

ORIGINAL ARTICLE

Effects of testosterone on sexual function in men: results of a meta-analysis

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Summary

Objectives The role of androgen decline in the sexual activity of adult males is controversial. To clarify whether sexual function would benefit from testosterone (T) treatment in men with partially or severely reduced serum T levels, we conducted a systematic review and meta-analysis of placebo-controlled studies published in the past 30 years. The aim of this study was to assess and compare the effects of T on the different domains of sexual life.

Data source A comprehensive search of all published randomized clinical trials was performed in MEDLINE, the Cochrane Library, EMBASE and *Current Contents* databases.

Review methods Guided by prespecified criteria, software-assisted data abstraction and quality assessed by two independent reviewers, a total of 17 randomized placebo-controlled trials were found to be eligible. For each domain of sexual function we calculated the standardized mean difference relative to T and reported the results of pooled estimates of T treatment using the random effect model of meta-analysis. Heterogeneity, reproducibility and consistency of the findings across studies were explored using sensitivity and meta-regression analysis.

Results Overall, 656 subjects were evaluated: 284 were randomized to T, 284 to placebo (P) and 88 treated in cross-over. The median study length was 3 months (range 1–36 months). Our meta-analysis showed that in men with an average T level at baseline below 12 nmol/l, T treatment moderately improved the number of nocturnal erections, sexual thoughts and motivation, number of successful intercourses, scores of erectile function and overall sexual satisfaction, whereas T had no effect on erectile function in eugonadal men compared to placebo. Heterogeneity was explored by grouping studies according to the characteristics of the study population. A cut-off value of 10 nmol/l for the mean T of the study population failed to predict the effect of treatment, whereas the presence of risk factors for vasculogenic erectile dysfunction (ED), comorbidities and

shorter evaluation periods were associated with greater treatment effects in the studies performed in hypogonadal, but not in eugonadal, men. Meta-regression analysis showed that the effects of T on erectile function, but not libido, were inversely related to the mean baseline T concentration. The meta-analysis of available studies indicates that T treatment might be useful for improving vasculogenic ED in selected subjects with low or low-normal T levels. The evidence for a beneficial effect of T treatment on erectile function should be tempered with the caveats that the effect tends to decline over time, is progressively smaller with increasing baseline T levels, and long-term safety data are not available. The present meta-analysis highlights the need, and pitfalls, for large-scale, long-term, randomized controlled trials to formally investigate the efficacy of T replacement in symptomatic middle-aged and elderly men with reduced T levels and ED.

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Introduction

Erectile dysfunction (ED) is a highly prevalent disorder among adult men and its incidence increases steadily with age.^{1–4} The ageing process in men is accompanied by a progressive decline in serum testosterone (T) levels and the various illnesses occurring in mid- to late adult life further contribute to lower circulating T independently of age.^{5–9} The use of androgen measurement in the evaluation of ED has shown that up to 35% of adults presenting with ED have reduced or borderline circulating androgen levels.^{5,10–14} This has prompted many physicians to prescribe T preparations to men with ED,¹⁵ even though a causal relationship between altered levels of androgens and erectile function has not yet been established.

Several studies have investigated the effect of androgen replacement on sexual function in hypogonadal^{16–25} and eugonadal^{26–33} men, often with disparate findings. In published reports, the lack of discrimination between androgenic effects on the different domains of sexual function – erectile function, sexual desire, orgasmic function, intercourse satisfaction and overall satisfaction – along with inadequate sample sizes and statistical power contributed to misconceptions and misuse of T in the treatment of ED. Animal studies have revealed

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that androgens regulate the expression of nitric oxide synthase (NOS) in the penis^{34,35} and, as demonstrated recently, also expression and function of the phosphodiesterase type 5 (PDE5) gene.³⁶ However, risk analysis on hormonal replacement therapy in postmenopausal women³⁷ has raised severe criticisms against unjustified and unconditional use of hormone therapy in ageing subjects, and many physicians have warned against repetition of similar mistakes in men.³⁸ The cost of a randomized controlled trial (RCT) designed to address safety of androgen supplementation in men is relevant,³⁹ and based on current data none of the pharmaceutical companies sharing the market of androgen replacement therapy would be aiming to sponsor a study that could yield counterproductive results. In addition, the Institute of Medicine clearly indicated that such a large-scale, long-term, risk-assessment study should be conducted only if clinically significant benefit has previously been documented.³⁹ A meta-analysis (MA) of placebo-controlled studies in the literature is an alternative or antecedent to multicentre studies that, while not replacing high-quality prospective RCTs, can at least provide physicians, and companies, with an objective and quantitative summary of all controlled data on the topic.

To this purpose we performed a review of randomized placebo-controlled trials of T supplementation, with separate analysis of the erectile function and sexual desire domains, respectively. In essence, this study sought to address the following questions: (i) 'Does testosterone improve erectile function and/or libido in hypogonadal subjects?' (ii) 'Does testosterone improve sexual function in eugonadal subjects?' (iii) 'Does testosterone supplementation have a role in the treatment of ED in the PDE5-inhibitors era?'. The method we applied to these questions is a systematic review of the literature and meta-analysis of randomized placebo-controlled trials.

Materials and methods

Data source identification and selection

A written in-house protocol stating the objectives of the study, the operational definition, the search methods, inclusion and exclusion criteria, the nature of clinically relevant findings, variables of interest, statistical power and period of time covered by the search was submitted to a local consensus panel (three academic board-certified andrologists) and this guided the review process, which was started in January 2003 and ended in December 2004. Studies were identified by a computerized search of MEDLINE, the Cochrane Library, EMBASE and *Current Contents* for the past 30 years (1975–2004), by searching the bibliographies of all retrieved articles and examining references of review articles found during the search to identify additional studies. The search was limited to RCTs published later than 1975, because at that time testosterone assays became widespread and more reliable. The database computerized search used the following key words and MESH terms: 'sexual function', 'impotence/erectile/dysfunction', 'clinical trial', 'randomized clinical trial', 'testosterone', 'testosterone therapy', 'androgen therapy'. Search was limited to trials performed with testosterone, including T esters, and dihydrotestosterone (DHT) preparations, independently of delivery. Inclusion criteria for eligible studies were: (1) the use of one active treatment group compared to a matched placebo group, (2) random-

ization of treatment and (3) adherence to a blinded protocol (double-blinded or blinded to patients only). Only articles published in peer-reviewed medical journals were used; no abstracts were used. Reports that compared active treatment with a control group (observation or not randomized) were excluded.^{40–44} Studies using clomiphene citrate,^{13,45} human chorionic gonadotrophin (hCG)⁴⁶ or oral mesterolone^{47,48} as active treatment were not included in the analysis because their effects could not be solely related to direct activation of the androgen receptor, they were synthetic compounds that could not be aromatized (important for CNS effects),⁴⁹ or they were not routinely used in clinical practice to treat androgen deficiency in adult males. Some of the studies of greater relevance in the field were reported for direct comparison with results obtained in the MA and for sensitivity analysis.^{13,45} Studies that used as active treatment a combination of androgens and PDE5-inhibitors were analysed separately.^{44,50,51} Exclusion of unpublished observations was not a major concern as the majority of RCTs were negative reports for at least one of the outcomes of interest. In several studies, issues of sexuality were not the primary outcome of the individual study, and in multiple comparison studies, an effort was made to retrieve those end-point measures cited in the materials and methods but not reported in the results section because they were lacking statistical significance.

Data abstraction

RCTs were abstracted using an in-house developed software. For each study included, the following data were abstracted: study reference details, number of subjects, withdrawals and drop-outs, mean and standard deviation (SD) (or standard error, SE) of baseline and post-treatment groups for all variables related to sexual function, dose and route of administration of all medications used, duration of treatment, prior treatments and discontinuation time, baseline and post-treatment androgen levels, and adverse effects. In some cases, the SD was not reported. If available, the SE was converted to an SD by multiplying it by the square root of the number of patients. Alternatively, the SD was calculated from the raw data points directly or measured in the error bars of a graphic display, or estimated using a regression model with the number of subjects, effect size and control group SD as predictors of missing treatment group SD values. When only ranges were provided, SD was obtained divided by the absolute interval by the square root of the number of patients. Sensitivity analysis was carried out with the exclusion of studies in which SD was calculated, in order to evaluate whether this approach had altered the findings. Table 1 presents some important aspects of each of the 17 clinical trials identified by the search.

Control of bias

Blindness and randomization were considered crucial factors because all scores of sexual activity, except nocturnal penile tumescence (NPT) measurements, were subjective measures. However, in those studies where a prescreening follow-up period discriminated patients with psychogenic ED, it has been shown that organic ED patients exhibit a modest placebo effect.⁴¹ The very high completion rate (> 95%) was reassuring because the lower the completion rate, the greater the chance for biases entering into the study. The quality

Table 1. Randomized placebo-controlled clinical trials of testosterone therapy on sexual function in adult men

