Treatment of female sexual dysfunction

A personalized medicine approach

Kim van Rooij

ISBN 978-94-6108-912-0

Printed in the Netherlands by Gildeprint, Enschede

Cover: drawing made by Kim van Rooij, 1998

Treatment of female sexual dysfunction

A personalized medicine approach

Behandeling van seksuele dysfuncties bij vrouwen: een gepersonaliseerde medische aanpak

(met een samenvatting in het Nederlands)

Proefschrift

ter verkrijging van de graad van doctor aan de Universiteit Utrecht op gezag van de rector magnificus, prof. dr. G.J. van der Zwaan, ingevolge het besluit van het college voor promoties in het openbaar te verdedigen op woensdag 18 februari 2015 des middags te 4.15 uur

door

Kim van Rooij

geboren op 25 januari 1980, te Amsterdam

Promotor:	Prof. dr. B. Olivier
Copromotoren:	Dr. J.J.A. Tuiten
	Dr. H.P.F Koppeschaar

De onderzoeken in dit proefschrift zijn gefinancierd door Emotional Brain B.V.

Dit proefschrift is mede gefinancierd door Emotional Brain B.V.

CONTENTS

Chapter 1

General introduction

GENERAL INTRODUCTION

This thesis describes the development of two different pharmacological treatments for women with low sexual desire and arousal, and who experience personal distress as the result of these problems. To date, no approved treatment is available for female sexual dysfunction.

The basis for this drug development trajectory is a personalized medicine approach; different treatment options related to different underlying causal mechanisms. These mechanisms are based on the assumption that sexual responding is the result of an interplay between activating excitatory and inhibitory processes involved in sexual functioning. Based on these underlying mechanisms we classified two different subtypes of women with low sexual desire: women who are low sensitive to sexual stimuli, and women in which sexual stimuli elicit a dysfunctional activation of sexual inhibitory mechanisms. For these subtypes two different drugs were developed: one with a combination of sublingual testosterone and a phosphodiesterase type 5 (PDE5)-inhibitor for women with low sensitivity for sexual stimuli, and another combination of sublingual testosterone and a serotonin-1A (5-HT_{1A}) receptor agonist for women with dysfunctional activation of sexual inhibitory mechanisms.

In this introduction the rationale behind this research will be described, including the subclassification used within our patient population. This will be followed by a description of the personalized medicine approach that was used in the drug development program.

Clinical background of the Research

Complaints regarding sexual functioning can negatively interfere with psychological and social wellbeing [1]. The most common sexual complaint in women is low sexual desire, which was formerly classified as Hypoactive Sexual Desire Disorder (HSDD) in the Diagnostic and Statistical Manual of Mental Disorders, 4th edition text revision [2]. In the same edition, complaints regarding sexual arousal were classified as Female Sexual Arousal Disorder (FSAD). In view of the fact that these diagnoses are difficult to separate, since sexual desire and

sexual arousal overlap [3], a combination of these two diagnoses is recently classified as Female Sexual Interest/Arousal Disorder (FSIAD) in the 5th edition of this manual [4]. Furthermore, an important criterion for diagnosing FSIAD is that sexual complaints of desire and arousal are accompanied by sexual dissatisfaction and personal distress.

The exact prevalence of women with HSDD/FSIAD[1] is difficult to establish from literature, because it varies between the populations that are surveyed (e.g., depending on differences in age, degree of distress, duration of sexual complaints, et cetera). Notwithstanding, high prevalence rates of sexual dysfunction in women are reported ranging from approximately 10% to 43% [5-9]. To date, there is no pharmacological treatment approved by the U.S. Food and Drug Administration (U.S. FDA) or European Medicines Agency (EMA).

The hypothesis that different underlying mechanisms are responsible for the sexual complaints in women with FSIAD, is partly based on an adapted version of the dual control model of Bancroft and Janssen. In this model they argue that individual differences in sexual responding are influenced by activation of excitatory and inhibitory mechanisms and processes [10,11]. In our view, the mechanisms regulating sexual excitation and sexual inhibition are influenced by the degree of sensitivity of the brain for sexual stimuli. In women with a relatively insensitive system for sexual cues, exposure to (internal or external) sexual stimuli might not lead to an appropriate or adequate activation of excitatory mechanisms involved in sexual motivation and sexual desire. The other mechanism (sexual inhibition) might become active in women with a normal or high sensitivity for sexual cues, but who have acquired a negative association with sexual events. For these women we postulate that the sexual complaints are related to the activity of the prefrontal cortex (PFC), which is involved in the inhibitory control of motivational behavior [12], including sexual behavior [13,14].

