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Treatment of pain in fibromyalgia patients with testosterone gel: Pharmacokinetics and clinical response

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ABSTRACT

To test our hypothesis that testosterone deficiency plays an important role in chronic pain, a Phase I/II pilot study was initiated with 12 fibromyalgia patients to verify that a daily dose for 28 days with transdermal testosterone gel would 1) significantly and safely increase mean serum testosterone concentrations from low baseline levels to mid/high-normal levels, and 2) effectively treat the pain and fatigue symptoms of fibromyalgia. Pharmacokinetic data confirmed that serum free testosterone concentrations were raised significantly above baseline levels, by assessment of maximum hormone concentration (C_{max}) and area under the curve (AUC) parameters: free testosterone C_{max} was significantly raised from a mean of 2.64 pg/mL to 3.91 pg/mL ($p < 0.05$), and 24 hour free testosterone AUC was significantly raised from a mean of 35.0 pg-hr/mL to 53.89 pg-hr/mL. Assessment of the typical symptoms of fibromyalgia by patient questionnaire and tender point exam demonstrated significant change in: decreased muscle pain, stiffness, and fatigue, and increased libido during study treatment. These results are consistent with the hypothesized ability of testosterone to relieve the symptoms of fibromyalgia. Symptoms not tightly related to fibromyalgia were not improved.

1. Introduction

Although fibromyalgia can strike at an early age, the mean age of onset in women has been reported as 44–53 years [1–3], similar to the mean age for menopause, 51 years [4]. Fibromyalgia affects 3.4% of women and 0.5% of men [5] or 2–4% of the population [6]. The incidence among women aged 50–80 is even higher at 6–7% [6]. Although criteria for diagnosing fibromyalgia in the clinic are well defined [7–9], the condition remains difficult to treat. The obvious therapeutic approaches for fibromyalgia or chronic pain all have risks: First, non-steroidal anti-inflammatory drug analgesics (NSAIDS), when used on an on-going basis for chronic conditions, can cause significant GI tract irritation. Secondly, the morphine-related opioid analgesic class of drugs such as oxycodone and hydromorphone can cause hyperalgesia [10], addiction, testosterone deficiency [11], and a lack of efficacy or "tolerance" develops over time, in which the morphine antinociceptive dose–response curve is displaced to the right [12]. Opioids are not recommended for people with fibromyalgia for these reasons. Third, the anti-depressants can have side effects that include sexual dysfunction and decreased libido [13]. Current practices for treatment also include non-pharmacologic therapies such as exercise, cognitive behavioral therapy, alternative medicine therapies and CNS neurostimulatory therapies [7,14]. All these approaches are less than optimal for providing relief either because of their well-recognized significant risks and/or suboptimal efficacy. Here we use fibromyalgia as an FDA preferred

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; AUC, area under the curve; BMI, body mass index; BP, blood pressure; BUN, blood urea nitrogen; CI, confidence intervals; C_{max}, maximum concentration; CNS, central nervous system; EIA, enzyme linked immunoassay; FSH, follicle-stimulating hormone; HDL, high density lipoprotein; HSDD, hypoactive sexual desire disorder; LDL, low density lipoprotein; N, normal; NSAID, nonsteroidal anti-inflammatory drug; SHBG, sex hormone binding globulin; SNRI, serotonin–norepinephrine reuptake inhibitor; TP, tender point; VAS, visual analog scale.

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model for the study of chronic pain [15] in recognition of the need for more targeted therapeutics. Due to a large body of evidence that links testosterone to pain, as detailed in a companion Commentary in this issue, it was hypothesized that testosterone therapy in these patients would significantly and effectively reduce pain without the side effects of the above commonly used therapeutics. Further, the side effects of androgen excess can be avoided when testosterone is delivered safely such that serum levels of bioavailable or free testosterone are within the reference range of the test used for detection.

In the phase I/II pilot study described here, we tested a novel hypothesis, that muscle pain and chronic fatigue, primary symptoms in women with fibromyalgia [7–9], are caused by testosterone deficiency, and that testosterone therapy can be safely delivered and it can significantly dampen chronic pain without the well known side effects of opioids, anti-depressants, NSAIDS or benzodiazepines, all of which have been prescribed for fibromyalgia.

The American College of Rheumatology diagnostic criteria for fibromyalgia [7–9] were used as a set of parameters for tracking the efficacy of testosterone replacement therapy in dampening pain in these patients. Fibromyalgia patients treated transdermally with a testosterone gel formulation once a day (8:00 AM) for 28 days responded well with respect to raising both free testosterone and total testosterone serum levels significantly and safely above baseline. In addition, clinical symptoms of muscle pain, stiffness and fatigue were significantly decreased, libido was significantly increased, and symptoms not tightly associated with fibromyalgia failed to show a response.

2. Patients, materials and methods

2.1. Patient selection

Women were recruited by advertising, and all study protocols were approved by the institutional review board. Subjects were aged 40–55 and diagnosed with fibromyalgia using the American College of Rheumatology criteria [7–9]. See Table 1 for an overview of patient selection criteria, and Table 2 for demographics. Women were included if they agreed to keep their medicines unchanged during the study, except for analgesics which were permitted to be decreased. Women who had been taking hormone replacement therapy were enrolled if they were off hormone therapy at least 2 weeks prior to, and for the duration of, the study. Pre- or peri-menopausal women were required to have adequate alternative contraception and a negative pregnancy test. Treatment was started within the early follicular (proliferative) phase of the cycle. Patients were included if they were willing to exercise 20 min a day, 5 days per week during therapy to promote the effects of testosterone, a requirement requested by the local Institutional Review Board despite the common understanding that exercise is difficult for individuals with fibromyalgia. A placebo-controlled study will be required to better evaluate the relative importance of testosterone vs exercise.

Table 1 Patient selection criteria.