Reference (Year of study)	No.	Age (years) (range)	Basal testosterone (nmol/l)	Regimen	Study period	Type of hypogonadism
Davidson <i>et al.</i> ¹⁶ (1979)	T: 6 P: 6	(39–61)	T < 5.2 P < 5.2	TE 400 mg/4-weeks	5 months	Primary: 33% Secondary: 77%
Skakkebaek <i>et al.</i> ¹⁷ (1981)	T: 12 P: 12	(22–50)	T: 1.5 ± 1.7 P: 1.5 ± 1.7	TU 160 mg/day	2 months	Primary: 50% Secondary: 50%
Bancroft and Wu ²⁰ (1983)	T: 8 P: 8	(21–57) 34.8 ± 11.9	T: 4.4 ± 3.6 P: 4.4 ± 3.6	TU 160 mg/day	2 months	Primary: 75% Secondary: 25%
Kwan <i>et al.</i> ¹⁹ (1983)	T: 6 P: 6	(24–69) 47.5 ± 10.2	T: 2.6 ± 0.1 P: 2.6 ± 0.1	TE 200–400 mg/month	3 months	Primary: 75% Secondary: 25%
Nankin <i>et al.</i> ²¹ (1986)	T: 10 P: 10	(51–72)	T: 13.7 ± 3.6 P: 11.1 ± 2.8	TC 200 mg/2-weeks	4 months	Primary: 10% Secondary: 90% Normogonadotropic
Carani <i>et al.</i> ^{22,27} (1990)	1 T: 6 P: 6 2 T: 8 P: 8	(21–57) 38 ± 6.2	T: 8.9 ± 5.2 P: 8.9 ± 5.2 T: 10.7 ± 4.9 P: 10.7 ± 4.9	TU 160 mg/day	6 weeks	Primary: 100% Secondary: 0%
Dobs <i>et al.</i> ²³ (1998)	T: 8 P: 5	41 ± 16	T: 6.1 ± 4.4 P: 5.8 ± 3.6	Buccal testosterone 10–20 mg/day	2 months	Primary: 46% Secondary: 54%
Seidman <i>et al.</i> ²⁴ (2001)	T: 13 P: 17	(35–71) 53.6 ± 10	T: 9.3 ± 6.8 P: 9.1 ± 5.9	TE 200 mg/week	6 weeks	Not known
Steidle <i>et al.</i> ²⁵ (2003)	T: 106 P: 99	56.8 ± 10.6	T: 8.1 ± 2.2 P: 7.9 ± 2.8	TG 100 mg/day	3 months	Primary: 7–8% Secondary: 93%
Benkert <i>et al.</i> ²⁶ (1979)	T: 13 P: 16	(45–75) 56.5 ± 5	T: 21.0 ± 6.8 P: 18.0 ± 7.4	TU 120 mg/day	8 weeks	Eugonadal men
O'Carroll and Bancroft ²⁷ (1984)	T: 20 P: 20	(19–64) 45.4 ± 13	T: 19.9 ± 6.2 P: 19.9 ± 6.2	Sustanon 250 mg fortnightly	3 months	Eugonadal men
Anderson <i>et al.</i> ²⁸ (1992)	T: 16 P: 15	(21–41)	T: 17.7 ± 7.2 P: 19.0 ± 8.9	TE 200 mg/week	4 weeks	Eugonadal men
Aydin <i>et al.</i> ²⁹ (1996)	T: 20 P: 18	38.7 ± 11.5		TE 120 mg/day	4 months	Eugonadal men
Schiavi <i>et al.</i> ³⁰ (1997)	T: 12 P: 12	(46–67) 60 ± 6.1	T: 15.32 ± 4.4 P: 13.6 ± 5.8	TE 200 mg/2-week	6 weeks	Eugonadal men
Snyder <i>et al.</i> ³¹ (1999)	T: 50 P: 46	73.1 ± 5.8	T: 12.7 ± 2.7 P: 12.8 ± 2.8	TS patch 6 mg/day	36 months	PADAM Normogonadotropic
Cavallini <i>et al.</i> ³² (2004)	T: 40 P: 45	(60–72) 63.4	T: 9.9 ± 1.8 P: 10.5 ± 2.3	TU 160 mg/day	6 months	PADAM Normogonadotropic
Svartberg <i>et al.</i> ³³ (2004)	T: 14 P: 15	(57–75) 66 ± 6.1	T: 21.6 ± 5.7 P: 20.5 ± 5.7	TE 250 mg/week	26 weeks	Eugonadal men

T, testosterone; P, placebo; TU, testosterone undecanoate; TE, testosterone enanthate; TC, testosterone cypionate; TP, testosterone propionate; TD, transdermal testosterone patch; TS, trans-scrotal testosterone; TG, transdermal testosterone gel; PADAM, partial androgen deficiency of the aging male.

of the individual studies was not similar in respect of different issues of sexual function, some studies being more focused on different primary outcomes (e.g. quality of life, body composition, NPT) than on libido or erectile function. In addition, many articles failed to report data discriminating results in the two domains, and only an overall sexual response could be abstracted. Differences among the studies involved the number of patients included, treatment regimen used, duration of treatment, and age and gonadal status of participating subjects. Crucial issues were prior androgen treatments and, when performed, the duration and the discontinuation (wash-out) prior to commencement of the trial. In general, we found that there was a trade-off between internal (how well the study was designed

to assess the issue of testosterone treatment) and external validity (to what extent the results from the study sample could be generalized to the population at large), so that the better the design, the less the study resembled the real world.⁵² The chance of publication bias was assessed using the funnel plot, Egger's test, Begg's test and the Rosenthal's 'file drawer' method.⁵³

Methods for data pooling

For each study, a treatment-effect size and the 95% confidence interval (CI) for the effect size were calculated using the method of Hedges and Olkin.⁵⁴ As most of the studies used different scales to

measure the effect of treatment, the mean difference for each study was divided by the pooled estimate of the SD, in order to express the effect size for each study in a common metric, namely the standardized mean difference (SMD). Unless otherwise specified, the overall effect sizes (EFS) are reported as units of SDs. This measure reflects how much active treatment affects the distribution of the outcome of interest compared to placebo. According to Cohen,⁵⁵ a small treatment-effect size is considered to be about 0.2, a medium effect size to be about 0.5, and a large effect size to be about 0.8. These guidelines can be used to judge the treatment-effect size of the individual studies as well as the treatment-effect size for all studies combined. Alternatively, the results expressed in SMDs can be multiplied by the SD of a known reference scale to obtain the same dimensional units. Because of the diversity in the sample populations with respect to their age, geographical origin, health and gonadal status, it could not be assumed that studies sampled the same population. It is more likely that there is a population of effect sizes, from which studies in the MA were randomly sampled;⁵⁶ for this reason summary statistics were reported using the EFS of the random effect model.

Analysis of heterogeneity

The presence of between-study heterogeneity was assessed using the standard χ^2 -test (denoted the *Q*-test) and quantified using the I^2 , which is a measure of the degree of inconsistency across studies, or the among-study variance (denoted τ^2).⁵⁷ Subgroup and sensitivity analysis was performed to explore the contribution of the different diagnostic categories to the overall effect. Comparison of the absolute values of the subgroup effect sizes should guide the physician to identify the kind of patients more likely to respond to the treatment. According to the predefined in-house protocol, between-study heterogeneity was also explored using meta-regression analyses to investigate the effect of the characteristics of the study populations, among which the inclusion criteria for age and gonadal status, the baseline T concentrations (using the mean 0900 h serum total T concentration), patient age (using mean age), the year of publication (using the year the study went to press), the length of study and the kind of preparation used. Meta-regression analysis was performed even if heterogeneity was not a significant issue in the majority of investigated variables. As the contribution of gonadal status to the effect of T therapy was a major point of interest of the present meta-analysis, additional analyses were performed. For each trial, an operational definition was provided by the reviewers based on the following criteria: when mean baseline T concentrations for both the placebo and the active treatment group were above 10 nmol/l, the study was defined as a 'study on eugonadal subjects' (for men aged > 50 years); on the contrary, if either the placebo or the active treatment group had a mean baseline serum T of < 10 nmol/l, the study was defined as a 'study on hypogonadal subjects'. However, such a defined cut-off might appear 'crude'. Therefore, we also divided the eligible studies into three categories: those with T > 12 nmol/l (eugonadal), those with T between 7 and 12 nmol/l (mixed), and those with T < 7 nmol/l (hypogonadal). We acknowledge that there may still be a consistent degree of uncertainty and heterogeneity in this classification, as well as in the diagnosis of hypogonadism based on a serum T specimen measured using different protocols; however,

this approach was found to be simple, consistent and reproducible. In addition, all studies with a basal mean T-value below 7 nmol/l were performed in hypogonadal men, while all studies with basal mean T above 12 nmol/l were performed in eugonadal men; this allows a direct comparison between the two groups of subjects. The test of interaction between groups was used to verify the hypothesis that both groups of studies were similar. The contribution of other variables, such as the presence of ED or other comorbidities (identified by questionnaires/enrolment criteria), was also investigated. Even though formal penile Doppler ultrasonography was not used in these studies, based on the clinical characteristics of the enrolled subjects (inclusion criteria), studies were arbitrarily categorized as likely to be performed in men with vasculogenic ED or not. A mixed model with restricted maximum likelihood ratio was used for meta-regression analysis.⁵³ All statistical analyses were carried out using Stata Statistical Software, release 8.2SE (Stata Corp., College Station, TX, USA).

Results

Study characteristics

Table 1 summarizes the 17 eligible reports that met all inclusion criteria. Five studies were performed on hypogonadal men (defined as having a study population baseline T-value below 7 nmol/l), seven on eugonadal (T > 12 nmol/l) and five on mixed hypo-eugonadal men. The 17 articles included in the analysis gave details of 656 patients over 20 years: 284 were randomized to testosterone, 284 were randomized to placebo and 88 were treated in a randomized cross-over design.^{16,19–22,27,30} The weighted mean age among studies was 57.5 years; the median study length was 3 months (range 1–36 months). No studies were large; most had less than 20 subjects in each arm (11 vs. 6 trials), and many were crossover studies (7 vs. 10). The studies varied widely in terms of T preparation, drug delivery and dosing, protocol design, measure of end-points, age and gonadal status of enrolled subjects, and presence and severity of sexual dysfunction at baseline. All eligible reports were described as randomized placebo-controlled trials (even though the methods of randomization were seldom reported). Fourteen trials blinded both the physicians and the patients, while three blinded only the patients. *P*-values were reported in the majority of studies. None reported a projected sample size or power calculation; and none used CIs. Figure 1 displays the 95% CI values for the results of each study. The width of the CI is inversely related to the power of the study to distinguish the testosterone group from the placebo group. Among all studies, eight trials investigated the effects of T on sexual thoughts and fantasies, six trials the effect on sexual desire and motivation, six trials the effect on night/morning erections, nine trials the number of successful intercourses per week, seven trials the number of total erections/ejaculations per week, seven trials the rating of erection/potency and seven trials the overall sexual satisfaction. In addition, eight reports gave details on the global assessment questions and response rates. Three studies^{19,23,32} reported the effects on NPT measurements. Sexual motivation, sexual thoughts and morning erections were either considered singularly or part of a wider group defined as the libido domain (LD), whereas the rating of