[1] In our future trials subjects diagnosed with FSIAD will also be required to have the symptoms "absent/reduced interest in sexual activity" and "absent/reduced sexual erotic thoughts or fantasies". These 2 indicators, which are included in DSM-5 Criterion A, match the main indicators of the former DSM-IV-TR diagnosis of HSDD. Thus, all women who meet these criteria are automatically diagnosed as having HSDD (but not vice versa). In order to avoid ambiguity, the population HSDD will be replaced by FSIAD throughout this thesis.

We hypothesized that in these women negatively valenced sexual events induce a phasic increase of serotonin activity in the PFC, which in turn lowers activity in the limbic areas responsible for sexual motivation, and thereby causing low sexual desire and arousal [15]. Based on these underlying mechanism we classified two different subtypes of women with FSIAD: women with low sensitivity to sexual stimuli and women with dysfunctional activation of sexual inhibitory mechanisms.

For these subtypes of women with FSIAD two different drugs were developed: a combination of sublingual testosterone and a PDE5-inhibitor for women with low sensitivity for sexual cues, and a combination of sublingual testosterone and a 5-HT_{1A} receptor agonist for women with dysfunctional activation of sexual inhibitory mechanisms.

Personalized Medicine Approach

A range of biological and psychological regulatory mechanisms control human social and sexual functions. However, these mechanisms vary between and within subjects. As stated before, there is still no approved pharmacological treatment for women with FSIAD. Up to date, pharmacological research has focused on a 'one size fits all' treatment assuming to be effective for all women diagnosed with FSIAD.

In the following paragraphs I will discuss the designing and development of two different drugs based on the above discussed mechanisms underlying the different subtypes of women diagnosed with FSIAD. Firstly, I want to mention the mechanism of action and the pharmacokinetic effects of sublingual administration of testosterone. Secondly, the effect of the combination of sublingual testosterone and a PDE5-inhibitor/5-HT_{1A} receptor agonist on sexual functioning will be described. Thirdly, a possible new indication of these treatments for women who use selective serotonin reuptake inhibitors (SSRIs) and experience sexual complaints will be discussed. At last, I will show the comparison of the pharmacokinetics of our first clinical prototype of a combination of sublingual testosterone and the 5-HT_{1A} receptor agonist buspirone versus an innovative fixed-combination tablet.

Sublingual testosterone

Testosterone is involved in social behavior [16,17] including sexual behavior [18,19]. The majority of studies regarding sexual behavior in women is done with chronic testosterone administration, including hormonal replacement therapy in naturally or surgically menopausal women [20-22]. Free testosterone (not bound to sex hormone binding globulin (SHBG)) is the biologically active testosterone [23]. The disadvantage of chronic administration is that it takes days to have an increase in free testosterone levels and most importantly, the sustained testosterone levels could have negative adverse events like increased hair growth or acne. The advantage of acute administration of testosterone by the sublingual route is the immediate uptake, a steep increase of free testosterone and fast return to baseline levels which is not possible by intramuscular or transdermal routes. Furthermore, acute administration with sublingual testosterone does not cause sustained elevated testosterone levels and therefore it is unlikely that this type of testosterone administration will result in androgenic adverse events.

The efficacy of both drugs is based on a delay in effect of about 4 hours of sublingual testosterone on behavior. In 2000, Tuiten et al. demonstrated that one single dose of 0.5 mg testosterone significantly increased vaginal vasocongestion and increased experiences of sexual lust and genital sensation in premenopausal women without sexual complaints [24]. These effects occurred approximately 4 hours after the observed testosterone peak and 2 hours after testosterone had returned to baseline plasma levels. This 4 hour delay in behavioral effect after sublingual testosterone administration has been frequently replicated in several other studies regarding social behavior and cognitive functions [25-37]. As an partly explanation for this 4 hour delay in effect after sublingual testosterone administration, van der Made et al. suggested an SHBG saturation threshold mechanism: an increased influx of testosterone into the body will occupy the testosterone binding sites of SHBG. When the binding sites are saturated, free testosterone (the biologically active testosterone [23]) and consequently the free fraction will increase, thereby inducing behavioral effects after approximately 4 hours [38]. The underlying mechanism for this delay in effect is not fully elucidated but androgenic

