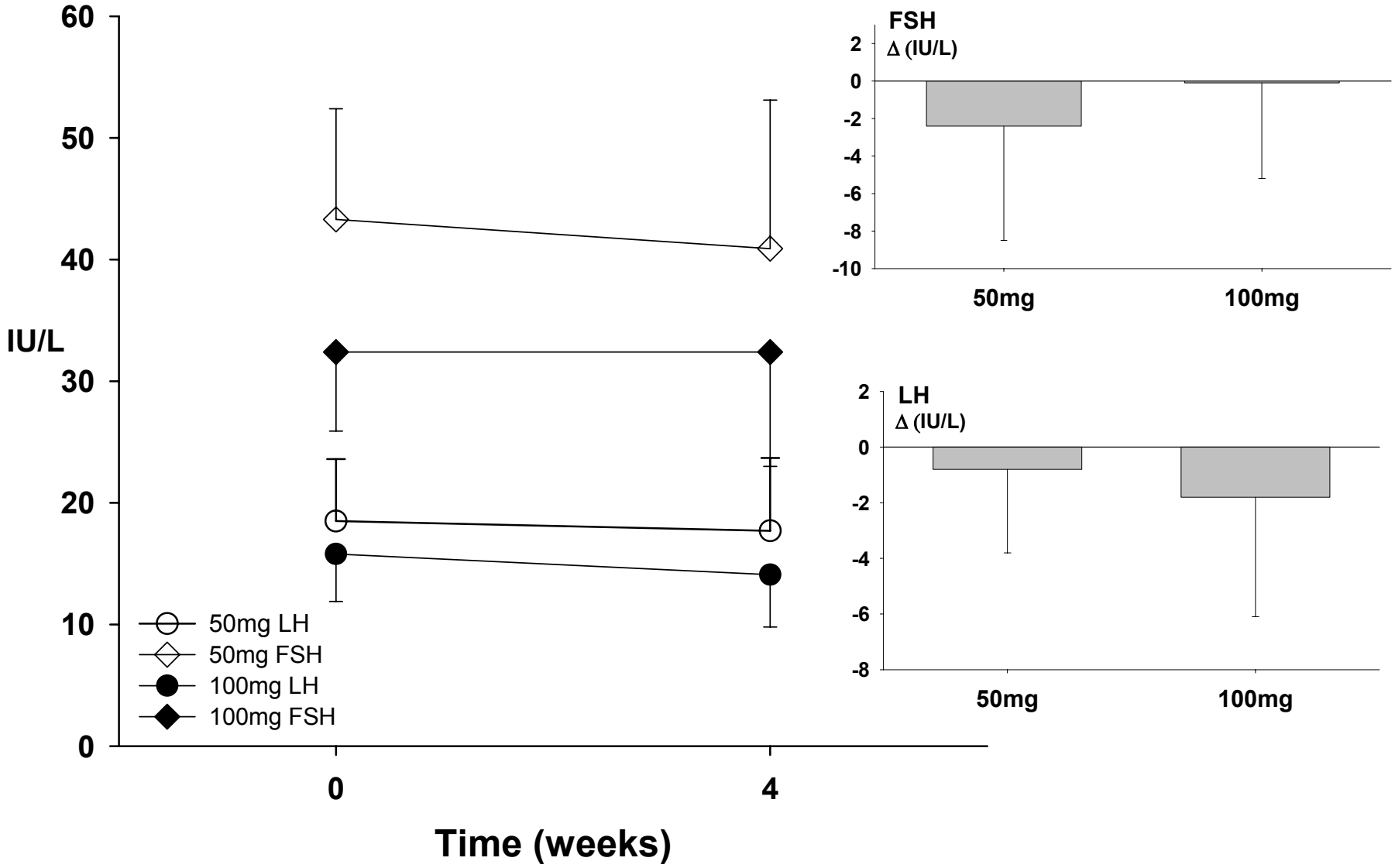

Testosterone
(nmol/L)
40
35
30
25
20
15
10
5
0
Week 0
Week 4
Week 12
0
2
4
6
8
10
12
14
16
18
20
22
24
Hours after application


Week 0
Testosterone (nmol/L)
50 mg/day (N=15)
100 mg/day (N=15)
0
5
10
15
20
25
30
35
40
0 2 4 6 8 10 12 14 16 18 20 22 24
Time (hours)
Week 4
Testosterone (nmol/L)
50 mg/day (N=15)
100 mg/day (N=15)
0 2 4 6 8 10 12 14 16 18 20 22 24
Time (hours)