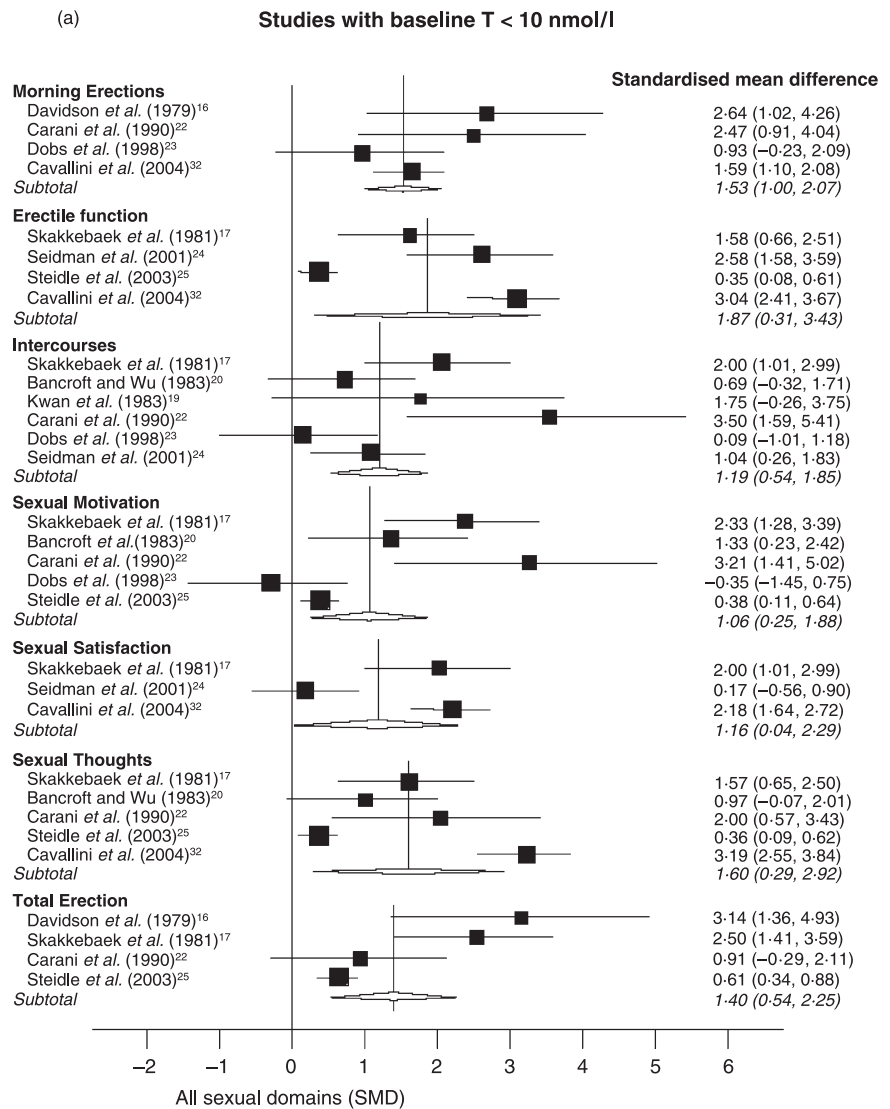


Fig. 1 Forest plot of the effects of testosterone (T) versus placebo (P) on different domains of sexual function in men with mean T levels (a) below 10 nmol/l and (b) above 10 nmol/l. The squares indicate the study-specific effect and the lines the 95% confidence interval. The rhombus represents the summary of the pooled studies.

erection, number of successful intercourses and total weekly erections were considered as part of the erectile function domain (EFD). Only two studies^{23,33} measured the SHBG levels; body mass index (BMI) was reported in only three studies and was ≤ 30 .^{25,31,33} Given the number of studies evaluated and the SD of the mean effect size, we had a 95% chance of detecting a medium-effect difference (0.5) of testosterone compared to placebo, with statistical significance. Thus, the 17 eligible studies, when pooled, provided adequate power for answering our primary question.

Pooled effects and subgroup analysis

The estimate of effects of T vs. P on different domains for all studies is reported in Fig. 1. The studies are classified according to the mean baseline T-value of each study population as having a mean T below (Fig. 1a) or above (Fig. 1b) 10 nmol/l, respectively. The first striking feature of these figures is that, although on absolute values the SMDs between the T-treated and the P-treated groups are small (i.e. the effects in some studies are close together) and vary widely between

studies, the T-treated groups always behaved better than the P-treated groups. This observation indicates that despite a substantial number of 'negative' studies (where statistical significance was not achieved), the cumulative addition of the individual treatment effects in an MA might be significant. Treatment effects were pooled according to the outcome of interest, with each trial providing 1–4 different outcomes. Both libido and erectile function domains increased significantly with T therapy compared to P. In the studies with a mean baseline T below 10 nmol/l, the pooled EFS for morning erection was 1.53 (95% CI 1.00–2.07), while in sexual motivation and sexual thoughts EFS was 1.06 (0.25–1.88) and 1.60 (0.29–2.29), respectively. The effects on erectile function were demonstrated by an improvement in erectile function (EFS 1.87; 0.31–3.43), episodes of successful intercourse (EFS 1.19; 0.54–1.85), sexual satisfaction (EFS 1.16; 0.04–2.29) and total erections/ejaculations (EFS 1.40; 0.54–2.25). Because most of these intervals are far from zero, testosterone treatment appears to be effective in most of studies performed in men with an average baseline T-value below 10 nmol/l. The overall pooled effect size of T on the number of intercourses was roughly

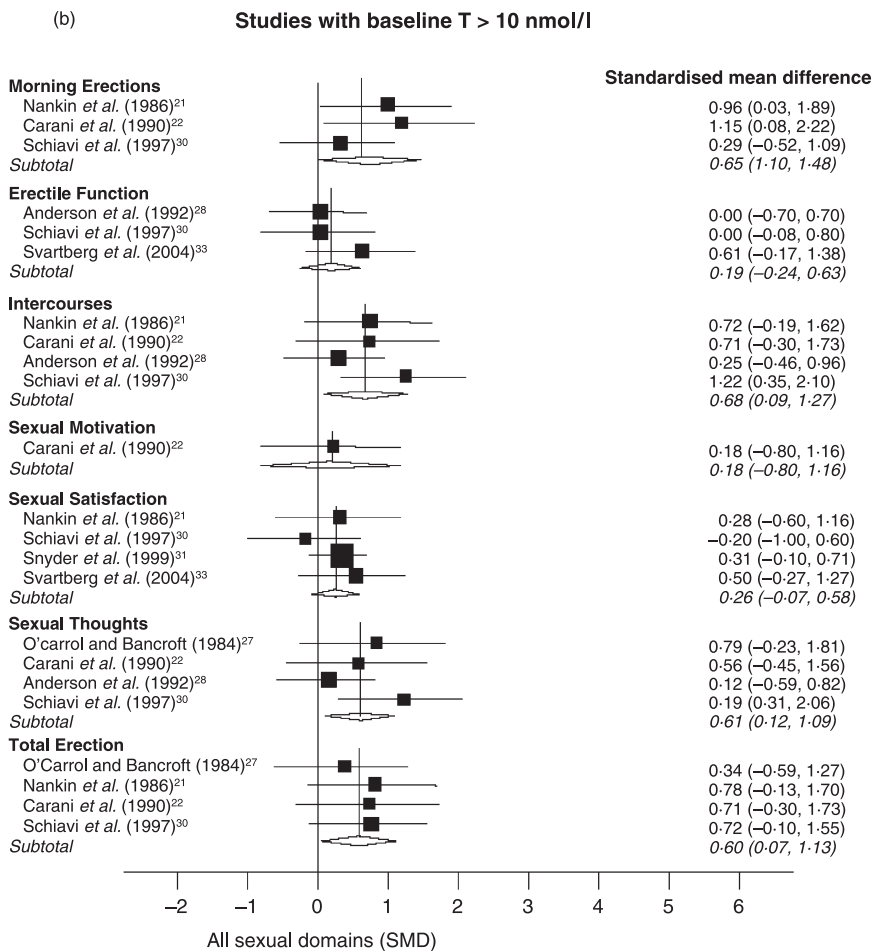


Fig. 1 Continued

equivalent to the doubling of successful sexual attempts per week (from 0.9 to 1.9 intercourses per week). However, a significant heterogeneity was found with up to 58% of variation of SMD attributable to heterogeneity (I^2). The estimate of between-study variance (τ^2) was 0.4. Only for morning erections were the results homogeneous ($I^2 = 30\%$). The observed heterogeneity found in most domains means that there is a possibility that separate subgroups of studies are being merged in the overall analysis. In addition, a considerable overlap in the CI of treatment-effect size between the two groups of studies was found (Table 2). For this reason we classified studies into smaller groups based more on homogeneous values of baseline T (Fig. 2). We then grouped studies according to the effects elicited in the two major domains of sexual function: libido and erectile function. The analysis revealed an equal magnitude of effect of T in both libido and erectile function in either studies performed on severely hypogonadal men (< 7 nmol/l; Fig. 2a) or men with partial androgen decline (7–12 nmol/l; Fig. 2b); on the contrary the effects of T in studies with basal T above 12 were much smaller or not significant (Fig. 2c). The findings in the latter group were homogeneous (I^2 between 11 and 43%). All subjects enrolled in the first group of studies were hypogonadal and all subjects included in the latter group were eugonadal according to the inclusion criteria of the original studies. Therefore, even though the studies are classified on the basis of mean T-values, Fig. 2 allows a direct comparison of

hypogonadal with eugonadal men (group 1 vs. group 3). The comparison between Figs 1 and 2 shows that a mean T-value of 12 nmol/l is a better cut-off than 10 nmol/l in predicting the response to treatment (see sensitivity analysis). The effects reported in Figs 1 and 2 are not adjusted for covariates and other confounding variables, and hence sensitivity and meta-regression analysis were also performed.

