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Sildenafil Improves Sleep-Related Erections in Hypogonadal Men: Evidence From a Randomized, Placebo-Controlled, Crossover Study of a Synergic Role for Both Testosterone and Sildenafil on Penile Erections

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ABSTRACT: To study the effects of sildenafil on human sleep-related erections according to the state of androgenization, we evaluated the effects of sildenafil on sleep-related erections in hypogonadal men before and during testosterone replacement treatment and in control subjects. We enrolled 24 hypogonadal men and 24 healthy men as a control group. All hypogonadal subjects had very low testosterone levels (<200 ng/dL [8.93 nmol/L]). All subjects underwent nocturnal penile tumescence and rigidity monitoring (NPTRM) for 3 consecutive nights and were randomly assigned to consume either 50 mg of sildenafil or placebo 1 hour before bedtime on the second or third night of nocturnal penile monitoring. The hypogonadal subjects were tested twice, first without replacement treatment (H−T) and then after at least 6 months of testosterone replacement therapy (H+T). The subjects of the control group (C) were tested once. The following parameters of sleep-related erections were analyzed: total number of valid erections, total duration of both rigidity greater than or equal 70% and increase in penile circumference greater than or equal 30 mm, maximum rigidity, and maximum increase in penile circumference. NPTRM parameters were reduced in hypogonadal men before testosterone treatment (H−T+P) when compared with control subjects taking placebo (C+P). NPTRM parameters after testosterone (H+T+P) and sildenafil (H−T+S) administration were similar to that of control subjects taking placebo (C+P). When the statistical analysis was restricted to the hypogonadal men before testosterone treatment, sildenafil alone significantly increased NPTRM parameters when compared with placebo (H−T+S vs H−T+P). Testosterone restored normal erections when administered to hypogonadal subjects (H+T+P vs H−T+P); in hypogonadal men, however, the combined treatment (sildenafil plus testosterone) resulted in the maximum positive effect on NPTRM parameters. When the increase from baseline was analyzed, the effects of testosterone plus sildenafil were greater than the sum of the effects of each drug used alone. In conclusion, sildenafil administered at bedtime improves sleep-related erections in hypogonadal men, suggesting that the nitric oxide pathway may be pharmacologically enrolled and enhanced despite low serum testosterone. Furthermore, these data strongly support the idea of a synergic effect on sleep-related erections of sildenafil and testosterone.

Key words: Nitric oxide pathway, androgen, hypogonadism, penile rigidity and tumescence, nocturnal erection, PDE5, NPT.

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Androgens are considered the major determinants of male sexual function, but the mechanism of their action on penile erection is still not completely understood in detail (Robbins, 1996). In animals there is evidence of a strong dependence of erectile function on androgens; in fact penile erections are lost after castration in rodents (Robbins, 1996; Shabsigh, 1997). Penile erections depend on androgens in men, as well, but this correlation is less definite. Androgens, particularly testosterone, promote and support psychogenic and reflexive erections (erections that occur during wake under the effects of psychogenic or sexual stimuli), but these erections may persist at a lower degree when circulating androgens are severely reduced (Kwan et al, 1983; Carani et al, 1995).

Hypogonadal men show impaired erections for both number and quality, but they may still have sexual intercourse (Bancroft and Wu, 1983) even when serum testosterone is completely lacking. Accordingly, androgen replacement restores full erectile function and sexual behavior in hypogonadal subjects (Cunningham et al, 1990).

Unlike psychogenic and reflexive erections, sleep-related penile erections are strongly androgen-dependent in men (Cunningham et al, 1990; Carani et al, 1995). Previous studies on male sexual behavior pointed out 2 thresholds for serum testosterone below which sexual be-

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havior is impaired: a first one of 350 ng/dL (15.63 nmol/L), below which sleep-related erections are usually normal and sexual behavior is impaired only in some subjects, and a lower one of 150 ng/dL (6.7 nmol/L), below which both sleep-related erections and sexual behavior are almost constantly impaired (Carani et al, 1996). In 1997 we demonstrated that sleep-related erections are constantly impaired when serum testosterone levels are below the threshold of 200 ng/dL (8.93 nmol /L) (Granata et al, 1997).

Even if some testosterone effects have been characterized in vivo, the molecular mechanisms by which androgens modulate the erectile function are far from being completely understood in men (Shabsigh, 2004; Aversa et al, 2004). On the other hand, studies on animals suggest that testosterone may regulate smooth muscle relaxation in the corpora cavernosa by acting at different sites of the nitric oxide (NO) pathway, including both NO synthase (Schirar et al, 1997; Shabsigh, 1997; Marin et al, 1999) and type 5 cyclic guanosine monophosphate (cGMP)-specific phosphodiesterase enzyme (PDE5) (Traish et al, 1999; Morelli et al, 2004), which are the key enzymes involved in the vascular control of erectile tissue. Furthermore, testosterone is considered a trophic agent for the penile tissue, being necessary for its normal ultrastructure at the level of both smooth muscles and collagenous fibers (Shen et al, 2003), and androgens may modulate the process of apoptosis within the rat corpora cavernosa, too (Zhang et al, 1999). Accordingly, in humans PDE5 gene expression is higher in corpora cavernosa than in other reproductive and nonreproductive male tissues, and this gene expression is androgen-dependent in the penis (Morelli et al, 2004).

Sildenafil is an oral selective inhibitor of PDE5, which is abundant in the corpora cavernosa, and its inhibition results in strengthening of the NO pathway. This pathway is crucial for penile smooth muscle relaxation and consequent penile vasodilatation, because it increases intracellular cGMP with a consequent decrease of intracellular Ca^{++} (Lue, 2000).

Sildenafil has been shown to be efficacious in men affected by erectile dysfunction (Boolell et al, 1996; Goldstein et al, 1998; Rendell et al, 1999; Lue, 2000). Sildenafil administered at bedtime also improves sleep-related erections in both eugonadal men affected by erectile dysfunction (Montorsi et al, 2000; Terradas et al, 2001) and healthy men (Rochira et al, 2002; Yaman et al, 2003).