metabolites, genomic mechanisms [16] or a combination of these factors could be responsible. The effect of testosterone dosing on SHBG saturation and the effects on plasma total and free testosterone concentrations have been further investigated in a pharmacokinetic study in which premenopausal sexual functioning women received three different dosages of sublingual testosterone (0.25, 0.50 and 0.75 mg). The results indicate the existence of the postulated SHBG saturation threshold for testosterone that determines the level of the free fraction of testosterone, which is described in chapter 2.

The combination of sublingual testosterone and a PDE5-inhibitor

As described earlier, Tuiten et al. demonstrated that 0.5 mg of sublingual testosterone increased vaginal vasocongestion about 4 hours after the testosterone pulse in women without sexual complaints [24]. Interestingly, in a study in women diagnosed with FSIAD in which we administered 0.5 mg testosterone sublingually no such delay in effect of sublingual testosterone on vaginal vasocongestion was observed [39]. However, an increase in vaginal vasocongestion was demonstrated when administration of sublingual testosterone was combined with vardenafil (a PDE5-inhibitor), in such a way that the plasma levels of vardenafil were increased during the behavioral window of the sublingual testosterone [39]. In a subsequent study Van der Made et al. investigated the efficacy of sublingual testosterone alone, the PDE5-inhibtor vardenafil alone and the combination of these drugs on preconscious attentional bias, genital and subjective indices of sexual functioning [38]. The results of this study demonstrated an effect on subjective indices of sexual functioning (i.e., vaginal sensation, sexual desire), after the administration of this combination of sublingual testosterone and a PDE5-inhibitor in women with FSIAD [38]. This experiment was based on the assumption that sublingual testosterone produces (consciously or unconsciously) activation of central sexual motivational systems in sexually functional women [24]. An increase in sexual motivation is necessary for the PDE5-inhibitor to be effective in increasing the amount of blood in the erectile tissues. For women with low desire, our hypothesis was that only the combination of sublingual testosterone and a PDE5-inhibitor would increase physiological sexual responding, and not either drug alone. Our hypothesis was confirmed in this study.

High and low sensitivity for sexual Cues

In the above mentioned study [39], it was found that women who reported the experience of childhood sexual abuse (CSA) showed a decrease in their originally high levels of preconscious attentional bias to sexual cues after sublingual testosterone administration, while the women without CSA showed an increase in this attentional bias. This difference was correlated with the effect of the combination of sublingual testosterone and a PDE5-inhibitor on physiological responding; women who had an originally low attention to sexual cues (and did not report a history of CSA) revealed an increase in their physiological responding to the combination of sublingual testosterone and the PDE5-inhibitor while women with a history of CSA did not show an increase (or even a decrease) in their response after administration of these drugs.

In the subsequent study, women with a history of CSA were excluded [38]. Again, preconscious attentional bias as well as physiological and subjective indices of sexual function were assessed during neutral and erotic visual stimulation. The women were stratified according to their baseline preconscious attentional bias for sexual cues. We re-conceptualized the women with a low and high preconscious attentional bias for sexual cues into women with a low and women with a high sensitive brain for sexual cues. The results of the sublingual testosterone administration on attentional bias reproduced our earlier findings; the low sensitive group showed an increase in attentional bias, while the high sensitive group showed the same level or a decrease. Furthermore, only in the low sensitive group the combination of sublingual testosterone and the PDE5-inhibitor produced an increase in physiological and subjective sexual responding, but not in the high sensitive group [38]. Although CSA was excluded, the high sensitive group reported more negative sexual experiences, which supported our hypothesis that a dysfunctional activity of sexual inhibitory systems resulted in low sexual desire in this subtype of women with FSIAD. These two studies form the basis for our subdivision of women with FSIAD in at least two subtypes: women who are low sensitive to sexual stimuli and women who have dysfunctional activation of sexual inhibitory mechanisms. See also chapter 3 for an extensive review of our hypotheses.