Sensitivity analysis

To explore the heterogeneity further, eligible trials were divided into a number of subgroups based on common study characteristics (Table 2). A 95% CI was calculated for each pooled subgroup. The only subgroup that reached statistical significance in this manner was connected with the presence of organic ED at enrolment, the presence of comorbidities (such as diabetes mellitus, hypertension, chronic obstructive pulmonary disease, dysthyroidism) and the duration of the study. Results of the most relevant subgroups are reported in Table 2. Sensitivity analysis, performed by calculating EfS by progressively adding studies by increasing sample size, revealed that the larger the sample of the study, the less T improved sexual function. Influence analysis performed calculating EfS by adding randomly one study at a time failed to identify any particular study as a major source of heterogeneity (data not shown).

Table 2. Sensitivity analysis. For each group and subgroup the pooled effect size and confidence interval are reported. The *P*-values refer to the test of heterogeneity between subgroups

	Mean T < 10 nmol/l		Mean T > 10 nmol/l	
Sexual domains (investigated outcomes)				
LD	0.8 (0.5–1.1)	} <i>P</i> = 0.09	0.5 (0.2–0.9)	} <i>P</i> > 0.1
EFD	1.0 (0.7–1.3)		0.3 (0.1–0.6)	
Erectile function at baseline				
No ED	0.4 (0.2–0.6)	} <i>P</i> < 0.01	0.2 (–0.0–0.5)	} <i>P</i> = 0.08
ED	1.4 (1.1–1.7)		0.5 (0.2–0.8)	
Comorbidities at enrolment				
Healthy	0.6 (0.4–0.9)	} <i>P</i> < 0.01	0.4 (0.2–0.6)	} –
Diseased	1.1 (0.7–1.4)		–	
Duration of treatment				
≤ 12 weeks	1.2 (0.9–1.5)	} <i>P</i> < 0.01	0.4 (0.2–0.6)	} <i>P</i> > 0.1
> 12 weeks	0.5 (0.3–0.7)		0.3 (–0.1–0.7)	
Mean age				
≤ 50 years	1.3 (0.9–1.8)	} <i>P</i> < 0.01	0.4 (0.1–0.7)	} <i>P</i> > 0.1
> 50 years	0.6 (0.4–0.8)		0.4 (0.1–0.8)	

LD, libido domain; EFD, erectile function domain; ED, erectile dysfunction.

Meta-regression analysis

Based on the in-house protocol, the baseline T concentration, year of publication and mean age were the only three continuous prespecified covariates to be investigated in the meta-regression analysis. Covariates were included in the model in a step-forward fashion, generating Table 3. Including baseline T-levels as covariate in the model suggests an approximately linear constant reduction of effect with increasing T concentration. The regression equation is $EfS = -0.07 \times T \text{ concentration} + 1.8$. The estimate of residual between-study variance was 0.5 (τ^2), suggesting that baseline T concentration explained little of the heterogeneity of treatment effects between studies. The presence of sexual dysfunction was subsequently added to the model (see Table 3) and revealed that studies including patients with ED revealed higher EF compared to those performed men without sexual disorders (see Table 3). Age did not add any significant contribution. Year of publication was subsequently added to the model and, although the data did not reach statistical significance (*P* = 0.09), earlier studies were revealed to have had higher EF

compared to more recent ones. After accounting for these sources of between-study heterogeneity, residual heterogeneity decreased, but remained within the between-study variance estimated as 0.3 (τ^2). To determine whether the residual differences could be due to the T preparation used, meta-regression analyses, including baseline T-values and type of T administered as covariate, were performed. T preparations were classified into five groups taking into account the number of studies available for the analysis: T injections, T oral (undecanoate), T gel, T patches, and other formulations. A numerical value ranging from 1 to 5 was randomly assigned to each group, generating five factorial new variables (*n* = 120). Each variable was then tested singularly in the model with the other covariates, to identify any potential effect modifier linked to the T preparation used. Five combinations (10 with their reciprocal) produced a small residual τ^2 , indicating that the type of T preparation contributes to the heterogeneity of these studies. The final regression equation shown in Table 3 is that with the average of scores given to these combinations for the different T preparation. In general, T undecanoate and T gel behaved better than T injections or T patches. However, these results are severely influenced by differences in the number/size of trials available for each preparation and therefore should not be used to compare drug effectiveness. Finally, to investigate whether the gonadal status contributed differently to the effect of T therapy on the libido and erectile function domains, two separate regression models of EfS on T were developed for LD and EFD with the regression equation as reported in Table 3. The model revealed that the effect of T on erectile function, but not on libido, was inversely related to baseline T concentration (Fig. 3).

Bias analysis

A Funnel plot for all studies included in the analysis examining any effect of T on sexual function was constructed to assess the degree of publication bias. This plot (Fig. 4) was highly asymmetrical, indicating that publication bias is likely to have an influence on the overall analysis. Egger's and Begg's tests confirmed the presence of significant publication bias even in subgroups of outcomes and gonadal status (*P* < 0.01, for both). However, when the Funnel plots were adjusted by the covariates described in the meta-regression analysis to remove any distortion caused by this known outcome modifier, a notable improvement in the visual impact of the Funnel plot was obtained and Egger's test revealed a *P* = 0.132 for risk of publication bias.

Table 3. Meta-regression analysis

Model	Covariates	Coefficients (SE)	<i>P</i> -value	Equation	τ^2
1	Basal testosterone (T)	–0.07 (0.02)	< 0.001	$EfS = -0.07T + 1.77$	0.5
2	Basal testosterone (T)	–0.06 (0.02)	< 0.001	$EfS = -0.06T + 0.69ED + 0.87$	0.3
	Erectile dysfunction (ED)	+0.69 (0.23)	< 0.001		
3	Basal testosterone (T)	–0.04 (0.01)	< 0.005	$EfS = -0.04T + 0.45ED - 0.27D + 1.7$	0.1
	Erectile dysfunction (ED)	+0.45 (0.18)	< 0.001		
	Treatment preparation (D)	–0.27 (0.11)	< 0.05		

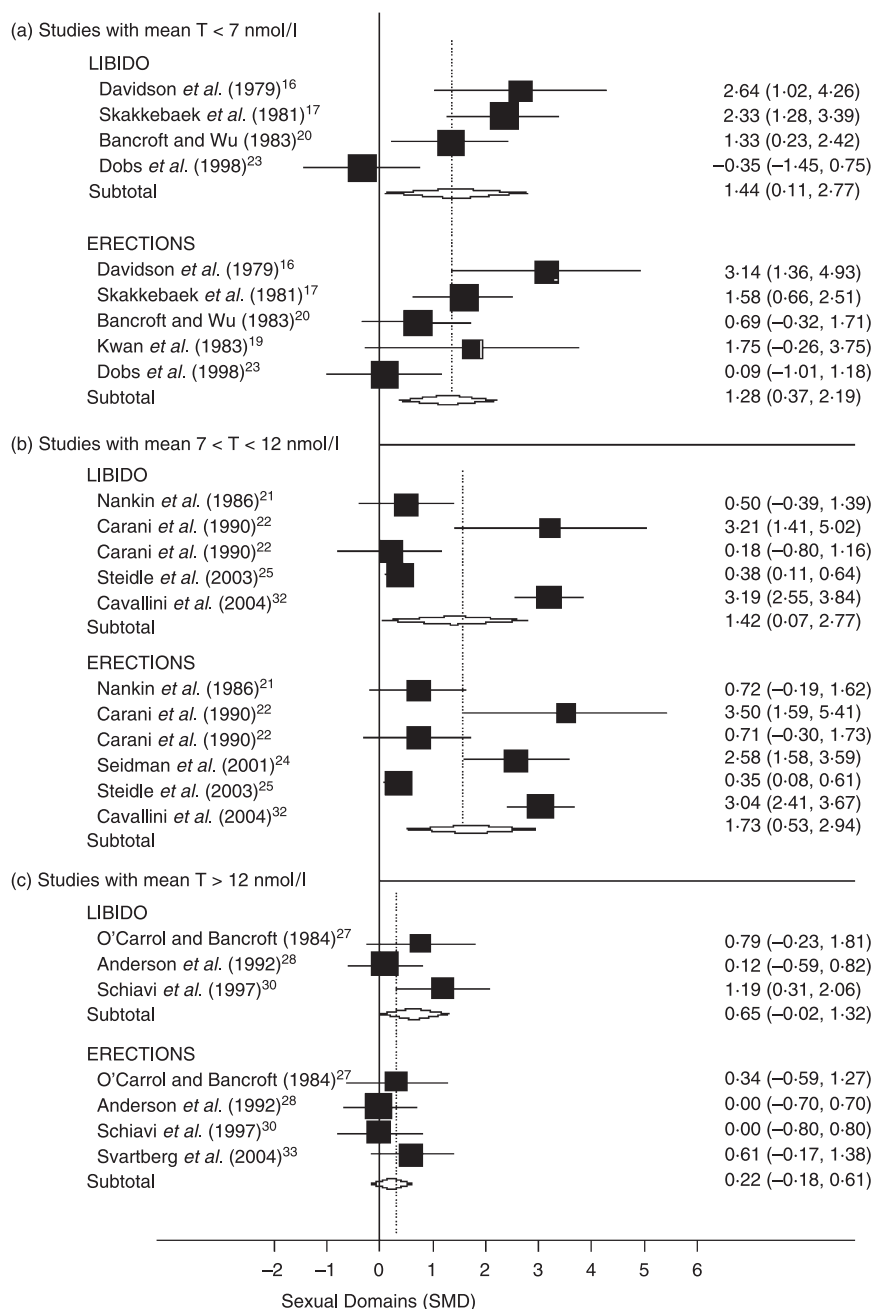


Fig. 2 Forest plot of the effects of testosterone (T) versus placebo (P) on the two major domains of sexual function: libido and erectile function. Studies were grouped according to baseline T concentrations in three groups: (a) serum T below 7 nmol/l, (b) serum T between 7 and 12 nmol/l and (c) serum T above 12 nmol/l.