A clinical study claimed that sildenafil is efficacious in the treatment of erectile dysfunction even when serum testosterone is slightly lower than normal, while a poor penile response to sildenafil has been suggested to occur when testosterone levels are very low (Guay et al, 2001). Recent evidence suggests that testosterone can modulate the erectile response to sildenafil administration in men with erectile dysfunction (Aversa et al, 2003; Aversa et al, 2004; Kalinchenko et al, 2003; Shabsigh, 2004). In patients affected by erectile dysfunction and with low to normal serum testosterone, a short-term testosterone treatment improved the erectile response to sildenafil evaluated by penile dynamic color duplex ultrasound (Aversa et al, 2003). Oral testosterone undecanoate administration restored erectile function in men with type 2 diabetes mellitus failing on sildenafil therapy alone (Kalinchenko et al, 2003). Until now, there have been no reports concerning the way sleep-related erections can be modified by sildenafil in hypogonadal men. There are 3 main reasons why sleep-related erections represent a valid tool to investigate the effects of sildenafil alone, testosterone alone, or the combined treatment on penile function. First, the continuous monitoring of sleep-related erections by means of a device provides qualitative and quantitative parameters of penile erections (Bradley, 1987; Kessler, 1988). Second, nocturnal erections are poorly or not affected by external factors (eg, embarrassment, anxiety, psychological correlates), which can interfere with penile erections when studied in awake subjects (Karacan, 1980; Bancroft, 1989; Granata et al, 1995). Third, sleep-related erections represent the ''gold standard'' to disclose the relationship between sildenafil and testosterone on penile function because they are the most androgen-dependent type of erection in men (Granata et al, 1997).

The aim of this study was to evaluate the effects of sildenafil on sleep-related erections with regard to the state of androgenization. To study the effect of sildenafil on sleep-related erections in men with low and normal serum testosterone levels, we evaluated sleep-related erections after a bedtime administration of sildenafil (50 mg) in hypogonadal men before and during testosterone replacement treatment and in healthy subjects using a randomized, placebo-controlled, crossover study (Figure 1).

Materials and Methods

Subjects

Twenty-four men who attended the Department of Endocrinology of Modena, Italy, because of hypogonadism were enrolled in the study. The characteristics (age, diagnosis, and serum testosterone level) of each hypogonadal man are summarized in Table 1. A serum testosterone level of 200 ng/dL (8.93 nmol/L) was used as a cut-off for the enrollment of hypogonadal subjects in the study protocol, because it is known that sleep-related erections are constantly impaired when serum testosterone is below this threshold (Granata et al, 1997). The subjects included 14 subjects who had been withdrawn from testosterone replacement treatment for at least 3 months and 10 hypogonadal subjects at their first diagnosis. Of the 14 subjects who had stopped testosterone treatment, 7 were hypogonadal men followed by our Unit who had stopped the replacement treatment spontaneously for

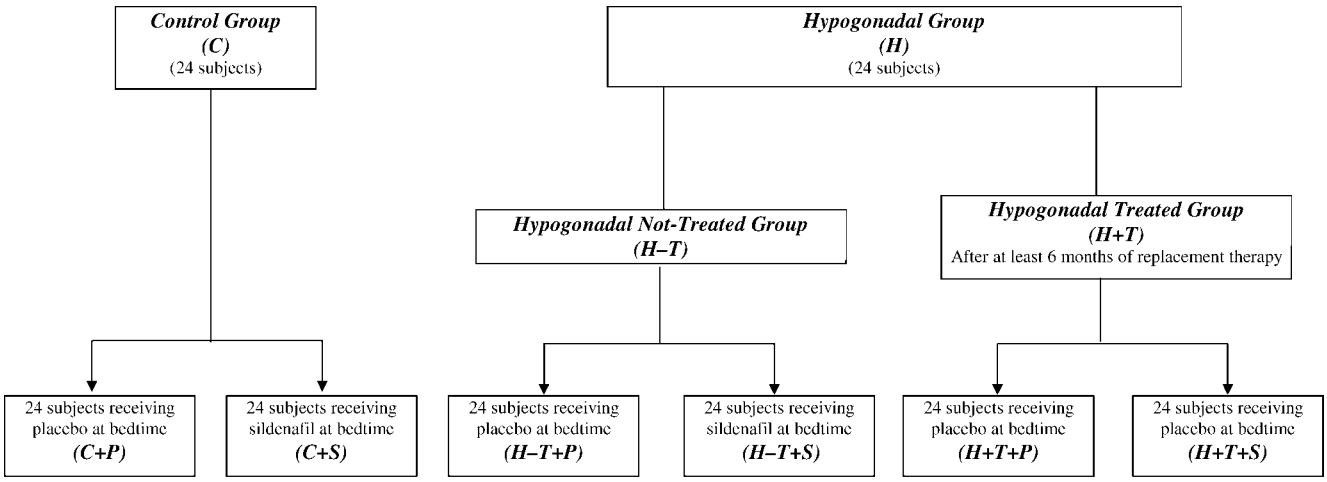

Figure 1. Study design.

more than 3 months. The other 7 subjects were affected by Klinefelter syndrome and were asked to stop the replacement treatment for 3 months to retest the remaining Leydig function. The subjects who stopped the replacement treatment were interviewed every 10 to 15 days for side effects due to the withdrawal; all complained of low libido after at least 50 days of withdrawal, 3 complained of reduced strength after at least 2 months, none complained of mood modifications, and none dropped out from the study. The protocol was arranged so that the 10 hypogonadal subjects at the first diagnosis with no previous replacement treatment started the replacement treatment as soon as possible (no longer than 20 days from the enrollment).