Primary screening:	
Sex, age	Female, 40–55
Diagnosis	ACR criteria for fibromyalgia
Exclusion criteria	Pregnant, on hormone therapy, undiagnosed vaginal bleeding, BMI > 30, ethanol or illicit drug abuse, active thrombophlebitis, breast cancer, hypertension, major skin disease, acne, hirsutism
Secondary screening:	
Cardiac exclusion criteria	Total fasting cholesterol > 240 mg/dL, HDL < 35 mg/dL, LDL > 210 mg/dL, triglycerides > 300 mg/L
Hepatic function exclusion criteria	ALT > 1.5 × N (normal at 0–40 U/L), alkaline phosphatase > 2 × N (normal at 40–120 U/L), AST > 1.5 × N (normal at 10–30 U/L), serum albumin > N (normal at 3.2–5.2 g/dL), total bilirubin > N (normal at 0.2–1.3 mg/dL), direct bilirubin > N (conjugated, soluble; normal at 0.0–0.3 mg/dL)
Kidney function exclusion criteria	BUN > 2 × N (normal at 8–18 mg/dL), serum creatinine > N (normal at 0.7–1.2 mg/dL)
Hematologic function	Normal CBC, normal hemoglobin (12–16 g/dL)
Additional serum tests	Total testosterone in the lower half of the reference range (excluded if >0.4 ng/mL); an FSH <22 IU/L indicated pre- or peri-menopausal status and thus the need for adequate contraception during therapy
Diet	Patient must agree to stop taking St. John's wort (induces catabolism of hormones by activating liver CYP3A)

Table 2
Patient demographics.

Pat ID#	Age	Ht (in)	Wt (lb)	BMI
001	54	66	155	25.1
002	54	62	139	25.9
005	51	64	150	26.0
006	53	60	130	25.5
007	54	62	160	29.3
009	53	67	160	25.1
010	45	67	175	27.5
011	50	62	135	24.8
012	54	62	122	22.4
016	55	64	140	24.3
017	45	62	140	25.7
018	42	64	130	22.4
Mean	51	63	145	25.3
Median	53	63	140	25.3
Minimum	42	60	122	22.4
Maximum	55	67	175	29.3

2.2. Screening by patient preliminary questionnaire

Pregnant women and women on hormone therapy, hormone contraceptives or infertility drugs were excluded. Women were excluded from the study if they reported undiagnosed vaginal bleeding, had a body mass index BMI > 30, admitted to ethanol or illicit drug abuse, had active thrombophlebitis, breast cancer, hypertension (BP > 160 systolic/95 diastolic with or without medication, after sitting for 5 min), or major skin disease, acne or hirsutism. Prior to serum testing, the most frequent exclusion criterion was for BMI >30.

2.3. Secondary screening. Sex steroid hormone status

The American Society for Reproductive Medicine's Princeton Consensus statement on female androgen insufficiency recommended therapy for women with serum free testosterone concentrations in the lowest quartile of the normal premenopausal range [16]. Since sufficient numbers of women with deficiency will skew the bottom of any test kit reference range downward, the study here enrolled women whose preliminary test demonstrated levels for total testosterone in the lower half of the reference range (16 of 18 fibromyalgia patients who were initially evaluated, see Section 2). Adequate estrogen status has also been recommended to be provided by the Princeton consensus statement panel, although, after the WHI studies [17,18], estrogen/progestin hormone replacement therapy remains a topic of discussion within the clinic and it was not evaluated here.

2.4. Physical exam and blood tests

Prior to enrollment, physical examination was performed and overnight fasting patient blood was collected and tested for cardiovascular risk factors (lipid levels), hepatic function, kidney function, hematologic function, FSH and total testosterone (details in Table 1). Blood was collected at 8:00 a.m., the zenith of the circadian cycle of circulating androgens [19]. Patients were cycled so that day 1 of

therapy was within the early proliferative phase of the 28 day cycle for premenopausal patients. Both total and free testosterone levels are known to peak just before the pre-ovulatory LH surge in normal premenopausal women [20]. Patients were excluded for serum total testosterone >0.4 ng/mL (the upper half of the reference range). Patients with FSH <22 IU/L (premenopausal status) were required to use non-hormonal contraception. Patients who had undergone bilateral oophorectomy were not tested for FSH and were not required to have adequate contraception. Patients were required to stop taking St. John's wort, since St. John's wort is known to induce catabolism of sex steroid hormones by activating CYP3A, a detoxifying enzyme complex in the liver [21,22]. Physical exam and blood tests were repeated at the end of the study to assess whether testosterone therapy adversely affected the general health of the study patient.

2.5. Study protocol

The study protocol was approved by the Dartmouth College Committee for Protection of Human Subjects. Written informed consent was obtained from study subjects prior to entry into the study, and the consent process was ongoing throughout the study. An independent Data Safety Monitoring board was in place. Twelve patients who met the eligibility criteria above were scheduled for physical exams including tender point assessment [9], verification of fibromyalgia diagnosis [7–9], and assessment of general health. On day 1, blood was drawn by venipuncture at 0, 1, 2, 3, 4, 6, 8, 10, 12 and 24 h for 24 hr pharmacokinetic profiling of testosterone serum concentrations. Testosterone gel, 0.75 g 1% w/w (0.75 mg bioavailable, see below), was applied by the patients to their lower abdominal skin just after the zero time point blood draw (8:00 a.m.). The patient also filled out a pain assessment questionnaire form (see pain assessment, below) and was given packets of testosterone gel for 8:00 a.m. daily application to lower abdominal skin, instructions for use and a patient medication log and exercise log for 28 days of therapy. On day 28, the blood draws for 24 hr pharmacokinetic measurements were repeated, and a follow-up exam was repeated at the end of the 28 days of therapy.

2.6. Formulation of testosterone gel and guidance for treatment

The gel used for this study was a 1% w/w testosterone gel, USP grade. The daily gel dose applied to the lower abdominal skin was 0.75 g of the 1% w/w testosterone gel; an expected bioavailability of 10% would deliver 0.75 mg testosterone over 24 h. The gel was formulated with women in mind by Bentley Pharmaceuticals, Inc. (North Hampton, NH; subsequently CPEX Pharmaceuticals, Inc.) using GLP conditions, and was colorless, comfortable on the skin with no irritation, quick drying and non-staining. The gel was produced at Bentley Pharmaceuticals under DEA license. Testosterone is a Schedule C-III controlled substance (Anabolic Steroid Control Act). Thus, all testosterone treatment samples were itemized, and accounted for at the end of the study.

The American Society for Reproductive Medicine's Princeton Consensus Statement on female androgen insufficiency has recommended treating women only if clinical symptoms such as diminished sense of well-being and chronic fatigue are clearly present [16]. The North American Menopause Society has also weighed in on this topic, recommending treatment for clinical symptoms of deficiency such as decreased sexual desire while monitoring blood levels to avoid supraphysiologic levels [23]. Most recently, the Endocrine Society evaluated androgen therapy for women, providing a clinical practice guideline [24]. Androgen therapy in healthy women was advised against, however deficiency symptoms of chronic pain were not considered. It was recommended that any woman who did receive testosterone therapy be monitored for signs and symptoms of androgen excess, a recommendation that was adhered to here.