Discussion

The results of the meta-analysis may be summarized as follows: i) a significant improvement in sexual function was found in studies performed in men with low and low-normal T levels, receiving standard replacement doses of testosterone compared to placebo; ii) the magnitude of effect on erectile function was inversely related to the baseline testosterone concentrations of the study population and detectable only in studies with basal T-values below 12 nmol/l; iii) no relevant effect of testosterone was found in studies with mean baseline levels above 12 nmol/l; iv) the presence of ED and other comorbidities (diabetes mellitus, hypertension, dyslipidaemia, etc.) the type of T preparation used and the length of follow-up assessment

all influenced the response to treatment; v) a significant heterogeneity in effect sizes and risk of publication bias was found in the currently available controlled studies on testosterone replacement and sexual function.

It is still a matter of debate whether the age-related changes in androgen levels^{9,58,59} play a role in the decline of sexual function of ageing men.^{5,60} Sexual activity declines even in old men who are healthy and have partners.⁶¹ There is a steady decline in orgasmic frequency,¹ sexual thoughts and enjoyment with age;⁶² prolonged detumescence, decreased vasocongestive responses and an increase in refractory period have also been described.⁶³ Regardless of the fact that a causal relationship is far from being demonstrated, androgen therapy for ED has proliferated, even in the PDE5-inhibitor era,⁶⁴ as

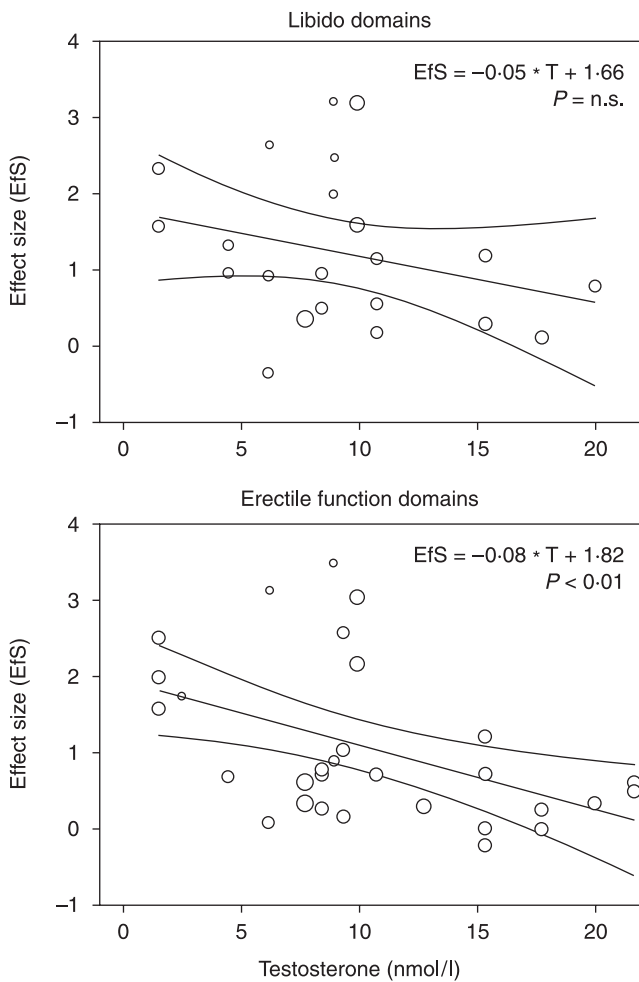


Fig. 3 Bubble plot showing the meta-regression analysis of the effects of testosterone on different domains of sexual function according to the baseline testosterone value of the study population. The size of the circle is inversely related to the variance of the study. The solid lines represent the linear regression and its 95% confidence interval for testosterone only.

an empirical treatment mainly based on personal experience and uncontrolled evidence. The physicians facing the decision to treat sexual dysfunction with androgens must be aware that only a few controlled data support this therapeutic option; nevertheless, by using adequate statistical techniques some conclusions can still be drawn.

MA has been used to address questions for which multiple data sources are in conflict or fail to reach a consensus. MA is particularly useful when there are a variety of reports with low statistical power; thus, pooling data can improve power and provide a convincing result. MA can also examine the body of literature as a whole in a way that a single investigation cannot. However, MA is most likely to provide a convincing answer when the studies are well designed and executed. In the present analysis four major topics of concern were identified. The first is on the quality of the studies retrieved. All the studies retrieved but one³¹ are short (6 months' duration or less), involve small numbers of participants (well under 500), and report data without adequate measures of variability or statistical considerations. The second concern is whether all relevant reports

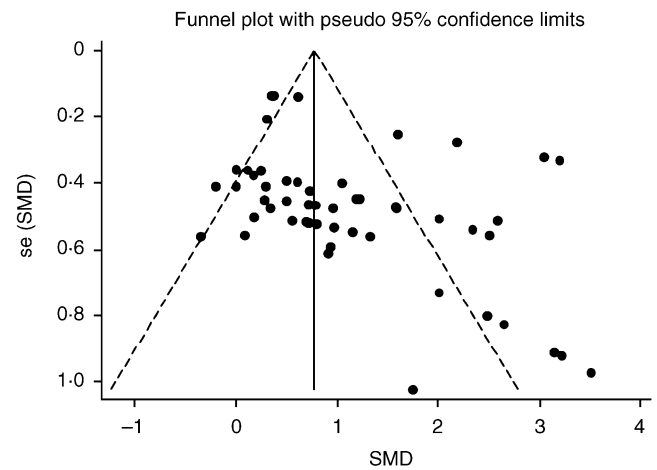


Fig. 4 Funnel plot for all studies included in the analysis.

were included. To overcome this issue, the most comprehensive search was performed and all suitable observations were included (with some trade-off in quality scores); then, we considered the size of a hypothetical unreported trial that would have substantially altered our conclusions. For the combined group of studies to reach a significant finding in favour of placebo, this trial would require such a large effect size that its existence can be reasonably rejected. The third concern regards pooling all the results together. Although all reports met the entrance criteria, they otherwise differed in age, size, aetiology of ED, drugs used, and just about every other characteristic. These differences may suggest that they should not be pooled. However, we were able to demonstrate that most of the heterogeneity of the reports was due to discrete aspects that explained with adequate statistical power the differences in effect sizes among trials. Although subdividing studies in diagnostic categories may appear a rational approach (see Table 2), providing a summary/indication for every group may lose applicability in clinical practice. Indeed, we have shown that meta-regression analysis of the eligible studies provided the best optimization for heterogeneity of data. The fourth concern is that the findings of this study are nonetheless prone to publication biases that may threaten their validity. Therefore, readers are invited not to focus only on the pooled measure, but to consider the whole forest plot reflecting the variation among all studies included. By using meta-regression analysis we tried to identify such pitfalls for future studies.⁵⁹

In adult males, a clear relationship between lower androgen levels and reduced sexual activity was first documented by Davidson *et al.*^{16,65} and Tsitouras *et al.*⁶⁶ Soon after, Slag *et al.*¹¹ and Spark *et al.*¹⁰ showed that ED and variable degrees of hypogonadism frequently coexist. In impotent men bioavailable testosterone (bio-T) is reduced far beyond that seen with age.^{67,68} Fabbri *et al.*⁶⁹ claimed a reduction in bioactive LH in psychogenic impotence, and Jannini and colleagues^{70,71} showed that T increases in men with ED who resume sexual activity using PDE5 inhibitors. However, Korenman *et al.*⁵ showed that hypogonadism, without compensatory gonadotrophin elevation, and impotence are both common in older men and distributed independently.

Because in young adults T levels as low as 7 nmol/l (200 ng/dl) are sufficient to restore sexual function, at least in the short term,^{18,72} it has been proposed that healthy males have much higher T levels than those required to maintain sexual function. Although this may apply *acutely* to young adults, several authors have proposed that this may not be the case *chronically* for elderly men. The age-related increase in adiposity,^{73,74} changes in the expression of androgen receptor in the genitalia and the polymorphism of CAG repeats may contribute to this hypothesis. Morley *et al.*⁷⁵ demonstrated that in healthy ageing males the decline in bio-T was the best predictor of age-correlated changes in cognitive and physical measures; but this still does not resolve the issue of whether older men respond in a favourable manner to testosterone 'replacement therapy', in terms of measurable domains of sexual function.⁷⁶

Effects of testosterone on nocturnal penile tumescence (NPT)

In nearly every study we reviewed on hypogonadal and eugonadal men, androgen supplementation resulted either in increased frequency, magnitude, duration or quality of NPT (EFS = 1.53 and 0.65). Furthermore, in eugonadal men, a single bed-time dose of exogenous T, sufficient to increase substantially circulating levels, produced more rigid and longer NPT than placebo.⁷⁷ Recently, it has been proposed that long-lasting pro-erectile drugs (PDE5i) may enhance nocturnal erections thus providing protection against hypoxia.⁷⁸ Therefore, the use of T to increase the number and duration of NPTs might be beneficial for the rehabilitation of long-standing sexual inactivity. The finding that erections after visual erotic stimuli (VES) continue despite androgen withdrawal^{20,49,79} led to the conceptual distinction between androgen-dependent and -independent responsiveness in men, with NPTs being seen as a 'window' into the neurophysiological substrate of central arousal, which relates to both spontaneous erections and sexual appetite,⁸⁰ whereas response to VES probably involves a different pathway. It now appears that such distinction is oversimplified, but still remains as androgen effects on NPT and spontaneous erections are much greater than erectile response to VES.⁸⁰ Indeed, even VES-induced erections can be affected by androgens in terms of duration of response, time to detumescence and degree of rigidity.⁸⁰