Table 1. *Age, diagnosis, and serum testosterone levels before and during testosterone treatment of the 24 hypogonadal subjects enrolled in the study*

			Testosterone Levels, ng/dL	
Subjects	Age	Diagnosis	Before Testosterone Treatment H−T	During Testosterone Treatment H+T
1	50	Panhypopituitarism	10	343
2	33	Klinefelter syndrome	170	633
3	31	Klinefelter syndrome	124	750
4	19	Partial hypopituitarism	96	799
5	50	Klinefelter syndrome	177	450
6	50	Klinefelter syndrome	115	499
7	34	Hypogonadotropic hypogonadism	25	590
8	50	Panhypopituitarism	10	490
9	30	Hypogonadotropic hypogonadism	40	552
10	41	Klinefelter syndrome	190	662
11	21	Hypogonadotropic hypogonadism	48	951
12	19	Panhypopituitarism	15	747
13	49	Panhypopituitarism	48	408
14	29	Hypogonadotropic hypogonadism	12	353
15	21	Hypogonadotropic hypogonadism	20	601
16	49	Klinefelter syndrome	120	558
17	30	Hypogonadotropic hypogonadism	50	552
18	41	Klinefelter syndrome	170	447
19	21	Hypogonadotropic hypogonadism	52	967
20	19	Hypogonadotropic hypogonadism	16	747
21	48	Panhypopituitarism	48	455
22	29	Kallman syndrome	14	390
23	31	Partial hypopituitarism	19	560
24	34	Panhypopituitarism	28	580
Mean	35		67	587
SD	12		61	171

Testosterone treatment consisted of the administration of testosterone enanthate 250 intramuscularly (IM) every 21 days in all subjects.

Twenty-four healthy eugonadal male volunteers not affected by erectile dysfunction were also enrolled as control group. General inclusion criteria were an age between 18 and 50 years and body mass index (BMI) less than 28; hypogonadal subjects and controls were matched for age (years ± SD = 34.54 ± 11.50 and 36.12 ± 7.11, respectively). Subjects affected by systemic diseases, pelvic or perineal trauma, pelvic surgery, and sleep disturbances were not included in the study. Depression and trait anxiety were previously excluded by self-filled questionnaires (Beck et al, 1961; Spielberger et al, 1970). Hypogonadal subjects with concomitant hyperprolactinemia (prolactin > 20 ng/mL) were also excluded since prolactin per se may negatively influence the erectile function (Carani et al, 1996). To perform nocturnal penile tumescence and rigidity monitoring (NPTRM), the circumference of the flaccid penis had to be at least 50 mm.

The subjects were not taking any medication, except the patients affected by panhypopituitarism or partial hypopituitarism who were on replacement therapy with L-thyroxine sodium, cortisone acetate, recombinant human growth hormone (r-hGH), or combined treatments as replacement therapy.

The subjects underwent NPTRM for 3 consecutive nights. Sildenafil or placebo was administered on the second night and vice versa on the third night.

Study Design

The study design is summarized in Figure 1. Control subjects (C) were tested once, and hypogonadal subjects were tested twice: before (H−T) and during testosterone treatment (H+T). The H+T involved all the hypogonadal subjects during testosterone replacement treatment for at least 6 consecutive months. NPTRM and blood collection were performed between the 6th and the 10th day from last testosterone injection.

Twelve subjects of the H−T, 12 of the H+T, and 12 of the control group were randomly assigned to receive 50 mg sildenafil tablet the second night, followed by the administration of placebo the third night. The rest of the subjects (12 H−T, 12 H+T, and 12 subjects C) were randomly assigned to receive placebo the second night, followed by the administration of 50 mg sildenafil tablet the third night (Figure 1). All subjects consumed the sildenafil or placebo 1 hour before starting NPTRM and at least 2 hours after their last meal as previously standardized (Rochira et al, 2002).

The study design did not include a placebo control for testosterone treatment because the protocol involved subjects at the first diagnosis of hypogonadism who needed testosterone replacement treatment; furthermore the effects of testosterone replacement treatment on sleep related erections have been previously demonstrated (Cunningham et al, 1990; Carani et al, 1995; Granata et al, 1997).

The study protocol was approved by our local ethical committee, and all subjects gave their written informed consent to the study.

Sleep-Related Erections Assessment

Each subject underwent a home NPTRM for 3 consecutive nights performed by means of RigiScan (Dacomed Corp, Minneapolis, Minn), which is known to be a valid tool to test nocturnal penile activity (Guay et al, 1996). The first night was regarded as time of adaptation. The results reported here come from the second and the third nights. Results from a single NPTRM monitoring may be adequate because RigiScan provides highly reproducible measurements of penile tumescence and rigidity (Burris et al, 1989). A single RigiScan was used to avoid unequal measurements from different devices (Munoz et al, 1993). Only the data recorded by the base loop of the RigiScan are here reported. The subjects went to bed at their usual time.

The following NPTRM parameters were analyzed according to our previous standardization (Granata et al, 1997):

Total Number of Valid Erections—This was defined as an increase in circumference of at least 30 mm from the baseline and with a rigidity of at least 60%, with both circumference increase and rigidity persisting for at least 5 minutes. The baseline for measurement of circumference increase was the minimum circumference that lasted at least 5 minutes.

Total Duration of Penile Rigidity Greater Than or Equal to 70%—This was defined as the sum of the time (minutes) during which penile rigidity is greater than or equal to 70% evaluated for each recorded erection in a single monitoring.

Total Duration of Increase in Penile Circumference Greater Than or Equal to 30 mm——This was defined as the sum of the time (minutes) during which penile circumference is greater than or equal 30 mm evaluated for each recorded erection in a single monitoring.

Maximum Penile Rigidity (%) Persisting for at Least 3 Minutes—This was defined as the maximum rigidity (%) lasting for a least 3 minutes in a single monitoring.

Maximum Increase in Penile Circumference Persisting for at Least 3 Minutes—This was defined as the maximum increase in circumference persisting for at least 3 minutes in a single monitoring.