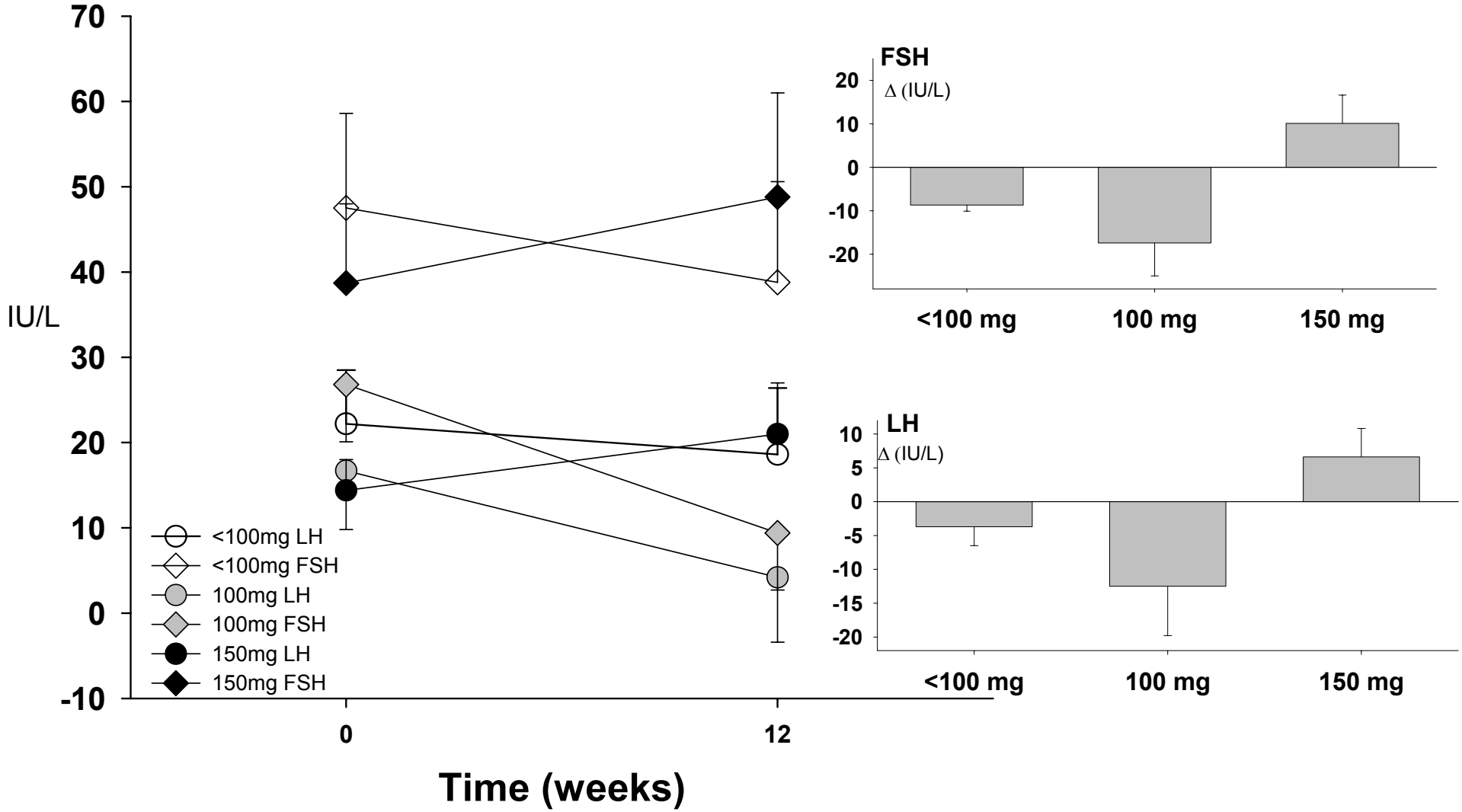
Week 12


Testosterone
(nmol/L)
40
35
30
25
20
15
10
5
0
<100 mg/day (N=5)
100 mg/day (N=15)
150 mg/day (N=10)
0
2
4
6
8
10
12
14
16
18
20
22
24
Time (hours)

Blood LH and FSH According to Initial Dose



Blood LH and FSH According to Final Dose



Appendix - Validation Of Testosterone Assay for Human Serum

Summary And Explanation Of The Assay

(Adapted from the manufacturer's product information)

Testosterone is the predominant androgen secreted by mammalian testes (1, 2, 3). Testicular testosterone production is principally controlled by pituitary LH and peripheral blood testosterone levels correlate well with net testicular androgen production as whole body testosterone clearance rate is relatively stable. The link with LH is particularly clear during the pubertal rise of testosterone levels in boys (4, 5, and 6). In women the circulating concentration of testosterone is roughly 10% of that in men, roughly equivalent to castrate males or prepubertal children. In women blood testosterone originates from the ovary and the adrenal cortex as well as by conversion of androgenic precursor steroids in peripheral tissues (2, 3). In males, testosterone measurement is used as a test of Leydig cell function. Patients with primary or secondary hypogonadism of various origins (Klinefelter's syndrome and other chromosomal alterations, hypopituitarism, enzymatic defects of androgen synthesis) show subnormal levels of testosterone (7). Delayed puberty can also be due to androgen deficiency. Testosterone and gonadotrophin determination may also be useful in the examination of erectile dysfunction since this syndrome is frequently the first sign of disease of the hypothalamic pituitary axis (8). In secondary hypogonadism and in male infants with cryptorchidism, an increase in testosterone levels after hCG administration indicates the presence of functional testicular tissue (9,10, 11).