2.7. Testosterone serum concentrations

Testosterone concentrations were determined by enzyme linked immunoassay (DSL-10-4900 Active Free Testosterone EIA; DSL-10-4000 Active [Total] Testosterone EIA; Diagnostic Systems Laboratories or DSL, Inc., Webster, Texas), where serum testosterone from study subjects competed with enzyme-linked testosterone bound to anti-testosterone mAb. This assay system was selected based on its ability to detect the lower concentrations of testosterone found in women as well as concentrations in the upper ranges. Free testosterone concentrations were determined by EIA using an anti-testosterone antibody that recognizes the unbound testosterone in the test sample, and has low affinity for sex hormone binding globulin and albumin.

Upper and lower limits for the DSL testosterone assay reference ranges are indicated by the dashed horizontal lines in Figs. 1 and 2, and are defined as follows: total testosterone for women aged 30–40 (generally premenopausal), 0.1–2.20 ng/mL, mean 0.50 ng/mL, StDev 0.54 ng/mL (n = 16); total testosterone for women aged 40–50 (this study, generally pre–peri–postmenopausal), 0.1–0.9 ng/mL, mean 0.31 ng/mL, StDev 0.23 ng/mL (n = 15); total testosterone for women aged 50–60 (this study, generally peri–postmenopausal), 0.1–0.69 ng/mL, mean 0.27 ng/mL, StDev 0.19 ng/mL (n = 16); free testosterone for premenopausal women 0.33–3.29 pg/mL, mean 1.42 pg/mL, StDev 0.09–0.22 pg/mL, (n = 38); and free testosterone for postmenopausal women 0.56–1.86 pg/mL, mean 1.28 pg/mL, St Dev 0.09–0.22 pg/mL, (n = 12). Total testosterone sensitivity was 0.04 ng/mL (n=20); free testosterone sensitivity was 0.16 pg/mL (n=20). Percent cross-reactivity for total testosterone was: testosterone 100%, 5-alpha-dihydrotestosterone 6.6%, and androstenedione 0.9%, all other steroids tested <2.2%. Percent cross-reactivity for free testosterone was: free testosterone 100%, 5-alpha-dihydrotestosterone not detectable, androstenedione 0.006%, all other steroids tested <0.3%.

For the purposes of determining mean testosterone concentrations, times were based on the nearest hour. Of the 240 time points taken for the pharmacokinetic data (10 time points per individual × 2 2 sets per individual × 12 individuals), 1 time point was missed (#012, 4 h point) and 3 additional time points were in-between the standard times for taking blood (#010, 8 h point; #012, 4 h and 10 h points). Values for these time points were derived by interpolation for the purposes of deriving mean testosterone concentrations.

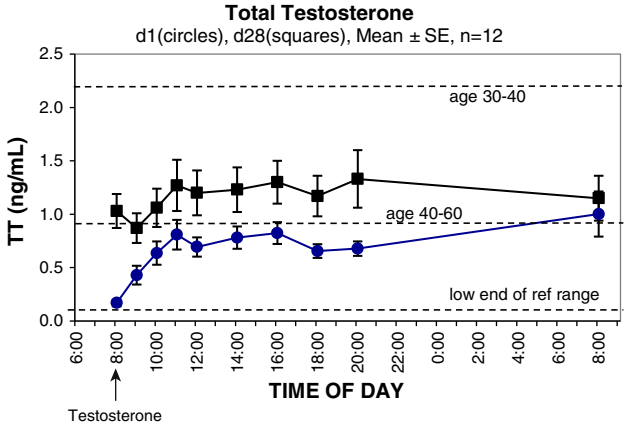

Fig. 1. Serum total testosterone concentrations in fibromyalgia patients are increased and normalized in response to testosterone gel therapy. Blood was taken by venipuncture from twelve fibromyalgia patients who fit the eligibility requirements, on day 1 at the times indicated and then again on day 28 of therapy (24 hr time scale on the horizontal axis; for each 24 hr profile, serum sampling started at 8:00 AM and ended the next day at 8:00 AM). Low and high ends of the reference range are as indicated by the dashed horizontal lines, and as specified in Section 2: low end, 0.1 ng/mL; high end for age 40–60 (this study), 0.90 ng/mL; high end for age 30–40, 2.20 ng/mL. Means ± SEM for day 1 (open symbols) vs day 28 (filled symbols). 95% CI (confidence interval) analysis demonstrated statistical significance for these data.

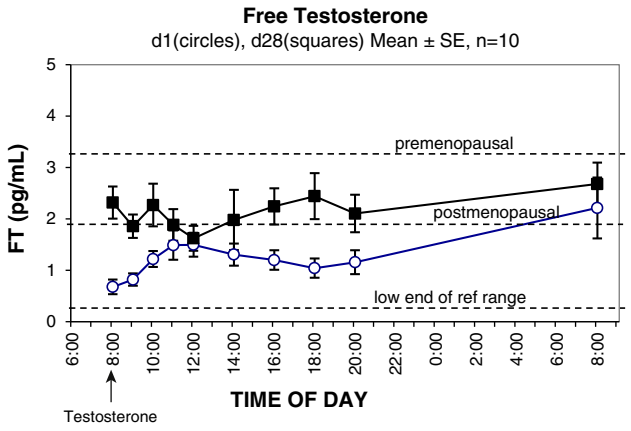

Fig. 2. Serum free testosterone concentrations in fibromyalgia patients are increased and normalized in response to testosterone gel therapy. Serum free testosterone concentrations were quantitated using methodology similar to Fig. 1. The low end of the reference range for pre- and post-menopausal women is 0.33 pg/mL, represented by the lower dashed horizontal line. The high end of the reference range for premenopausal women is 3.3 pg/mL, represented by the upper dashed horizontal line; and the high end for postmenopausal women is 1.9 pg/mL, represented by the middle dashed horizontal line. Means ± SEM for day 1 (open symbols) vs day 28 (filled symbols). 95% CI analysis demonstrated statistical significance for these data.