The data concerning penile circumference are directly related to penile tumescence.

Hormonal Assessment

An overnight fasting venous blood sample was taken from each subject by a cannula placed in the antecubital vein at 8:00 AM after the end of the second night test and between the 6th and 10th day from last testosterone injection. Basal serum levels of testosterone, luteinizing hormone (LH), follicle-stimulating hormone (FSH), and prolactin were assayed. Only the results of testosterone are reported in the text (Table 1). All blood samples were allowed to clot at room temperature, centrifuged, and the serum stored frozen at −20°C until assayed. Serum testosterone was assayed by a chemiluminescence method (ACS: 180 [R] SE Automated Chemiluminescence System, Bayer, Milan, Italy). The intra- and interassay coefficients of variation were respectively 3.9% and 3.7%.

Statistical Analysis

All the variables that resulted were normally distributed. The one-way analysis of variance (ANOVA) was used for the comparison among the 6 groups (C+P; C+S; H−T+P; H−T+S; H+T+P; H+T+S) (Figure 1), and it was followed by Tukey's post-hoc test, with a .05 level of significance.

To determine differences between the 4 groups of hypogonadal men and over time, we performed an ANOVA with repeated measures on hypogonadal subjects using a conservative (Greenhouse-Geisser) F test, because 4 different series of measurements has been obtained from each subject (H−T+P; H−T+S; H+T+P; H+T+S) (Figure 1). An ANOVA univariate followed by Tukey's post-hoc test was performed to establish differences among each single group of the 4 groups of hypogonadal men. The level of significance was .05.

To compare the effects of each treatment alone (sildenafil or testosterone) with that of the combined treatment (testosterone plus sildenafil), the percentage increase from baseline was calculated for each NPTRM measurement in all hypogonadal subjects, considering as baseline the initial condition without any medication: hypogonadal subjects before testosterone treatment taking placebo (H−T+P). A Student's *t* test for paired data was used for the analysis of differences among the percentage increase from baseline with a level of significance of .05.

To compare the effect of sildenafil alone in the presence of very low serum testosterone with that of sildenafil alone in the presence of normal serum testosterone, the percentage increase of penile circumference from baseline was calculated for each NPTRM measurement in all hypogonadal subjects assuming as basal condition the placebo session. We obtained the percentage increase from baseline in hypogonadal men before testosterone treatment and while taking sildenafil (H−T+S) compared with the same subjects taking placebo (H−T+P), as well as the percentage increase from baseline in hypogonadal men during testosterone treatment while taking sildenafil (H+T+S) compared with the same subjects taking placebo (H+T+P). A Student's *t* test for paired data was performed for the analysis of differences among the percentage increase of penile circumference from baseline with a level of significance of .05.

Because some NPTRM parameters in hypogonadal men are often severely impaired (Carani et al, 1995; Granata et al, 1997) and may be equal to 0, the value was approximated to be 0.1 for the increments from baseline when this was the case.

This study had a cross-over design for the day of administration of placebo and sildenafil (Figure 1). ANOVA for repeated measures was performed to reveal a possible day effect for the data with placebo and with sildenafil for all the parameters. No significant differences were observed with this analysis, suggesting that our results are independent from the day of administration (the 2nd or the 3rd night) of the drug in the protocol (data not shown) as previously described (Rochira et al, 2002).

Statistical analyses were conducted using SPSS Statistical Software for Windows, version 11.0 (SPSS Inc, Chicago, Ill).

Results

In hypogonadal men serum testosterone was significantly lower before (H−T) than during testosterone treatment (H+T) (Table 1). Serum testosterone was significantly higher in controls (C) (mean ± SD: 612.78 ± 159.65 ng/dL) than in untreated hypogonadal men (H−T). No differences in serum testosterone were found between controls and treated hypogonadal men (H+T).

All NPTRM of the protocol lasted at least 6 hours.

The ANOVA univariate among and within the 6 groups provided strong evidence of significant differences among groups for all NPTRM parameters ($P < .0001$). Differences among the groups were established by means of Tukey's test, and results are summarized in Table 2 as means ± SE.

Except for maximum rigidity and maximum increase of circumference, sildenafil administration significantly increased nocturnal erectile parameters in both control subjects (C+S) and hypogonadal men during testosterone treatment (H+T+S) when compared with control subjects taking placebo (C+P) (Table 2).

All NPTRM parameters were significantly higher in C+P than in hypogonadal men before testosterone treatment and taking placebo (H−T+P) (Table 2).

All NPTRM parameters were significantly higher in C+S than in H−T+P, hypogonadal men before testosterone treatment taking sildenafil (H−T+S), and hypogonadal men during testosterone treatment taking placebo (H+T+P), except for maximum rigidity and maximum increase of circumference in the latter comparison (Table 2).

No differences were found when C+P were compared with H−T+S for all parameters except maximum increase of circumference (Table 2). Additionally, no differences were found when C+P were compared with H+T+P, or when C+S were compared with H+T+S (Table 2).

Multivariate analysis (ANOVA for repeated measures) performed on hypogonadal subjects in 4 different series of measurements on each subject (H−T+P, H−T+S; H+T+P; H+T+S) (Figure 1) showed a significant difference among groups for all 5 NPTRM parameters ($P < .0001$).

Some differences showed by statistics performed on all 6 groups of subjects were confirmed by the comparison among the 4 groups of hypogonadal men (ANOVA univariate with Tukey's post-hoc test), but the latter comparison showed new significant differences for some parameters, because the analysis was restricted to hypogonadal men. Particularly it allowed the disclosure of the effects of each single treatment (sildenafil alone or testosterone alone) or of the combination of both treatments versus the basal condition.