Effects of testosterone on libido

Many investigators^{17,20,22,42} found that androgen administration to hypogonadal men increased 'all measures of sexual interest and behaviour'. Although ageing men more often complain of erectile failure than of loss of libido, Hajjar *et al.*⁸¹ reported a dramatic improvement in self-assessed libido in hypogonadal elderly men receiving T therapy compared to controls. The study, however, was retrospective and, despite the positive results, one-third of patients discontinued the therapy.⁸¹ Conversely, in old hypogonadal men receiving testosterone for ED, Nankin *et al.*²¹ showed only borderline significant changes in sexual motivation. Dobs *et al.*²³ found more impressive effects of buccal T on erectile function compared to libido. Thus, T replacement seems to enhance the frequency of sexual thoughts and the intensity of sexual feelings, with a larger effect

reported in young hypogonadal men than in ageing men. Indeed, in old men with hypoactive sexual disorders but adequate androgen levels, T supplementation failed to improve sexual life, suggesting that T may not be effective when other psychological factors are present. Our data support this hypothesis. In studies with a mean T below 10 nmol/l, we found a significant effect of T replacement on sexual thoughts (EFS = 1.60) and sexual motivation (EFS = 1.06), with lower effects sizes than those obtained in the erectile function domains (EFS = 1.87 and 1.19). Surprisingly, even in studies with a mean T above 10 nmol/l, testosterone administration produced a significant effect in sexual thoughts (EFS = 0.61). A possible explanation to combine the findings from experimental studies and clinical trials is that the effects of T on libido are not closely related to the achievement of a 'universal threshold' value, but rather due to a 'relative' and 'transient increase' in circulating sex hormones. This hypothesis would explain why an improvement in libido can be achieved by either treating GnRH analogue-induced hypogonadal men with subphysiological replacement doses⁸² or eugonadal subjects with suprphysiological doses,^{28,83} but not by treating eugonadal men with standard replacement doses. Finally, some investigators^{84,85} found that anxiety and irritability decreased in chronically hypogonadal men receiving androgen replacement, suggesting that the effects of androgens on human emotional traits and behaviour are complex.

Effects of testosterone on erectile function

In most of the studies on hypogonadal men, the number of erectile events per day, penile rigidity and the number of erections successful for intercourse increased significantly during T therapy compared to placebo. Patients' orgasm and satisfaction also improved. The findings from the RCTs reviewed in this MA are consistent with other uncontrolled studies showing a 33–75% improvement in sexual attitudes and performance in hypogonadal impotent patients who received T or clomiphene.^{13,45,86–88} On the contrary, we found that physiological doses of T administered to men with a mean T-value above 10 nmol/l failed to improve rigidity. However, the number of successful intercourses improved significantly in this group, although to a much smaller extent compared to hypogonadal men. By subgroup analysis we were able to show that the effects of T on erectile function are still detectable in studies with mean basal T-values between 7 and 12 nmol/l, but no longer in studies with a mean T above 12 nmol/l. The direct comparison between Figs 1 and 2 reveals that a mean T-value of 12 nmol/l is a better discriminant of the effect of treatment than 10 nmol/l. The meta-regression analysis showed that men with the lowest total serum T concentrations had the greatest response in erectile function, with some effect still detectable up to 12 nmol/l, and that a single cut-off value of 10 nmol/l (300 ng/dl) for serum total T could not accurately predict the success of treatment (sensitivity analysis). These analyses highlight the need for more controlled trials to formally investigate the efficacy of T replacement in men with baseline T concentrations between 7 and 12 nmol/l. It is not known whether erectile changes produced by T primarily involve central (cortical or limbic) or peripheral mechanisms.⁸⁹ It is likely that sex hormones have multiple roles, acting as pleiotropic facilitators that enable synergy and coordination of

events at every site involved in sexual response.⁷⁵ It has been suggested that androgen effects may act to enhance the 'pleasurable awareness of sexual response', acting on both penile sensation and the autonomic nervous system innervations of penile vascular structures.⁹⁰ It is likely that sex steroids lower the threshold to initiate, maintain and conclude sexual acts. Androgens are known to be essential for normal NOS and PDE5 expression in the corpora cavernosa.^{34–36} We found that men with vasculogenic ED had higher responses to T administration. A possible explanation for this apparently surprising finding is that in men with psychogenic ED, T supplementation has a smaller or no clinical effect. Another issue of concern regards the values of free or bioavailable T. Only two studies measured SHBG levels and therefore this covariate could not be included in our analysis.

Salmimies *et al.*¹⁸ and Gooren *et al.*⁷² have tentatively investigated which level of circulating T is critical for sexual functioning in adult men. Upon administration of parental T esters, this level was found to lie between 5 and 12 nmol/l, with a clear intersubject variation. However, such an approach cannot be used to address the issue of androgen therapy in the ageing male. Gooren *et al.*⁷² demonstrated that an acute decline of sex steroid levels do not affect the entire spectrum of sexuality. Only the subjective quality of sexual acts, the frequency of sexual thoughts, and nonsexual elements such as anxiety, vigour and fatigue were adversely affected, but will that stand for chronic androgen deprivation in the ageing male? Once again, the threshold concept appears not to be convincingly demonstrated. Our data would suggest that if such a threshold exists, it would be lower for the behavioural components of sexual function (i.e. libido, sexual desire and motivation) than for the erectile function domains. In elderly men, T levels required to produce maximal effect on erectile capacity may even lie above the lower end of normal reference range. Our previous data on androgen supplementation to men failing PDE5 inhibitors are consistent with this proposal.^{50,91}

In conclusion, clinical evaluation and clinical response should guide the physician treating ED in hypogonadal men; we therefore recommend evaluating patients individually. The National Institute on Ageing Advisory Panel has indicated that the strongest statement that can be made based on existing epidemiological research is that overt hypogonadism (e.g. total T below 250 ng/ml or 8 nmol/l) is associated with osteoporosis and decreased sexual function.⁹² The present study shows that T treatment might be beneficial for sexual function in selected subjects with low (< 7 nmol/l) and low-normal androgen levels (< 12 nmol/l) especially when comorbidities are present. In those men who failed to restore adequate androgen levels using pro-erectile drugs,^{70,71} testosterone treatment might be useful to rehabilitate a patient's sexual life by increasing nocturnal erections, the number of successful intercourses and recovering a full response to PDE5 inhibitors.^{50,51} However, this analysis also indicates the need for more research based on validated methods for the evaluation of the sexual function (IIEF, GAQ and ADAM questionnaires; evaluation of NPTs and penile Color Doppler Ultrasound). In this view, a large-scale, long-term RCT to formally investigate the efficacy of T replacement in symptomatic middle-aged and elderly men with reduced T levels and erectile dysfunction would represent a next step to overcome the limitations of the literature and to better substantiate the role of T replacement therapy in men with sexual dysfunction.

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References

- Morley, J.E. & Kaiser, F.E. (1989) Sexual function with advancing age. *Medical Clinics of North America*, **73**, 1483–1495.
- Feldman, H.A., Goldstein, I., Hatzichristou, D.G., Krane, R.J. & McKinlay, J.B. (1994) Impotence and its medical and psychosocial correlates: results of the Massachusetts Male Aging Study. *Journal of Urology*, **151**, 54–61.
- Fabbri, A., Aversa, A. & Isidori, A. (1997) Erectile dysfunction: an overview. *Human Reproduction Update*, **3**, 455–466.
- Aversa, A., Isidori, A.M., Greco, E.A., Giannetta, E., Gianfrilli, D., Spera, E. & Fabbri, A. (2004) Hormonal supplementation and erectile dysfunction. *European Urology*, **45**, 535–538.
- Korenman, S.G., Morley, J.E., Mooradian, A.D., Davis, S.S., Kaiser, F.E., Silver, A.J., Viosca, S.P. & Garza, D. (1990) Secondary hypogonadism in older men: its relation to impotence. *Journal of Clinical Endocrinology and Metabolism*, **71**, 963–969.
- Gray, A., Feldman, H.A., McKinlay, J.B. & Longcope, C. (1991b) Age, disease, and changing sex hormone levels in middle-aged men: results of the Massachusetts Male Aging Study. *Journal of Clinical Endocrinology and Metabolism*, **73**, 1016–1025.
- Turner, H.E. & Wass, J.A. (1997) Gonadal function in men with chronic illness. *Clinical Endocrinology*, **47**, 379–403.
- Morales, A., Heaton, J.P. & Carson, C.C. III (2000) Andropause: a misnomer for a true clinical entity. *Journal of Urology*, **163**, 705–712.
- Feldman, H.A., Longcope, C., Derby, C.A., Johannes, C.B., Araujo, A.B., Coviello, A.D., Bremner, W.J. & McKinlay, J.B. (2002) Age trends in the level of serum testosterone and other hormones in middle-aged men: longitudinal results from the Massachusetts Male Aging Study. *Journal of Clinical Endocrinology and Metabolism*, **87**, 589–598.
- Spark, R.F., White, R.A. & Connolly, P.B. (1980) Impotence is not always psychogenic. Newer insights into hypothalamic–pituitary–gonadal dysfunction. *Journal of the American Medical Association*, **243**, 750–755.
- Slag, M.F., Morley, J.E., Elson, M.K., Trencle, D.L., Nelson, C.J., Nelson, A.E., Kinlaw, W.B., Beyer, H.S., Nuttall, F.Q. & Shafer, R.B. (1983) Impotence in medical clinic outpatients. *Journal of the American Medical Association*, **249**, 1736–1740.
- Nickel, J.C., Morales, A., Condra, M., Fenemore, J. & Surridge, D.H. (1984) Endocrine dysfunction in impotence: incidence, significance and cost-effective screening. *Journal of Urology*, **132**, 40–43.
- Guay, A.T., Bansal, S. & Heatley, G.J. (1995) Effect of raising endogenous testosterone levels in impotent men with secondary hypogonadism: double blind placebo-controlled trial with clomiphene citrate. *Journal of Clinical Endocrinology and Metabolism*, **80**, 3546–3552.
- Govier, F.E., McClure, R.D. & Kramer-Levien, D. (1996) Endocrine screening for sexual dysfunction using free testosterone determinations. *Journal of Urology*, **156**, 405–408.