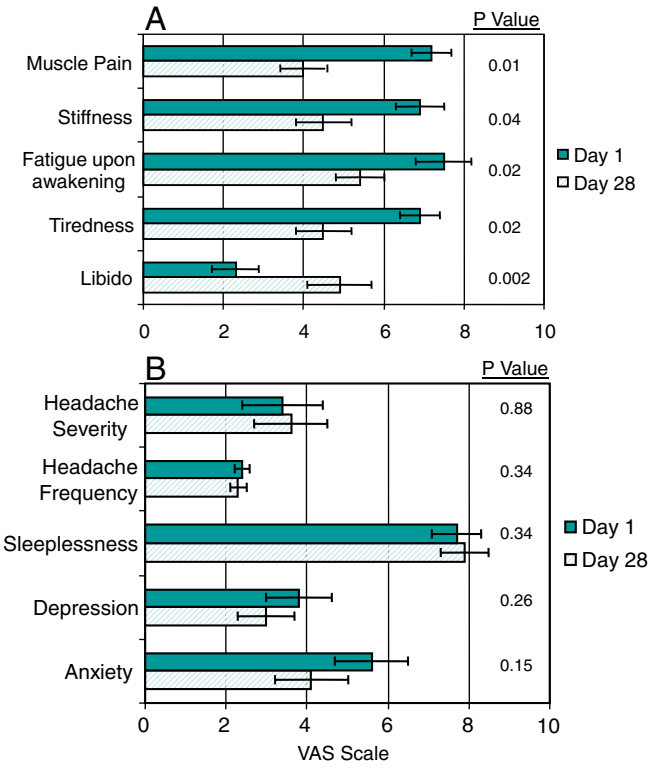

Fig. 3. All typical symptoms of fibromyalgia were improved in fibromyalgia patients after testosterone gel therapy. Patients were administered a Patient Questionnaire Form by the study coordinator to assess their feelings of pain. For the categories of muscle pain, stiffness, fatigue upon awakening and tiredness, the number 10 represents the worst severity of symptoms (decreases expected in response to therapy), with the exceptions that headache frequency was measured on a scale of 1–4, and "sleeplessness" was a "fatigue upon awakening" background information parameter for the number of hours of sleep each individual thought was optimal for themselves. For libido, the number 10 represents the strongest feelings of libido (sex drive; increases expected in response to therapy). Day 1, dark bars. Day 28, hatched bars. A, parameters that are highly prevalent in the fibromyalgia patient population [29]; B, parameters of headache, depression and anxiety are more weakly prevalent in the fibromyalgia patient population. Panel A, P values were statistically significant for all parameters, $p < 0.05$. Panel B, P values demonstrate lack of statistical significance for all parameters, $p > 0.05$.

A noncompartmental pharmacokinetic analysis using WinNonlin Pro (Pharsight, Mountain View, California) used the exact time points recorded for all the patients.

Two subjects (#010 and #016) tested abnormally and unrealistically high for free testosterone, well above the reference range for women, likely due to interference with the EIA antibody by some unknown serum factor present in those individuals. Justification for exclusion of those data in Fig. 2 and Table 4, provided in Section 3.2, includes the fact that those individuals had normal total testosterone levels and did not have any clinical signs of hyper-androgenization such as hirsutism which they would have had if their free testosterone levels had actually been that high. Because those data points were excluded from Fig. 2, the data are provided here: For day 1/day 28, subject #010: 0 h, 5.25/7.75 pg/mL; 1 h, 4.48/6.32 pg/mL; 2 h, 6.86/6.47 pg/mL; 3 h, 5.52/5.99 pg/mL; 4 h, 3.40/6.60 pg/mL; 6 h, 3.85/7.14 pg/mL; 8 h, 4.19/6.46 pg/mL; 10 h, 3.75/6.00 pg/mL; 12 h, 4.93/6.48 pg/mL; and 24 h, 6.65/10.88 pg/mL. For day 1/day 28, subject #016: 0 h, 6.45/7.08 pg/mL; 1 h, 6.60/5.77 pg/mL; 2 h, 4.77/6.83 pg/mL; 3 h, 4.46/5.98 pg/mL; 4 h, 6.39/8.649 pg/mL; 6 h, 6.32/7.44 pg/mL; 8 h, 5.05/6.41 pg/mL; 10 h, 3.77/7.01 pg/mL; 12 h, 7.49/4.73 pg/mL; and 24 h, 6.98/9.63 pg/mL. Test reagents were extensively controlled by positive and negative controls, comparison assays, intra-assay and inter-assay controls, etc. to ensure all other testosterone EIA testing performed as expected.

2.8. Pain assessment

Patients were administered questionnaire forms on day 1 and again at the end of therapy on day 28 to assess pain and the other symptoms of fibromyalgia over the past week. The patient questionnaire was based on a published and validated Fibromyalgia Impact Questionnaire as well as other accepted criteria for fibromyalgia patient assessment [9,25,26] and included the following parameters: muscle pain, stiffness, fatigue upon awakening, tiredness, libido, depression and anxiety. A verbally anchored 100 mm visual analog scale was used (VAS, denoted as 0–10 in Fig. 3, and see Results for verbal anchors used). Tender point exams were administered by a qualified rheumatologist experienced in treating women with fibromyalgia, and involved applying approximately 9 lb (4 Kg) of pressure at each tender point, asking whether the patient felt pain, and scoring pain on a 10 point scale with verbal anchors of "no pain" and "severe pain", data in Fig. 4. This practice was in accordance with criteria specified by the American College of Rheumatology. Exams were administered just prior to Day 1 of therapy (and therefore designated as "pretreatment") and at the end of therapy. The pretreatment tender point assessment was performed on all patients within 1 week before the start of therapy. Dolorimeter readings were taken from the bilateral second costochondral junction and trapezius tender points, for comparison, in 11 of the 12 study subjects, and the resultant data supported the non-dolorimetry tender point exam results (dolorimetry data not shown).

2.9. Statistical analysis

Pharmacokinetic analysis of serum testosterone concentration vs time data was carried out using WinNonlin Pro software using the noncompartmental model with extravascular input. Differences between Day 1 and Day 28 C_{max} and AUC were assessed by calculating individual subject Day 28 minus Day 1 data and estimating 95% confidence intervals of this difference to determine if significance ($p < 0.05$) was reached. Tender point evaluations were analyzed by Student's t test (paired, 2-tailed) after summing all 18 tender point values for each individual at baseline vs d28 (end of study).