Sildenafil alone significantly increased all NPTRM parameters when compared with placebo in hypogonadal men before testosterone treatment (H−T+S vs H−T+P) except for total duration of increase of circumference greater than or equal 30 mm (Figures 2 through 6). In hypogonadal men testosterone treatment alone significantly increased all NPTRM parameters (H+T+P vs H−T+P) (Figures 2 through 6).

Testosterone plus sildenafil (H+T+S) significantly in-

Table 2. *Parameters of NPTRM expressed as mean and standard error (in parenthesis) of all six groups: Control Group (C), hypogonadal not-treated (H−T) and treated (H+T) men taking placebo (P) or sildenafil (S)**

	Control Group		Hypogonadal Not Treated Group		Hypogonadal Treated Group	
	Placebo C+P	Sildenafil C+S	Placebo H−T+P	Sildenafil H−T+S	Placebo H+T+P	Sildenafil H+T+S
Number of valid erections	3.17 (0.26)	4.17 (0.27)	0.76 (0.22)	2.71 (0.18)	2.79 (0.27)	4.29 (0.24)
Total duration of rigidity > 70%, min	60.66 (7.98)	108.04 (11.43)	10.57 (3.83)	43.59 (5.14)	52.02 (8.74)	105.48 (13.02)
Total duration of increase of circumference ≥ 30 mm, min	51.78 (5.84)	103.49 (10.42)	6.67 (1.83)	30.14 (4.26)	38.63 (5.01)	100.02 (11.45)
Maximum rigidity, %	89.46 (2.11)	94.04 (1.39)	60.83 (3.83)	79.21 (3.44)	84.00 (2.10)	91.75 (1.54)
Maximum increase of circumference, mm	40.50 (1.38)	44.58 (1.45)	25.79 (1.45)	33.46 (0.89)	40.00 (1.70)	43.58 (1.31)
Number of Valid Erections						
C+P vs C+S: $P < .05$	C+P vs H−T+S: n.s.		H−T+P vs H−T+S: $P < .0001$			
C+P vs H+T+S: $P < .05$	C+P vs H+T+P: n.s.		H−T+P vs H+T+S: $P < .0001$			
C+P vs H−T+P: $P < .0001$	C+S vs H+T+S: n.s.		H−T+P vs H+T+P: $P < .0001$			
C+S vs H−T+P: $P < .0001$			H−T+S vs H+T+S: $P < .0001$			
C+S vs H−T+S: $P < .0001$			H+T+P vs H+T+S: $P < .0001$			
C+S vs H+T+P: $P = .001$						
Total Duration of Rigidity ≥ 70%, min						
C+P vs C+S: $P < .005$	C+P vs H−T+S: n.s.		H−T+P vs H+T+P: $P < .05$			
C+P vs H+T+S: $P = .005$	C+P vs H+T+P: n.s.		H−T+P vs H+T+S: $P < .0001$			
C+P vs H−T+P: $P = .001$	C+S vs H+T+S: n.s.		H−T+S vs H+T+S: $P < .0001$			
C+S vs H−T+P: $P < .0001$			H−T+P vs H+T+S: $P < .0001$			
C+S vs H−T+S: $P < .0001$						
C+S vs H+T+P: $P < .0001$						
Total Duration of Increase of Circumference ≥ 30 mm, min						
C+P vs C+S: $P < .0001$	C+P vs H−T+S: n.s.		H−T+P vs H+T+P: $P < .05$			
C+P vs H+T+S: $P < .0001$	C+P vs H+T+P: n.s.		H−T+P vs H+T+S: $P < .0001$			
C+P vs H−T+P: $P < .0001$	C+S vs H+T+S: n.s.		H−T+S vs H+T+S: $P < .0001$			
C+S vs H−T+P: $P < .0001$			H+T+P vs H+T+S: $P < .0001$			
C+S vs H−T+S: $P < .0001$						
C+S vs H+T+P: $P < .0001$						
Maximum Rigidity, %						
C+P vs H−T+P: $P < .0001$	C+P vs C+S: n.s.		H−T+P vs H−T+S: $P < .0001$			
C+S vs H−T+P: $P < .0001$	C+P vs H−T+S: n.s.		H−T+P vs H+T+P: $P < .0001$			
C+S vs H−T+S: $P = .0001$	C+P vs H+T+P: n.s.		H−T+P vs H+T+S: $P < .0001$			
	C+P vs H+T+S: n.s.		H−T+S vs H+T+S: $P < .01$			
	C+S vs H+T+P: n.s.					
	C+S vs H+T+S: n.s.					
Maximum Increase of Circumference, mm						
C+P vs H−T+P: $P < .0001$	C+P vs C+S: n.s.		H−T+P vs H−T+S: $P = .001$			
C+P vs H−T+S: $P < .005$	C+P vs H+T+P: n.s.		H−T+P vs H+T+P: $P < .0001$			
C+S vs H−T+P: $P < .0001$	C+P vs H+T+S: n.s.		H−T+P vs H+T+S: $P < .0001$			
C+S vs H−T+S: $P < .0001$	C+S vs H+T+P: n.s.		H−T+S vs H+T+P: $P < .05$			
	C+S vs H+T+S: n.s.		H−T+S vs H+T+S: $P < .0001$			

* The comparisons among groups were performed by means of one-way analysis of variance, and results are reported above. The level of significance was .05. All comparisons between control groups and hypogonadal men are reported in the table, even if they are not significant. For comparisons among hypogonadal men, only those that were significant are reported, because more detailed and appropriate statistical analyses have been performed (see the text). NPTRM indicates nocturnal penile tumescence and rigidity monitoring; n.s., not significant; C+P, controls taking placebo; C+S, controls taking sildenafil; H−T+P, hypogonadal men before testosterone treatment taking placebo; H−T+S, hypogonadal men before testosterone treatment taking sildenafil; H+T+P, hypogonadal men during testosterone treatment taking placebo; and H+T+S, hypogonadal men during testosterone treatment taking sildenafil. Both placebo and sildenafil were taken at bedtime.