- 15 Jain, P., Rademaker, A.W. & McVary, K.T. (2000) Testosterone supplementation for erectile dysfunction: results of a meta-analysis. *Journal of Urology*, **164**, 371–375.
- 16 Davidson, J.M., Camargo, C.A. & Smith, E.R. (1979) Effects of androgen on sexual behavior in hypogonadal men. *Journal of Clinical Endocrinology and Metabolism*, **48**, 955–958.
- 17 Skakkebaek, N.E., Bancroft, J., Davidson, D.W. & Warner, P. (1981) Androgen replacement with oral testosterone undecanoate in hypogonadal men: a double blind controlled study. *Clinical Endocrinology*, **14**, 49–61.
- 18 Salmimies, P., Kockott, G., Pirke, K.M., Vogt, H.J. & Schill, W.B. (1982) Effects of testosterone replacement on sexual behavior in hypogonadal men. *Archives of Sexual Behavior*, **11**, 345–353.
- 19 Kwan, M., Greenleaf, W.J., Mann, J., Crapo, L. & Davidson, J.M. (1983) The nature of androgen action on male sexuality: a combined laboratory–self-report study on hypogonadal men. *Journal of Clinical Endocrinology and Metabolism*, **57**, 557–562.
- 20 Bancroft, J. & Wu, F.C. (1983) Changes in erectile responsiveness during androgen replacement therapy. *Archives of Sexual Behavior*, **12**, 59–66.
- 21 Nankin, H.R., Lin, T. & Osterman, J. (1986) Chronic testosterone cypionate therapy in men with secondary impotence. *Fertility and Sterility*, **46**, 300–307.
- 22 Carani, C., Zini, D., Baldini, A., Della, C.L., Ghizzani, A. & Marrama, P. (1990) Effects of androgen treatment in impotent men with normal and low levels of free testosterone. *Archives of Sexual Behavior*, **19**, 223–234.
- 23 Dobs, A.S., Hoover, D.R., Chen, M.C. & Allen, R. (1998) Pharmacokinetic characteristics, efficacy, and safety of buccal testosterone in hypogonadal males: a pilot study. *Journal of Clinical Endocrinology and Metabolism*, **83**, 33–39.
- 24 Seidman, S.N., Spatz, E., Rizzo, C. & Roose, S.P. (2001) Testosterone replacement therapy for hypogonadal men with major depressive disorder: a randomized, placebo-controlled clinical trial. *Journal of Clinical Psychiatry*, **62**, 406–412.
- 25 Steidle, C., Schwartz, S., Jacoby, K., Sebree, T., Smith, T. & Bachand, R. (2003) AA2500 testosterone gel normalizes androgen levels in aging males with improvements in body composition and sexual function. *Journal of Clinical Endocrinology and Metabolism*, **88**, 2673–2681.
- 26 Benkert, O., Witt, W., Adam, W. & Leitz, A. (1979) Effects of testosterone undecanoate on sexual potency and the hypothalamic–pituitary–gonadal axis of impotent males. *Archives of Sexual Behavior*, **8**, 471–479.
- 27 O'Carroll, R. & Bancroft, J. (1984) Testosterone therapy for low sexual interest and erectile dysfunction in men: a controlled study. *British Journal of Psychiatry*, **145**, 146–151.
- 28 Anderson, R.A., Bancroft, J. & Wu, F.C. (1992) The effects of exogenous testosterone on sexuality and mood of normal men. *Journal of Clinical Endocrinology and Metabolism*, **75**, 1503–1507.
- 29 Aydin, S., Odabas, O., Ercan, M., Kara, H. & Agargun, M.Y. (1996) Efficacy of testosterone, trazodone and hypnotic suggestion in the treatment of non-organic male sexual dysfunction. *British Journal of Urology*, **77**, 256–260.
- 30 Schiavi, R.C., White, D., Mandeli, J. & Levine, A.C. (1997) Effect of testosterone administration on sexual behavior and mood in men with erectile dysfunction. *Archives of Sexual Behavior*, **26**, 231–241.
- 31 Snyder, P.J., Peachey, H., Hannoush, P., Berlin, J.A., Loh, L., Lenrow, D.A., Holmes, J.H., Dlewati, A., Santanna, J., Rosen, C.J. & Strom, B.L. (1999) Effect of testosterone treatment on body composition and muscle strength in men over 65 years of age. *Journal of Clinical Endocrinology and Metabolism*, **84**, 2647–2653.
- 32 Cavallini, G., Caracciolo, S., Vitali, G., Modenini, F. & Biagiotti, G. (2004) Carnitine versus androgen administration in the treatment of sexual dysfunction, depressed mood, and fatigue associated with male aging. *Urology*, **63**, 641–646.
- 33 Svartberg, J., Aasebo, U., Hjalmsen, A., Sundsfjord, J. & Jorde, R. (2004) Testosterone treatment improves body composition and sexual function in men with COPD, in a 6-month randomized controlled trial. *Respiratory Medicine*, **98**, 906–913.
- 34 Lugg, J.A., Rajfer, J. & Gonzalez-Cadavid, N.F. (1995) Dihydrotestosterone is the active androgen in the maintenance of nitric oxide-mediated penile erection in the rat. *Endocrinology*, **136**, 1495–1501.
- 35 Mills, T.M., Reilly, C.M. & Lewis, R.W. (1996) Androgens and penile erection: a review. *Journal of Andrology*, **17**, 633–638.
- 36 Morelli, A., Filippi, S., Mancina, R., Luconi, M., Vignozzi, L., Marini, M., Orlando, C., Vannelli, G.B., Aversa, A., Natali, A., Forti, G., Giorgi, M., Jannini, E.A., Ledda, F. & Maggi, M. (2004) Androgens regulate phosphodiesterase type 5 expression and functional activity in corpora cavernosa. *Endocrinology*, **145**, 2253–2263.
- 37 Beral, V. (2003) Breast cancer and hormone-replacement therapy in the Million Women Study. *Lancet*, **362**, 419–427.
- 38 Snyder, P.J. (2004) Hypogonadism in elderly men – what to do until the evidence comes. *New England Journal of Medicine*, **350**, 440–442.
- 39 Liverman, C.T., Blazer, D.G., National Research Council (U.S.) & Clinical Trials of Testosterone Replacement Therapy (2004) *Testosterone and Aging: Clinical Research Directions*. National Academies Press, Washington, DC.
- 40 Clopper, R.R., Voorhess, M.L., MacGillivray, M.H., Lee, P.A. & Mills, B. (1993) Psychosexual behavior in hypopituitary men: a controlled comparison of gonadotropin and testosterone replacement. *Psychoneuroendocrinology*, **18**, 149–161.
- 41 Schultheiss, D., Hiltl, D.M., Meschi, M.R., Machtens, S.A., Truss, M.C., Stief, C.G. & Jonas, U. (2000) Pilot study of the transdermal application of testosterone gel to the penile skin for the treatment of hypogonadotropic men with erectile dysfunction. *World Journal of Urology*, **18**, 431–435.
- 42 Boyanov, M.A., Boneva, Z. & Christov, V.G. (2003) Testosterone supplementation in men with type 2 diabetes, visceral obesity and partial androgen deficiency. *Aging Male*, **6**, 1–7.
- 43 Park, N.C., Yan, B.Q., Chung, J.M. & Lee, K.M. (2003) Oral testosterone undecanoate (Andriol) supplement therapy improves the quality of life for men with testosterone deficiency. *Aging Male*, **6**, 86–93.
- 44 Kalinchenko, S.Y., Kozlov, G.I., Gontcharov, N.P. & Katsiya, G.V. (2003) Oral testosterone undecanoate reverses erectile dysfunction associated with diabetes mellitus in patients failing on sildenafil citrate therapy alone. *Aging Male*, **6**, 94–99.
- 45 Guay, A.T., Jacobson, J., Perez, J.B., Hodge, M.B. & Velasquez, E. (2003) Clomiphene increases free testosterone levels in men with both secondary hypogonadism and erectile dysfunction: who does and does not benefit? *International Journal of Impotence Research*, **15**, 156–165.
- 46 Liu, P.Y., Wishart, S.M. & Handelsman, D.J. (2002) A double-blind, placebo-controlled, randomized clinical trial of recombinant human chorionic gonadotropin on muscle strength and physical function and activity in older men with partial age-related androgen deficiency. *Journal of Clinical Endocrinology and Metabolism*, **87**, 3125–3135.
- 47 Cooper, A.J. (1980) A clinical and endocrine study of mesterolone in secondary impotence. *Journal of Psychosomatic Research*, **24**, 275–279.