Principle Of The Assay

The DELFIA testosterone assay is a solid phase fluoroimmunoassay based on competition between europium-labelled testosterone and sample testosterone for polyclonal anti-testosterone antibodies (derived from rabbit). Standard, control and patient samples containing testosterone inhibit the binding of the europium-labelled testosterone to the antibody molecules. The blocking agent in the testosterone assay buffer facilitates the release of testosterone from binding proteins in the sample. A second antibody, directed against rabbit IgG, is coated to the solid phase and binds the IgG-testosterone complex, giving convenient separation of antibody-bound and free antigen. Thus the assay requires only one incubation step.
Enhancement solution dissociates europium ions from the labelled testosterone into solution, where they form highly fluorescent chelates with components of the Enhancement solution. The fluorescence from each sample is inversely proportional to the concentration of testosterone in the sample (12,13,14,15).

Specificity: Cross Reactions

Cross-reactions of related steroids were determined by direct incubation of the steroids with the testosterone antibody used in the assay. The percent cross reactivity was determined at a level of 50% bound for significant cross reactants (see Table 1)

TABLE 1 Cross reactivity at the 50% displacement level

Substance	Cross reactivity %
5α-Dihydroxytestosterone	12.0
5α-Androstan-3β,17β-diol	0.8
5α-Androstan-3α,17β-diol	0.3
Androstanedione	0.2
Androstenediol	0.2
Cortisol	0.02
Androsterone	<0.01
Dehydroisoandrosterone	<0.01

Estradiol-17β	<0.01

Method

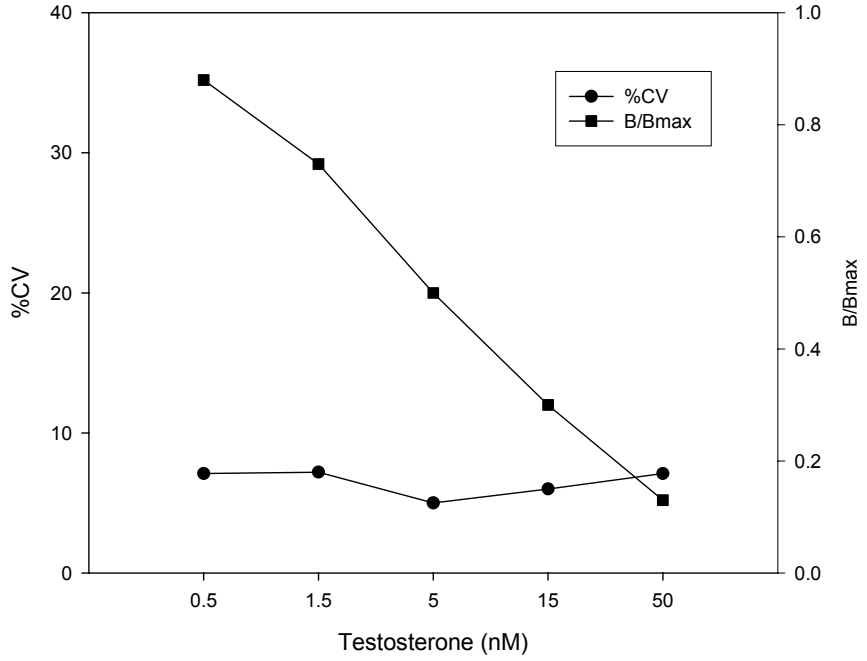
Testosterone was measured in unextracted male blood samples using the DELFIA Testosterone kit (catalogue number A050-101).

Validation Results And Discussion

Standard Curve

A standard curve and precision profile obtained with the DELFIA Testosterone assay are shown below. The precision profile was calculated from 300 duplicate measurements of standards and serum specimens using the MultiCalc data management program. All %CV in the range covered by the standard curve easily fall below 10%.

Graph 1
Performance Characteristics



Calibration

Testosterone standards in the range 0 to 50nM provided with the kit are calibrated using gravimetric methods.

Precision

The variation of the DELFIA assay was determined in 9 runs with 14 replicates and the analysis of variance approach was used to calculate the results in Table 2.