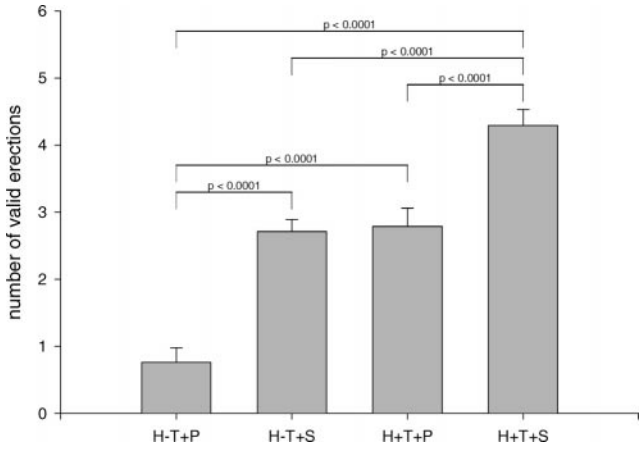

Figure 2. Number of valid erections in hypogonadal subjects. H−T+P indicates hypogonadal men before testosterone treatment taking placebo at bedtime; H−T+S, hypogonadal men before testosterone treatment taking sildenafil at bedtime; H+T+P, hypogonadal men during testosterone treatment taking placebo at bedtime; and H+T+S, hypogonadal men during testosterone treatment taking sildenafil at bedtime.

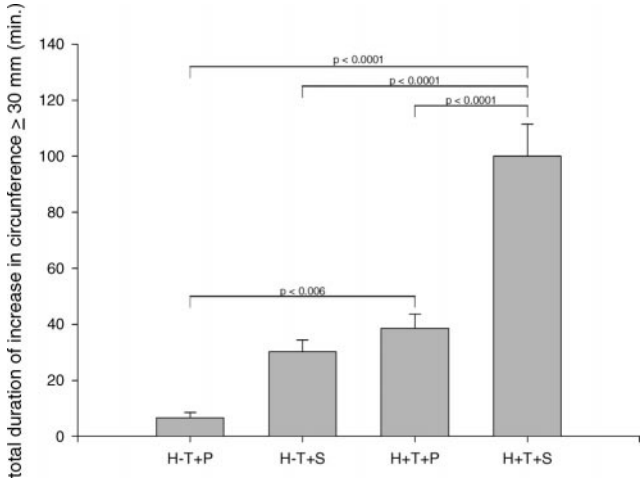

Figure 4. Total duration of increase in circumference greater than or equal to 30 mm (min) in hypogonadal subjects. H−T+P indicates hypogonadal men before testosterone treatment taking placebo at bedtime; H−T+S, hypogonadal men before testosterone treatment taking sildenafil at bedtime; H+T+P, hypogonadal men during testosterone treatment taking placebo at bedtime; and H+T+S, hypogonadal men during testosterone treatment taking sildenafil at bedtime.

creased all NPTRM parameters when compared with 1) basal condition (H−T+P), 2) sildenafil alone (H−T+S), and 3) testosterone alone (H+T+P) (except for maximum rigidity and maximum increase in circumference in the latter comparison) (Figures 2 through 6). When testosterone alone (H+T+P) was compared with sildenafil alone (H−T+S) in hypogonadal men, no significant differences were found for all the parameters, except for maximum increase of circumference (Figures 2 through 6).

When the first measurement (H−T+P) was considered as basal condition for hypogonadal men, the percentage increase from baseline disclosed the effects of testosterone or sildenafil alone compared with that of combined treatment. In this view for the parameters total duration of rigidity greater than or equal to 70% and total duration of increase in circumference greater than or equal to 30 mm, the percentage increase from baseline (H−T+P) due to the administration of both testosterone and sildenafil (combined treatment) was significantly greater ($P < .05$) than the sum of the percentage increase from baseline (H−T+P) due to sildenafil alone and to testosterone alone, the comparison between these last 2 situations not showing significant differences (data not shown). For the remaining NPTRM parameters the effect of testosterone plus sildenafil (expressed as increase from baseline) was

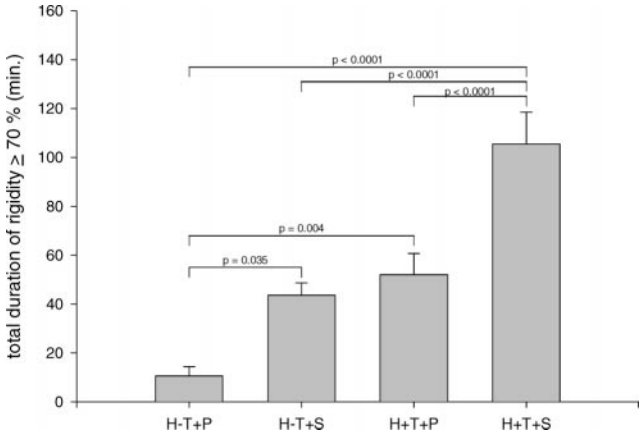

Figure 3. Total duration of rigidity greater than or equal to 70% (min) in hypogonadal subjects. H−T+P indicates hypogonadal men before testosterone treatment taking placebo at bedtime; H−T+S, hypogonadal men before testosterone treatment taking sildenafil at bedtime; H+T+P, hypogonadal men during testosterone treatment taking placebo at bedtime; and H+T+S, hypogonadal men during testosterone treatment taking sildenafil at bedtime.