- 48 Vogel, W., Klaiber, E.L. & Broverman, D.M. (1985) A comparison of the antidepressant effects of a synthetic androgen (mesterolone) and amitriptyline in depressed men. *Journal of Clinical Psychiatry*, **46**, 6–8.
- 49 Luisi, M. & Franchi, F. (1980) Double-blind group comparative study of testosterone undecanoate and mesterolone in hypogonadal male patients. *Journal of Endocrinological Investigations*, **3**, 305–308.
- 50 Aversa, A., Isidori, A.M., Spera, G., Lenzi, A. & Fabbri, A. (2003) Androgens improve cavernous vasodilation and response to sildenafil in patients with erectile dysfunction. *Clinical Endocrinology*, **58**, 632–638.
- 51 Shabsigh, R., Kaufman, J.M., Steidle, C. & Padma-Nathan, H. (2004) Randomized study of testosterone gel as adjunctive therapy to sildenafil in hypogonadal men with erectile dysfunction who do not respond to sildenafil alone. *Journal of Urology*, **172**, 658–663.
- 52 Streiner, D.L. (2002) The 2 'Es' of research: efficacy and effectiveness trials. *Canadian Journal of Psychiatry*, **47**, 552–556.
- 53 Sutton, A.J. (2000) *Methods for Meta-analysis in Medical Research*. Wiley, Chichester.
- 54 Hedges, L.V. & Olkin, I. (1985) *Statistical Methods for Meta-analysis*. Academic Press, Orlando.
- 55 Cohen, J. (1977) *Statistical Power Analysis for the Behavioral Sciences*. Academic Press, New York.
- 56 Hedges, L.V. & Vevea, J.L. (1998) Fixed and random-effects models in meta-analysis. *Psychological Methods*, **3**, 486–504.
- 57 Higgins, J.P., Thompson, S.G., Deeks, J.J. & Altman, D.G. (2003) Measuring inconsistency in meta-analyses. *British Medical Journal*, **327**, 557–560.
- 58 Tenover, J.S., Matsumoto, A.M., Plymate, S.R. & Bremner, W.J. (1987) The effects of aging in normal men on bioavailable testosterone and luteinizing hormone secretion: response to clomiphene citrate. *Journal of Clinical Endocrinology and Metabolism*, **65**, 1118–1126.
- 59 Gray, A., Berlin, J.A., McKinlay, J.B. & Longcope, C. (1991a) An examination of research design effects on the association of testosterone and male aging: results of a meta-analysis. *Journal of Clinical Epidemiology*, **44**, 671–684.
- 60 Kim, Y.C. (1999) Testosterone supplementation in the aging male. *International Journal of Impotence Research*, **11**, 343–352.
- 61 Martin, C.E. (1981) Factor affecting sexual functioning in 60–79-year-old married males. *Archives of Sexual Behavior*, **10**, 399–420.
- 62 Davidson, J.M., Chen, J.J., Crapo, L., Gray, G.D., Greenleaf, W.J. & Catania, J.A. (1983) Hormonal changes and sexual function in aging men. *Journal of Clinical Endocrinology and Metabolism*, **57**, 71–77.
- 63 Bhasin, S. (1992) Androgen treatment of hypogonadal men. *Journal of Clinical Endocrinology and Metabolism*, **74**, 1221–1225.
- 64 Basaria, S. & Dobs, A.S. (2001) Hypogonadism and androgen replacement therapy in elderly men. *American Journal of Medicine*, **110**, 563–572.
- 65 Davidson, J.M., Stefanick, M.L., Sachs, B.D. & Smith, E.R. (1978) Role of androgen in sexual reflexes of the male rat. *Physiological Behavior*, **21**, 141–146.
- 66 Tsitouras, P.D., Martin, C.E. & Harman, S.M. (1982) Relationship of serum testosterone to sexual activity in healthy elderly men. *Journal of Gerontology*, **37**, 288–293.
- 67 Nankin, H.R. & Calkins, J.H. (1986) Decreased bioavailable testosterone in aging normal and impotent men. *Journal of Clinical Endocrinology and Metabolism*, **63**, 1418–1420.
- 68 Schiavi, R.C., Schreiner-Engel, P., White, D. & Mandeli, J. (1988) Pituitary-gonadal function during sleep in men with hypoactive sexual desire and in normal controls. *Psychosomatic Medicine*, **50**, 304–318.
- 69 Fabbri, A., Jannini, E.A., Ulisse, S., Gnassi, L., Moretti, C., Frajese, G. & Isidori, A. (1988) Low serum bioactive luteinizing hormone in nonorganic male impotence: possible relationship with altered gonadotropin-releasing hormone pulsatility. *Journal of Clinical Endocrinology and Metabolism*, **67**, 867–875.
- 70 Carosa, E., Benvenega, S., Trimarchi, F., Lenzi, A., Pepe, M., Simonelli, C. & Jannini, E.A. (2002) Sexual inactivity results in reversible reduction of LH bioavailability. *International Journal of Impotence Research*, **14**, 93–99.
- 71 Carosa, E., Martini, P., Brandetti, F., Di Stasi, S.M., Lombardo, F., Lenzi, A. & Jannini, E.A. (2004) Type V phosphodiesterase inhibitor treatments for erectile dysfunction increase testosterone levels. *Clinical Endocrinology*, **61**, 382–386.
- 72 Gooren, L.J. (1987) Androgen levels and sex functions in testosterone-treated hypogonadal men. *Archives of Sexual Behavior*, **16**, 463–473.
- 73 Isidori, A.M., Caprio, M., Strollo, F., Moretti, C., Frajese, G., Isidori, A. & Fabbri, A. (1999) Leptin and androgens in male obesity: evidence for leptin contribution to reduced androgen levels. *Journal of Clinical Endocrinology and Metabolism*, **84**, 3673–3680.
- 74 Isidori, A.M., Strollo, F., More, M., Caprio, M., Aversa, A., Moretti, C., Frajese, G., Riondino, G. & Fabbri, A. (2000) Leptin and aging: correlation with endocrine changes in male and female healthy adult populations of different body weights. *Journal of Clinical Endocrinology and Metabolism*, **85**, 1954–1962.
- 75 Morley, J.E., Kaiser, F., Raum, W.J., Perry, H.M. III, Flood, J.E., Jensen, J., Silver, A.J. & Roberts, E. (1997) Potentially predictive and manipulable blood serum correlates of aging in the healthy human male: progressive decreases in bioavailable testosterone, dehydroepiandrosterone sulfate, and the ratio of insulin-like growth factor 1 to growth hormone. *Proceedings of the National Academy of Sciences of the United States of America*, **94**, 7537–7542.
- 76 Tenover, J.L. (1997) Testosterone and the aging male. *Journal of Andrology*, **18**, 103–106.
- 77 Carani, C., Scuteri, A., Marrama, P. & Bancroft, J. (1990) The effects of testosterone administration and visual erotic stimuli on nocturnal penile tumescence in normal men. *Hormones and Behavior*, **24**, 435–441.
- 78 Aversa, A., Basciani, S., Visca, P., Arizzi, M., Gnassi, L., Frajese, G. & Fabbri, A. (2001) Platelet-derived growth factor (PDGF) and PDGF receptors in rat corpus cavernosum: changes in expression after transient in vivo hypoxia. *Journal of Endocrinology*, **170**, 395–402.
- 79 Carani, C., Bancroft, J., Granata, A., Del Rio, G. & Marrama, P. (1992) Testosterone and erectile function, nocturnal penile tumescence and rigidity, and erectile response to visual erotic stimuli in hypogonadal and eugonadal men. *Psychoneuroendocrinology*, **17**, 647–654.
- 80 Bancroft, J. (1995) Are the effects of androgens on male sexuality noradrenergically mediated? Some consideration of the human. *Neuroscience and Biobehavioral Reviews*, **19**, 325–330.
- 81 Hajjar, R.R., Kaiser, F.E. & Morley, J.E. (1997) Outcomes of long-term testosterone replacement in older hypogonadal males: a retrospective analysis. *Journal of Clinical Endocrinology and Metabolism*, **82**, 3793–3796.
- 82 Buena, F., Swerdloff, R.S., Steiner, B.S., Lutchmansingh, P., Peterson, M.A., Pandian, M.R., Galmarini, M. & Bhasin, S. (1993) Sexual function does not change when serum testosterone levels are pharmacologically varied within the normal male range. *Fertility and Sterility*, **59**, 1118–1123.
- 83 Bagatell, C.J., Heiman, J.R., Matsumoto, A.M., Rivier, J.E. & Bremner, W.J. (1994) Metabolic and behavioral effects of high-dose,

- exogenous testosterone in healthy men. *Journal of Clinical Endocrinology and Metabolism*, **79**, 561–567.
- 84 O'Carroll, R., Shapiro, C. & Bancroft, J. (1985) Androgens, behaviour and nocturnal erection in hypogonadal men: the effects of varying the replacement dose. *Clinical Endocrinology*, **23**, 527–538.
- 85 O'Connor, D.B., Archer, J. & Wu, F.C. (2004) Effects of testosterone on mood, aggression, and sexual behavior in young men: a double-blind, placebo-controlled, cross-over study. *Journal of Clinical Endocrinology and Metabolism*, **89**, 2837–2845.
- 86 Arver, S., Dobs, A.S., Meikle, A.W., Allen, R.P., Sanders, S.W. & Mazer, N.A. (1996) Improvement of sexual function in testosterone deficient men treated for 1 year with a permeation enhanced testosterone transdermal system. *Journal of Urology*, **155**, 1604–1608.
- 87 Morales, A., Johnston, B., Heaton, J.P. & Lundie, M. (1997) Testosterone supplementation for hypogonadal impotence: assessment of biochemical measures and therapeutic outcomes. *Journal of Urology*, **157**, 849–854.
- 88 Wang, C., Swedloff, R.S., Iranmanesh, A., Dobs, A., Snyder, P.J., Cunningham, G., Matsumoto, A.M., Weber, T. & Berman, N. (2000) Transdermal testosterone gel improves sexual function, mood, muscle strength, and body composition parameters in hypogonadal men. Testosterone Gel Study Group. *Journal of Clinical Endocrinology and Metabolism*, **85**, 2839–2853.
- 89 Cunningham, G.R., Hirshkowitz, M., Korenman, S.G. & Karacan, I. (1990) Testosterone replacement therapy and sleep-related erections in hypogonadal men. *Journal of Clinical Endocrinology and Metabolism*, **70**, 792–797.
- 90 Davidson, J.M., Kwan, M. & Greenleaf, W.J. (1982) Hormonal replacement and sexuality in men. *Clinical Endocrinology and Metabolism*, **11**, 599–623.
- 91 Aversa, A., Isidori, A.M., De Martino, M.U., Caprio, M., Fabbrini, E., Rocchietti-March, M., Frajese, G. & Fabbri, A. (2000) Androgens and penile erection: evidence for a direct relationship between free testosterone and cavernous vasodilation in men with erectile dysfunction. *Clinical Endocrinology*, **53**, 517–522.
- 92 National Institute on Aging Advisory Panel (2001) Report of the National Institute on Aging Advisory Panel on Testosterone Replacement in Men. *Journal of Clinical Endocrinology and Metabolism*, **86**, 4611–4614.