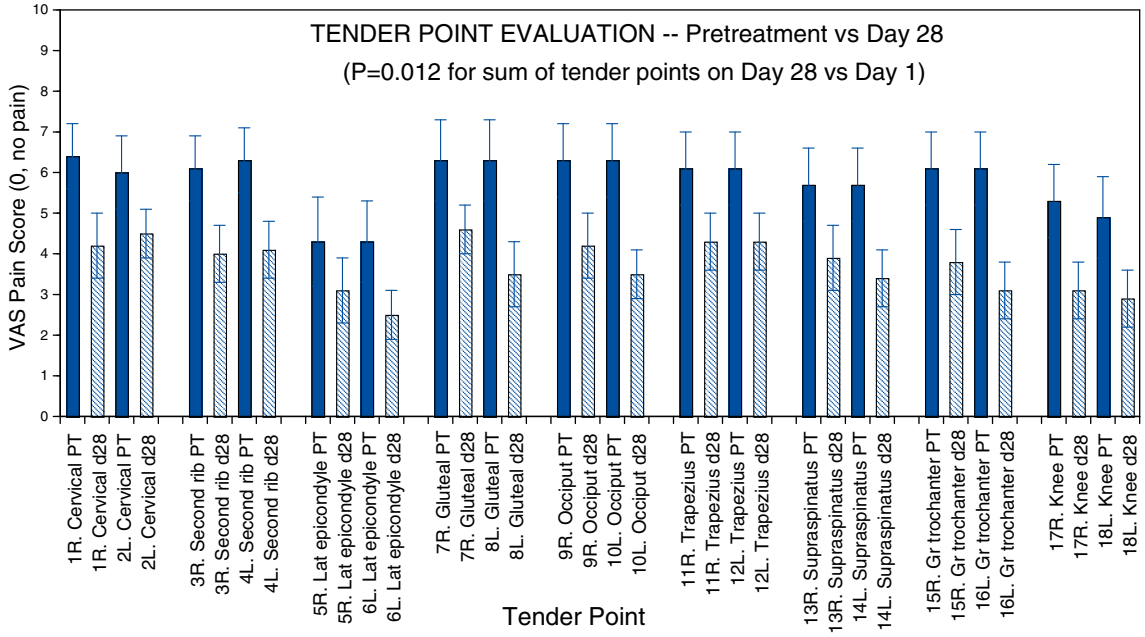

Fig. 4. Tender point pain was decreased in fibromyalgia patients after testosterone gel therapy. Tender point exams were administered by the study rheumatologist. Pain was assessed on a visual analog scale VAS of 0–10, with zero being no pain, and 10 being the most pain. Means of scores ± SEM are shown. The p value, determined by summing all tender point values for each patient at the baseline vs d28 time points and assessing by paired Student's t test, was $p = 0.012$. Dark bars, pretreatment (PT). Hatched bars, Day 28. R, right side tender point; L, left side tender point.

3. Results

3.1. Serum total testosterone concentrations were significantly increased in fibromyalgia patients in response to treatment with testosterone gel hormone replacement therapy

Twelve fibromyalgia patients 40–55 years of age who met the study eligibility criteria (Table 1, Section 2) were enrolled for this study and treated. Patient demographics for age, height and weight are shown in Table 2. All patients were white Caucasian females, reflective of demographics in northern New England. Serum total testosterone concentrations vs time data for Day 1 and Day 28 are shown in Fig. 1. Reference ranges for the concentration of total testosterone in serum from women 40–50 years of age and 50–60 years of age are provided in Section 2, and denoted by dashed horizontal lines in Fig. 1. The day 1 zero time point for total testosterone confirmed that these patients initially had total testosterone concentrations in the lower half of the reference range. The mean serum concentration of total testosterone 24 h after application of the first dose of hormone on Day 1 was significantly higher than the mean serum concentration for time zero on Day 1 (Fig. 1, $p = 0.01$). Steady state serum concentrations were reached quickly in the aggregate by 24 h on day 1, as confirmed on day 28 via similar mean concentrations at both the beginning and end of the 24 h sampling. Upon treatment, mean total testosterone values were raised to just above the reference range for women aged 40–60 (42–55 was the age range for this study). All values for day 1 or day 28, however, were well below the premenopausal reference range for women aged 30–40, and also well below the upper end of the total testosterone reference range for men (5–10 ng/mL, using the same DSL test kit). Furthermore, it is the free testosterone serum levels (see Section 4) that are important for defining safe levels since free testosterone (not total testosterone) is an appropriate biomarker for bioavailable testosterone.

Summary pharmacokinetic parameter analysis demonstrated significantly increased mean total testosterone maximum concentration in response to testosterone therapy: C_{max} was 1.92 ng/mL on day 28 compared with 1.21 ng/mL on day 1 (Table 3). Significantly increased mean total testosterone area under the curve values (assessed over the 24 hr profiling time period) were also found: AUC was 28.75 ng-h/mL on day 28 compared with 18.36 ng-h/mL on day 1, $p < 0.05$. The differential C_{max} and AUC values for day 28 after subtraction of day 1 baselines are provided in the right panel of Table 3, with 95% CI analysis demonstrating statistical significance for these data. The data, taken together, demonstrate that with therapy, mean serum total testosterone concentrations initially rose quickly over the first 3 h and were then sustained over time. In

Table 3
Total testosterone pharmacokinetic parameters.

Total testosterone d1 (n = 12)					Total testosterone d28 (n = 12)				d28 − d1 differential		
	Tmax	Cmax	Cmin	AUC (0–24 h)	Tmax	Cmax	Cmin	AUC (0–24 h)	Cmax	Cmin	AUC (0–24 h)
	h	ng/ml	ng/mL	ng·h/ml	h	ng/ml	ng/mL	ng·h/ml	ng/ml	ng/mL	ng·h/ml
Mean		1.21	0.17	18.36		1.92	0.72	28.75	0.7	0.55	10.38
SD		0.71	0.10	7.10		0.90	0.44	13.91	0.96	0.42	12.61
Median	16.11	1.08	0.18	16.88	10.17	1.79	0.61	26.71			
Min	2.33	0.53	0.00	10.35	1.62	0.63	0.21	11.29			
Max	24.75	3.05	0.37	30.95	23.92	3.13	1.67	59.56			
							95% CIs For diff	CI high -> CI low ->	1.36 0.06	0.79 0.31	19.23 1.55

addition, mean serum total testosterone concentrations were raised from baseline levels (day 1, 8:00 am) at the lower boundary of the reference range to just above the upper end of the reference range for women aged 40–60 and below the upper end of the reference range for premenopausal women.