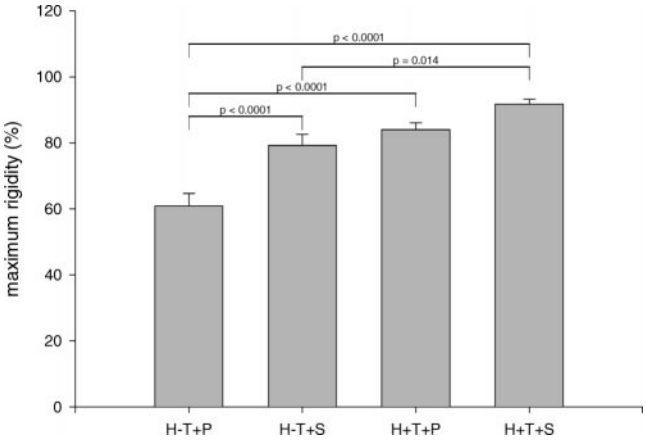

Figure 5. Maximum rigidity (%) in hypogonadal subjects. H−T+P indicates hypogonadal men before testosterone treatment taking placebo at bedtime; H−T+S, hypogonadal men before testosterone treatment taking sildenafil at bedtime; H+T+P, hypogonadal men during testosterone treatment taking placebo at bedtime; and H+T+S, hypogonadal men during testosterone treatment taking sildenafil at bedtime.

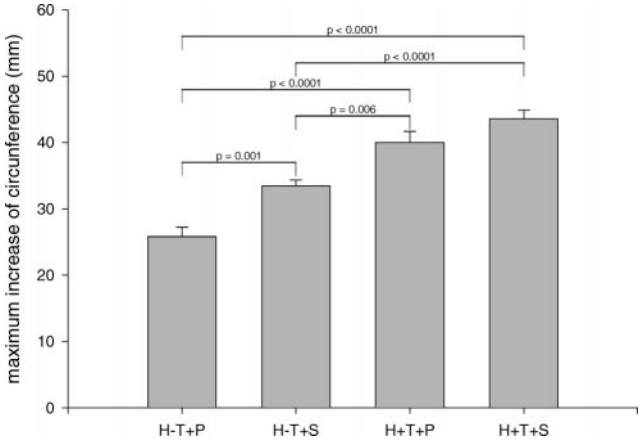

Figure 6. Maximum increase of circumference (min) in hypogonadal subjects. H−T+P indicates hypogonadal men before testosterone treatment taking placebo at bedtime; H−T+S, hypogonadal men before testosterone treatment taking sildenafil at bedtime; H+T+P, hypogonadal men during testosterone treatment taking placebo at bedtime; and H+T+S, hypogonadal men during testosterone treatment taking sildenafil at bedtime.

not significantly different from the sum of the effects of sildenafil alone and testosterone alone.

When the placebo session was considered as baseline for both untreated (H−T) and treated (H+T) hypogonadal men, respectively, the percentage increase from baseline was related only to the pure effect of sildenafil alone in 2 different conditions (normal or low circulating testosterone, respectively). In this view, for all NPTRM parameters the percentage increase from baseline in H−T+S versus H−T+P was significantly greater than the percentage increase from baseline in H+T+S versus H+T+P ($P < .05$) except for the maximum rigidity, which showed only a trend for a higher percentage value (data not shown). However this very significant difference for all the NPTRM parameters was due to very low values (quite near 0) for all these parameters in the hypogonadal men before testosterone replacement.

Discussion

The first major outcome of this study is that sildenafil administered at bedtime improves sleep-related erections in men affected by hypogonadism, as in healthy subjects (Rochira et al, 2002; Yaman et al, 2003) and in subjects affected by erectile dysfunction (Montorsi et al, 2000; Terradas et al, 2001). Particularly, sildenafil increased all NPTRM parameters, except for total duration of increase in circumference, (Figures 2, 3, 5, 6) in hypogonadal men (H−T+S vs H−T+P), similar to the result when testosterone alone was administered, since no differences were found between H−T+S and H+T+P groups for all parameters (Figures 2 through 6). Accordingly, sildenafil restored normal values of NPTRM parameters as shown by the comparison with control group with placebo (H−T+S vs C+P) (Table 2), except for the maximum increase in circumference, which was lower in hypogonadal men.

The advantage to testing erectile function through the study of the effects of testosterone and sildenafil (as single or combined treatment) on sleep-related erections is that these erections are poorly or not affected by external factors (eg, embarrassment, state anxiety) in contrast to psychogenic, awake erections and erections during sexual intercourse (Karacan, 1980; Bancroft, 1989). Additionally, while erections in rodents depend completely on the presence of androgens and are reduced and often eliminated after castration (Mills et al, 1994; Robbins, 1996), in men only sleep-related erections are clearly androgen dependent (Carani et al, 1995; Granata et al, 1997). Therefore, sleep-related erections in men are a highly appropriate model of investigation to assess the effect of sildenafil on penile erection, according to the degree of circulating serum testosterone levels. Thus, this randomized, placebo-controlled, crossover study has demonstrated for the first time that sildenafil can recruit the NO pathway even when serum testosterone is very low (66.6% of hypogonadal men enrolled had serum testosterone less than 55 ng/dL, Table 1). Hence PDE5 inhibitors maintain their positive effect on penile erection in the presence of low circulating androgens, and sildenafil does not necessarily require the restoration of normal serum testosterone for its efficacy in hypogonadal men. Additionally, the positive effects of sildenafil on sleep-related erections in hypogonadal men who are not of advanced age (like the subjects in this study) are also evident when serum testosterone is lower than normal or is very low (nearly undetectable). A recent study led to similar results in hypogonadal men complaining of erectile dysfunction (Guay et al, 2001). Guay et al showed an impairment of erectile quality during sexual intercourse, even after sildenafil consumption, but at the same time they raised the question of a possible failure of sildenafil when serum testosterone is severely impaired (Guay et al, 2001). From our data it is possible to conclude that sildenafil is able to restore normal erections in hypogonadal men, because the effects of sildenafil on NPTRM parameters are similar to those seen with testosterone (Figures 2 through 6) and because sleep-related erections in hypogonadal men taking sildenafil before testosterone treatment (H−T+S) did not significantly differ from controls (C+P) (Table 2). In addition, in this study sildenafil also improved nocturnal erections when normal circulating levels of testosterone were restored by testosterone replacement treatment (H+T+S).