TABLE 2 - Assay precision

Serum sample	Total mean value nM	Intra-assay variation %CV	Inter-assay variation %CV	Total variation %CV
1	2.0	8.2	9.9	12.8
2	11.4	3.7	4.5	5.8
3	24.8	3.7	3.9	5.3

Limit Of Detection
The assay limit of detection was determined by measuring in one assay 24 replicates of the zero standard. The dose that is equivalent to the mean response minus 2 standard deviations is defined as the limit of detection. This was determined to be 0.1nM.

Recovery
Spiked serum samples were prepared by adding varying levels of testosterone to pooled serum specimens containing known amount of testosterone. Recoveries were in the range 83%-115% with a mean value of 99% (n=5).

Description Of Analytical Run
Each testosterone assay contained a full standard curve including zero samples together with quality controls intermixed amongst the patient samples.

Description Of Quality Controls
Quality control samples for testosterone are serum samples containing varying levels of testosterone. They are made according to standard operating procedure to last at least three years. Commercial controls are not used so that the laboratory can be assured of a long-term supply of material that has not been supplemented with exogenous calibrants.

Assay Acceptance Criteria
These criteria that follow are based on control pools, replicates, standard curve parameters and recoveries. The senior supervisor or manager reviews each assay and results. In addition to easily definable assay parameters, assays are evaluated in terms of whether the results make clinical sense and in terms of expected levels for the patient's age, condition, treatment and other relevant parameters. Additional criteria may be specified by a study sponsor for acceptance of results or the re-assay of samples. These criteria may not be less stringent than those established for the routine use of the assay.

Results Evaluation
1. The run should have an acceptable standard curve
2. Two control pools may not exceed 95% (+/- 2SD) confidence interval. If two control pools do exceed this limit, corrective action must be taken. Corrective action consists of repeating all or some samples, or re-evaluating the assay using a previous acceptable curve.
3. Internal repeats differences should be comparable to the control pool limits in the sample range. Samples with variation greater than these limits should be repeated.
4. Samples greater than 50nM should be repeated with appropriate dilutions.
5. The minimum reportable limit is 0.1nM.

Results Reporting
Review procedures and result reporting procedures have been established to avoid dissemination of erroneous results. Assay readings are transferred electronically from the fluorescence meter to a computer that calculates results using MultiCalc, a program provided by Perkin Elmer. Only after all assay quality control parameters and each individual patient or study result is reviewed are results released.

Robustness
This Delfia method has been in continuous use in the laboratory since 1998. Assay control data is collected and summarised on a regular basis for control reports. These reports demonstrate the long-term stability of this procedure over the previous 12 to 18 months

Specimen Requirements

Blood collected by venipuncture and allowed to clot can be separated by centrifugation and serum collected for assaying. Plasma containing EDTA or citrate cannot be used due to chelating effects on europium. Heparinised plasma can also be used. If testosterone values are higher than 50nM, the samples should be diluted with DELFIA zero diluent and the result multiplied by the appropriate factor. Samples can be stored for 12 hours at room temperature (+20 - +25C) or 3 days at +2- +8C. For longer periods store samples at -20C. Repeated freezing and thawing should be avoided.

Expected Values

Reference ranges were measured from 127 apparently healthy men (aged 17- 65 years). These men had a mean value of 20nM (range 8.7 – 33nM).

Sample Integrity

While some hormones are stable for long periods at room temperature or refrigerated, others are not. Therefore we maintain samples frozen to ensure specimen integrity. Samples that are not shipped by the client directly are picked-up and shipped frozen by couriers. All samples except whole bloods are transported frozen on dry ice. Monitoring systems are maintained to track shipping delays ands thawed boxes in order to resolve problems that may occur. Specimen processing includes at least the following steps.

1. Boxes are received and checked. Boxes are opened and checked to make sure dry ice is still in the container.
2. Unusual or thawed samples are placed on dry ice in a separate query box. This system is used to review and follow up possible problems.
3. Individual samples are associated with their appropriate paper work
4. A unique lab number is given to each sample
5. When samples are processed for assaying lab numbers, volumes and sample integrity are checked

Quality Control

Procedures are maintained by the laboratory to assure accuracy of test results. All assays are run with a standard curve with each batch. Assay quality control pools, standards and blanks are monitored with each run and quality control data are calculated and reviewed by experienced personnel. Rejection criteria are established for all assays. Long-term supplies of control are prepared to provide better long-term monitoring of assay variation.

A comprehensive preventative maintenance is maintained on all critical equipment such as centrifuges, counters, fluorometers, pipettors and diluters. Calibration of thermometers, pipetting devices and other small lab instruments are performed regularly.

Long-term frozen storage of samples facilitates any required repeat or additional testing on difficult specimens. Samples are never discarded without consultation with clients or sponsors.

Service to assist clients with interpretation of data is available from technical staff as well as nationally recognised physicians.

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