3.2. Low serum free testosterone concentrations were safely raised to premenopausal concentrations in fibromyalgia patients in response to testosterone gel hormone replacement therapy, and the differences in increased serum levels vs baseline levels were statistically significant

Concentrations of free testosterone in serum were similarly analyzed, Fig. 2. Similar results were obtained, but with the following particular findings. Two individuals had unrealistically high concentrations of free testosterone prior to and throughout the course of therapy despite normal total testosterone levels (#010 and #016), suggesting serum interference with the free testosterone testing antibody in these two cases. The only medication or supplement reported by both study subjects #010 and #016 and not by any other subject was ginger root; and the anti-depressant Trazodone was taken by both individuals. Preliminary tests in vitro suggested that ginger root appears to interfere with the enzyme linked immunoassay for free testosterone, analogous to the finding by others that DHEA-S [27,28] can interfere with testosterone immunoassays. Free testosterone data for these two outliers were therefore dropped due to interference with the EIA, whether due to ginger root alone or in combination with Trazodone, for the following reasons: 1) extremely high levels of free testosterone were found at baseline on day 1 at zero time (prior to therapy) for only these two subjects, 2) these high free testosterone levels were not possible given the total testosterone levels found in these same patients (consistent with some factor interfering with the antibody used in the assay), and 3) by the lack of hirsutism and other clinical symptoms that would have occurred in these two subjects if these high baseline free testosterone levels had been real. Individual profiles for the remainder of the patients showed mean serum free testosterone concentrations that significantly increased from the baseline at the lower end of the postmenopausal range to the middle-to-upper half of the premenopausal reference range in response to therapy, and remained safely within the upper end of the premenopausal reference range (Fig. 2).

Summary pharmacokinetic parameter analysis showed a mean free testosterone C_{max} of 3.91 pg/mL on day 28 compared with 2.64 pg/mL on day 1, and a mean free testosterone AUC of 53.89 pg-h/mL on day 28 compared with 35.0 pg-h/mL on day 1, Table 4, with 95% CI analysis demonstrating statistical significance for these data. Thus, free testosterone C_{max} and AUC were significantly increased with therapy on day 28 compared with day 1 baseline values (day 1, zero time).

3.3. Testosterone gel therapy reduced pain in fibromyalgia patients

Pain parameters were evaluated by patient questionnaire using a visual analog scale (VAS) from 0 to 10, and with verbal anchors, along with other parameters (Fig. 3A and B). Muscle pain (0 = "no muscle pain", 10 = "incapacitating muscle pain"), stiffness (0 = "no stiffness", 10 = "very stiff"), fatigue upon awakening (0 = "awoke well rested", 10 = "awoke very tired") and tiredness (0 = "no tiredness", 10 = "very tired/bedridden") were all significantly decreased during testosterone treatment, Fig. 3A. For muscle pain, a defining symptom for fibromyalgia, 42% of patients had 30% or greater decrease in pain, and 33% of patients had 50% or greater decrease in pain after 4 weeks of therapy. Muscle pain VAS score improvement was not dependent on whether the patient had previously had a clinical relationship with the study rheumatologist and therefore might have wanted to please the doctor. Libido (sex drive, 0 = "lowest level", 10 = "highest level") was also significantly increased in response to testosterone treatment. Symptoms not tightly associated with fibromyalgia failed to show any significant response to treatment, Fig. 3B: headache severity and frequency, sleeplessness, depression and anxiety. P values are provided in Fig. 3.

These findings are consistent with our hypothesis that restoration of premenopausal serum testosterone concentrations was expected to relieve symptoms that most specifically relate to testosterone deficiency, for example loss of muscle function, increased fatigue and loss of sexual desire [29]. Further, the results confirm the correct dose was used, necessary information for designing a placebo controlled study. Blood tests and physical exam at the end of the study verified testosterone therapy did not adversely affect the general health of the study patient, and no study patient reported any adverse events that were attributable to the treatment.

Patients were also evaluated for tender point pain prior to the beginning of therapy and at the end of therapy by a rheumatologist (Section 2), with results shown in Fig. 4. Using a pain scale of 0 to 10, where zero is "no pain", 10 is "severe pain", there were mean decreases in pain for every tender point, with statistical significance achieved after summing values across all 18 tender points ($p = 0.012$), a finding that compares favorably to other studies using calcium channel blocker or SNRI therapeutics for fibromyalgia patients [30–33]. Using a dolorimeter at the same office visit to control for the ability to accurately apply pressure by using mechanical means, pain responses were quantitated for the bilateral second costochondral junction and bilateral trapezius tender points. The resultant dolorimetry findings supported the non-dolorimetry tender point data (data not shown).

4. Discussion and conclusions

4.1. Overview of findings

In the prospective study here, the hypothesis that testosterone therapy can decrease pain responses is supported by the clinical data: 28 days of once-a-day therapy with 0.75 g 1% (w/w) testosterone gel 1) raised serum concentrations of total and, most importantly, free testosterone in fibromyalgia patients from baseline to high-normal concentrations for premenopausal women, and 2) significantly reduced the pain and fatigue symptoms of fibromyalgia patients without any

Table 4
Free testosterone pharmacokinetic parameters.

	Free testosterone d1 (n = 10)				Free testosterone d28 (n = 10)				d28 − d1 differential		
	Tmax	Cmax	Cmin	AUC (0–24 h)	Tmax	Cmax	Cmin	AUC (0–24 h)	Cmax	Cmin	AUC (0–24 h)
	h	pg/ml	pg/mL	pg·h/ml	h	pg/ml	pg/mL	pg·h/ml	pg/ml	pg/mL	pg·h/ml
Mean		2.64	0.54	35.0		3.91	1.29	53.89	1.27	0.75	18.9
SD		1.70	0.41	18.3		1.23	0.54	18.71	1.71	0.72	27.1
Median	6.38	2.19	0.41	31.8	9.13	4.16	1.12	51.49			
Min	1.92	0.95	0.09	13.7	0.00	1.81	0.71	27.95			
Max	24.8	6.86	1.35	64.1	24.05	5.58	2.27	90.08			
							95% CIs	CI high	2.33	1.19	35.6
							For diff	CI low	0.21	0.30	2.10

evidence of short term risk as determined by blood tests for cardiovascular, hepatic, kidney or hematologic function, by patient logs and by physical exam. Our findings of increased levels of testosterone taken together with improvements observed for clinical symptoms, including muscle pain, stiffness, fatigue and libido, support our hypothesis that testosterone therapy given to chronic pain patients can reduce pain, down-modulate an inflamed nociceptive nervous system (as discussed in the related Commentary in this issue) and help restore feelings of well-being.