On the basis of all these results in clinical practice, it is possible to consider sildenafil treatment as a crucial therapeutic choice in male hypogonadism alone or in

combination with androgen supplementation. Particularly, hypogonadal men for whom androgen supplementation is not suitable (eg, men with pharmacological hypogonadism because of prostate cancer and hypogonadism in the elderly) may take advantage of a specific therapy for sexual symptoms, such as PDE5 inhibitors, especially if a concomitant vascular or neurological injury in the penile tissue is absent.

The second outcome of this study is that sildenafil plus testosterone replacement treatment is more effective in improving sleep-related erections than the sum of the effects of sildenafil alone and of testosterone alone, in particular when the time of duration of both rigidity and circumference are considered. Thus, a synergic effect between testosterone and sildenafil on sleep-related erections may be supposed. Recently an interaction between sildenafil and testosterone has been largely emphasized on the basis of data coming from men affected by erectile dysfunction (Aversa et al, 2004; Shabsigh, 2004). Sildenafil improved awake erections also when testosterone was administered to men with arteriogenic erectile dysfunction, but with low to normal baseline serum testosterone (Aversa et al, 2003). Similarly, oral testosterone undecanoate was effective in restoring both normal serum testosterone and sildenafil efficacy in patients with both erectile dysfunction and type 2 diabetes mellitus, with these patients having lower serum testosterone than controls and being unresponsive to sildenafil before androgen supplementation (Kalinchenko et al, 2003). More recently, a randomized study found that the combined therapy with both testosterone gel and sildenafil restored a good response to sildenafil in hypogonadal men affected by erectile dysfunction who previously failed with sildenafil alone (Shabsigh et al, 2004).

The failure of sildenafil in men with both hypogonadism and erectile dysfunction (Kalinchenko et al, 2003; Shabsigh et al, 2004) seems to go against the major outcome of the present study, but the following statements should be noted. Factors such as erectile dysfunction or diabetes as well as a greater age (in previous studies the mean age is greater than 44) represents both confounding and worsening conditions, and these previous studies considered only awake erections. Actually, the evidence from this study of a synergic effect on sleep-related erections due to the combined treatment (testosterone plus sildenafil) reinforces the concept that the association treatment may restore full penile erections even when the erectile function is severely affected, as happens in the case of severe (Aversa et al, 2003) or multifactorial (Kalichenko et al, 2003; Shabsigh et al, 2004) erectile dysfunction. In the future this association may be promising for the treatment of erectile dysfunctions in some particular conditions even when serum testosterone level is normal (Aversa et al, 2004; Shabsigh, 2004).

In a pathophysiological perspective, these data suggest that the NO pathway may also be enrolled in hypogonadal men despite lower or nearly undetectable serum testosterone and that sildenafil plus testosterone may strengthen their actions, reinforcing each other their activity. Evidence does exist on the interrelationship between testosterone and the NO pathway (Schirar et al, 1997; Marin et al, 1999). Experimental studies on animals demonstrated that testosterone deficiency cuts down NO availability in the corpus cavernosum (Reilly et al, 1997) by modulating the synthesis and the activity of NO synthase, since both NO synthase expression and activity are reduced in castrated rats (Reilly et al, 1997; Marin et al, 1999; Baba et al, 2000). These studies suggest that the NO pathway is severely impaired when testosterone is absent. We do not know if the same mechanisms operate in humans, as well, but even if it is possible that testosterone modulates positively the NO pathway in men, in humans NO still remains available as our study demonstrates that in hypogonadal men the inhibition of PDE5, obtained by sildenafil administration, leads to an increase in NPTRM parameters. As a matter of fact, in men the erections are only partially androgen dependent (Robbins, 1996) while in animals testosterone represents a prerequisite for the occurrence of penile erections (Robbins, 1996; Mills et al, 1994).

On the other hand in animals it has been demonstrated that testosterone promotes both the activity (Traish et al, 1999) and the expression of PDE5 (Morelli et al, 2004), and some evidence in the same direction exists in humans (Morelli et al, 2004). Accordingly, a consensus sequence for the androgen receptor has been identified in the 5′-flanking region of the PDE5 promoter and this may represent the physical substrate for the modulation of PDE5 expression by androgens (Lin et al, 2001).

These results suggest that testosterone may enhance sildenafil activity because it increases expression and activity of PDE5, which represents the substrate for the inhibitory action of sildenafil. Thus, the increased availability of NO in the penile tissue, due to the direct action of testosterone on NO synthase on one hand together with the coexisting increase and higher level of PDE5 inhibition on the other hand, accounts for the strengthening of sleep-related erections in hypogonadal men. This may explain the synergic effect of the combined treatment, when compared to the single treatments.

This study confirms that sleep-related erections are strongly androgen-dependent. As previously shown (Cunningham et al, 1990; Granata et al, 1997), all NPTRM parameters are severely impaired in hypogonadal men before testosterone treatment when compared with controls (C+P vs H−T+P) (Table 2). As expected (Cunningham et al, 1990; Carani et al, 1996; Granata et al, 1997) testosterone replacement treatment in hypogonadal men re-

stored normal sleep-related erections (H−T+P vs H+T+P) (Figures 2 through 6), which did not differ from controls (C+P vs H+T+P) (Table 2).

The positive effect on sleep-related erections of sildenafil administered at bedtime in healthy subjects (C+S vs C+P) (Table 2) confirms that sildenafil strengthens nocturnal erections by enhancing the NO pathway in the penile tissue in normal subjects (Rochira et al, 2002).

In conclusion sildenafil administered at bedtime improves sleep-related erections in hypogonadal men. Furthermore the NO pathway may be pharmacologically enrolled and enhanced by a PDE5 inhibitor in hypogonadal men despite low testosterone serum levels. Again, on the basis of our data, a synergic effect of sildenafil and testosterone on sleep-related erections is suggested. These data may have some important implications in clinical practice.

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