4.2. Delivery of testosterone with a beneficial safety profile

It is the free and/or bioavailable testosterone blood levels (not total testosterone) that indicate the degree of safety because complex metabolic feedback mechanisms normally ensure relatively constant levels of free and bioavailable testosterone even though total testosterone and SHBG levels can vary substantially depending on health status and pharmacologic input [34]. This generally holds true even when total testosterone and SHBG levels are perturbed by drugs or disorders that affect steroidogenesis [34].

The unique gel formulation used here demonstrated steady state blood levels by 24 h of day 1, and even delivery over a 24 hr profile period without spiking due to unsafe delivery of "too-much too-fast" early in the 24 hr period, a significant problem that gels modeled after male formulations, the patch and injection delivery are susceptible to. Our superior delivery is due, in part, to the adipose effect that this particular transdermal gel formulation provides, and it provides a prolonged half-life. The importance of keeping free testosterone levels stable, without spiking, and safely within the reference range is driven home by the morbidities found in individuals with testosterone levels abnormally above the reference range, for example in women with virilizing tumors [35], breast cancer [36], in female-to-male transgenders treated with testosterone [37], and in East German athlete doping [38]. Cause and effect between hyperandrogenism and cardiovascular risk factors has been demonstrated when the hyperandrogenism was corrected resulting in a decreased cardiovascular risk profile, for example [37]. That testosterone can be delivered safely to women was suggested by a five year study from BioSante Pharmaceuticals in postmenopausal women with elevated cardiovascular risk and hypoactive sexual desire disorder (HSDD) who were treated with a testosterone gel (LibiGel) and assessed for cardiovascular and breast cancer risk [39]. The FDA accepted that study as demonstrating safety [40], although LibiGel failed to achieve primary endpoints for efficacy in HSDD patients [41].

4.3. Overall conclusion

In conclusion, patients with chronic pain states, whose baseline blood testosterone levels are in the lower half of the reference range, will likely be responsive to testosterone replacement therapy, clearly supporting the rationale for conducting further studies of testosterone replacement therapy to treat fibromyalgia patients in a formal double blind placebo-controlled clinical trial.

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Potential conflicts of interest

HDW and TDR own shares of White Mountain Pharma, Inc.; RJG was Vice President of Scientific Innovation at Bentley Pharmaceuticals Inc., now CPEX Pharmaceuticals Inc. Neither the New Hampshire Industrial Research Center nor Bentley Pharmaceuticals Inc. had a role in the writing of this report or the decision to submit the paper for publication, and neither has biased the opinions expressed in this manuscript. HDW had overall responsibility for the study design and for the collection, analysis and interpretation of data; has full control of all of the data in this study; and takes complete responsibility for the integrity of the data analyses.

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References

[1] M.A. Cathey, F. Wolfe, S.M. Kleinheksel, D.J. Hawley, Socioeconomic impact of fibrositis. A study of 81 patients with primary fibrositis, Am. J. Med. 81 (1986) 78–84.
[2] J. Waxman, S.M. Zatzkis, Fibromyalgia and menopause. Examination of the relationship, Postgrad. Med. 80 (1986) 165–167.
[3] M.B. Yunus, F. Inanici, Fibromyalgia syndrome: clinical features, diagnosis, and biopathophysiologic mechanisms, in: E.S. Rachlin, I.S. Rachlin (Eds.), Myofascial Pain and Fibromyalgia, Second ed.Mosby Elsevier Science, St. Louis 2002, pp. 3–31.
[4] L.J.B. Jaszmann, Epidemiology of the climacteric syndrome, in: S. Campbell (Ed.), The Management of the Menopause and Postmenopausal Years, University Park Press, Baltimore 1976, pp. 11–23.
[5] S. Chakrabarty, R. Zoorob, Fibromyalgia, Am. Fam. Physician 76 (2007) 247–254.
[6] F. Wolfe, K. Ross, J. Anderson, I.J. Russell, L. Hebert, The prevalence and characteristics of fibromyalgia in the general population, Arthritis Rheum. 38 (1995) 19–28.
[7] D.J. Clauw, Fibromyalgia: a clinical review, JAMA 311 (2014) 1547–1555.
[8] F. Wolfe, D.J. Clauw, M.A. Fitzcharles, D.L. Goldenberg, R.S. Katz, P. Mease, et al., The American College of Rheumatology preliminary diagnostic criteria for fibromyalgia and measurement of symptom severity, Arthritis Care Res. 62 (2010) 600–610.
[9] F. Wolfe, H.A. Smythe, M.B. Yunus, R.M. Bennett, C. Bombardier, D.L. Goldenberg, et al., The American College of Rheumatology 1990 criteria for the classification of fibromyalgia. Report of the Multicenter Criteria Committee, Arthritis Rheum. 33 (1990) 160–172.
[10] J. Mao, Opioid-induced abnormal pain sensitivity, Curr. Pain Headache Rep. 10 (2006) 67–70.
[11] R. Abs, J. Verhelst, J. Maeyaert, J.P. Van Buyten, F. Opsomer, H. Adriaensen, et al., Endocrine consequences of long-term intrathecal administration of opioids, J. Clin. Endocrinol. Metab. 85 (2000) 2215–2222.
[12] J.Y. Xie, D.S. Herman, C.O. Stiller, L.R. Gardell, M.H. Ossipov, J. Lai, et al., Cholecystokinin in the rostral ventromedial medulla mediates opioid-induced hyperalgesia and antinociceptive tolerance, J. Neurosci. 25 (2005) 409–416.
[13] R.S. Gregorian, K.A. Golden, A. Bahce, C. Goodman, W.J. Kwong, Z.M. Khan, Antidepressant-induced sexual dysfunction, Ann. Pharmacother. 36 (2002) 1577–1589.
[14] S.F. Carville, S. Arendt-Nielsen, H. Bliddal, F. Blotman, J.C. Branco, D. Buskila, et al., EULAR evidence-based recommendations for the management of fibromyalgia syndrome, Ann. Rheum. Dis. 67 (2008) 536–541.
[15] FDA CDER, Meeting of the Arthritis Advisory Committee on Fibromyalgia, Transcript, June 23, 2003 (http://www.fda.gov/ohrms/dockets/ac/03/transcripts/3967T1.htm).
[16] G. Bachmann, J. Bancroft, G. Braunstein, H. Burger, S. Davis, L. Dennerstein, et al., Female androgen insufficiency: the Princeton consensus statement on definition, classification, and assessment, Fertil. Steril. 77 (2002) 660–665.
[17] S.W. Fletcher, G.A. Colditz, Failure of estrogen plus progestin therapy for prevention, J. Am. Med. Assoc. 288 (2002) 366–368.
[18] Writing Group for the Women's Health Initiative, Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results from the Women's Health Initiative randomized controlled trial, J. Am. Med. Assoc. 288 (2002) 321–333.
[19] G.C. Lachelin, M. Barnett, B.R. Hopper, G. Brink, S.S. Yen, Adrenal function in normal women and women with the polycystic ovary syndrome, J. Clin. Endocrinol. Metab. 49 (1979) 892–898.
[20] I. Sinha-Hikim, S. Arver, G. Beall, R. Shen, M. Guerrero, F. Sattler, et al., The use of a sensitive equilibrium dialysis method for the measurement of free testosterone levels in healthy, cycling women and in human immunodeficiency virus-infected women, J. Clin. Endocrinol. Metab. 83 (1998) 1312–1318.
[21] J.M. Wentworth, M. Agostini, J. Love, J.W. Schwabe, V.K. Chatterjee, St John's wort, a herbal antidepressant, activates the steroid X receptor, J. Endocrinol. 166 (2000) R11–R16.
[22] L.B. Moore, B. Goodwin, S.A. Jones, G.B. Wisely, C.J. Serabjit-Singh, T.M. Willson, et al., St. John's wort induces hepatic drug metabolism through activation of the pregnane X receptor, Proc. Natl. Acad. Sci. U. S. A. 97 (2000) 7500–7502.

[23] North-American-Menopause-Society, The role of testosterone therapy in postmenopausal women: position statement of The North American Menopause Society, Menopause 12 (2005) 497–511.
[24] M.E. Wierman, W. Arlt, R. Basson, S.R. Davis, K.K. Miller, M.H. Murad, et al., Androgen therapy in women: a reappraisal: an endocrine society clinical practice guideline, J. Clin. Endocrinol. Metab. 99 (2014) 3489–3510.
[25] D. Goldenberg, M. Mayskiy, C. Mossey, R. Ruthazer, C. Schmid, A randomized, double-blind crossover trial of fluoxetine and amitriptyline in the treatment of fibromyalgia, Arthritis Rheum. 39 (1996) 1852–1859.
[26] C.S. Burckhardt, S.R. Clark, R.M. Bennett, The fibromyalgia impact questionnaire: development and validation, J. Rheumatol. 18 (1991) 728–733.
[27] A.H. Heald, A. Butterworth, J.W. Kane, J. Borzomato, N.F. Taylor, T. Layton, et al., Investigation into possible causes of interference in serum testosterone measurement in women, Ann. Clin. Biochem. 43 (2006) 189–195.
[28] M.H. Warner, J.W. Kane, S.L. Atkin, E.S. Kilpatrick, Dehydroepiandrosterone sulphate interferes with the Abbott Architect direct immunoassay for testosterone, Ann. Clin. Biochem. 43 (2006) 196–199.
[29] G.A. McCain, Fibromyalgia and myofascial pain syndromes, in: P.D. Wall, R. Melzack (Eds.), Textbook of Pain, Third ed.Churchill Livingstone, New York 1994, pp. 475–493.
[30] L.M. Arnold, P.E. Keck Jr., J.A. Welge, Antidepressant treatment of fibromyalgia. A meta-analysis and review, Psychosomatics 41 (2000) 104–113.
[31] P.G. O'Malley, E. Balden, G. Tomkins, J. Santoro, K. Kroenke, J.L. Jackson, Treatment of fibromyalgia with antidepressants: a meta-analysis, J. Gen. Intern. Med. 15 (2000) 659–666.
[32] L.M. Arnold, Y. Lu, L.J. Crofford, M. Wohlreich, M.J. Detke, S. Iyengar, et al., A double-blind, multicenter trial comparing duloxetine with placebo in the treatment of fibromyalgia patients with or without major depressive disorder, Arthritis Rheum. 50 (2004) 2974–2984.
[33] L.J. Crofford, M.C. Rowbotham, P.J. Mease, I.J. Russell, R.H. Dworkin, A.E. Corbin, et al., Pregabalin for the treatment of fibromyalgia syndrome: results of a randomized, double-blind, placebo-controlled trial, Arthritis Rheum. 52 (2005) 1264–1273.
[34] P. Felig, L.A. Frohman, Endocrinology and Metabolism, 4th ed. McGraw Hill, 2001. 646–647.
[35] C. Regnier, A. Bennet, D. Malet, T. Guez, M. Plantavid, P. Rochaix, et al., Intraoperative testosterone assay for virilizing ovarian tumor topographic assessment: report of a Leydig cell tumor of the ovary in a premenopausal woman with an adrenal incidentaloma, J. Clin. Endocrinol. Metab. 87 (2002) 3074–3077.
[36] C.C. Ho, M. Rohaizak, S.Z. Zulkifli, M.A. Siti-Aishah, U. Nor-Aini, S.H. Sharifah-Noor-Akmal, Serum sex hormone levels in pre- and postmenopausal breast cancer patients, Singap. Med. J. 50 (2009) 513–518.
[37] L.J. Gooren, E.J. Giltay, Review of studies of androgen treatment of female-to-male transsexuals: effects and risks of administration of androgens to females, J. Sex. Med. 5 (2008) 765–776.
[38] W.W. Franke, B. Berendonk, Hormonal doping and androgenization of athletes: a secret program of the German Democratic Republic government, Clin. Chem. 43 (1997) 1262–1279.
[39] W.B. White, D. Grady, L.C. Giudice, S.M. Berry, J. Zborowski, M.C. Snabes, A cardiovascular safety study of LibiGel (testosterone gel) in postmenopausal women with elevated cardiovascular risk and hypoactive sexual desire disorder, Am. Heart J. 163 (2012) 27–32.
[40] BioSante-Pharmaceuticals-Inc., BioSante Pharmaceuticals Reports Positive LibiGel Safety Data Review in Phase III Program, http://finance.yahoo.com/news/BioSante-Pharmaceuticals-bw-3282504237.html October 11, 2011.
[41] BioSante-Pharmaceuticals-Inc., BioSante Pharmaceuticals Announces Results from LibiGel® Efficacy Trials, http://www.reuters.com/article/2011/12/14/idUS226971+14-Dec-2011+BW20111214 December 14, 2011.