The differentiation of the two subtypes in women with FSIAD was further investigated in a randomized, double-blind, placebo-controlled cross-over study in a larger population of women with FSIAD. As expected, the combination of sublingual testosterone (0.5 mg) and the PDE5-inhibitor sildenafil (50 mg orally) increased sexual satisfaction and other subjective and physiological measures of sexual responding in women with a low sensitivity to sexual stimuli (based on the emotional Stroop task). The results of this study are described in chapter 4.

The next paragraph describes the results of an experiment in women who were high sensitive to sexual stimuli (and - as we assume – have a dysfunctional activation of sexual inhibitory systems).

The combination of sublingual testosterone and a 5-HT_{1A} receptor agonist

Based on the earlier studies in which the high sensitive women (or women with a dysfunctional activation of sexual inhibitory mechanisms) reported no effect of the combination of sublingual testosterone and the PDE5-inhibitor, it was hypothesized that these women could benefit from a combination of testosterone and a 5-HT_{1A} receptor agonist. Sublingual testosterone increases the sensitivity of the brain in these high sensitive women (as it does in the low sensitive women), and because these women are more prone to sexual inhibition (for example, when negative sexual experiences occurred in the past), we hypothesized that would exhibit strong inhibitory activity in the prefrontal cortex (PFC) under sublingual testosterone exposure. Motivation and emotions, and the execution of behavior [12], including sexual behavior [13,14], is partly under the control of parts of the PFC. An important function of the prefrontal cortex is inhibition of inappropriate and undesirable behavior provoked by the motivational system. 5-HT is an important neurotransmitter involved in inhibitory control mechanisms in the brain [40], and exerts its inhibitory effects at least partly via the PFC [41]. A 5-HT_{1A} receptor agonist, like buspirone, binds to somatodendritic autoreceptors of the raphe nuclei in the midbrain. This binding activates 5-HT_{1A} auto receptors, which inhibits serotonin release from the presynaptic terminal [42], and subsequently decreases firing activity in the PFC [43]. Thus, the inhibition of serotonin release from the presynaptic terminal reduces extracellular serotonin levels and serotonin firing activity in the PFC

[44]. This effect is only accomplished if a 5-HT_{1A} receptor agonist, such as buspirone, is given as an acute treatment, whereas chronic administration of buspirone has the opposite effect [44]. Accordingly, acute treatment with a 5-HT_{1A} receptor agonist is hypothesized to decrease the sexual stimuli induced phasic increase of serotonin in the PFC and could thereby result in a reduced inhibitory response to sexual cues in women with FSIAD who are prone to sexual inhibition. Thus, we expected that these women would benefit sexually when sublingual testosterone and buspirone were taken in combination. This hypothesis was tested during a randomized, double-blind, placebo-controlled cross-over study in which women took 'on-demand' placebo, sublingual testosterone combined with a PDE5-inhibitor (sildenafil, 50 mg orally), and sublingual testosterone combined with a 5-HT_{1A} receptor agonist (buspirone, 10 mg orally) for one month each, during three consecutive months. Under the condition of sublingual testosterone combined with sildenafil, some women reported a decrease in sexual functioning. Based on their inhibitory response on sexual satisfaction and other indices of sexual functioning during the treatment with sublingual testosterone combined with sildenafil, we were able to distinguish between women with low inhibition and women with high inhibition. Subsequently, we demonstrated that the women with high inhibition (i.e. with decreased sexual responses) during treatment with sublingual testosterone combined with sildenafil, significantly improved their sexual functioning when treated with sublingual testosterone combined with buspirone. See chapters 5 and 6 for the results of this study.

Our subdivision within the FSIAD population may also be of interest to treat other indications where women experience sexual difficulties. One of our hypotheses is that women who develop sexual complaints after the start of antidepressants, such as selective serotonin receptor inhibitors (SSRIs), could also be subtyped into these classifications and might benefit from sublingual testosterone combined with a PDE5-inhibitor or a 5-HT_{1A} receptor agonist. These potential treatments for this indication will be described in the next section.

SSRI-induced Sexual Dysfunction

According to the DSM-IV-TR and DSM-5, the diagnosis FSIAD cannot be held if the etiology of the sexual complaints is attributed to a medical condition or a substance. As described earlier, we hypothesize that in the FSIAD population different underlying mechanisms are of influence in the development of the sexual complaints. As said above, there is another population of women in which the sexual complaints are attributed to the use of antidepressants. Out of all types of antidepressants the SSRIs are the most notorious for causing sexual dysfunction with an estimated prevalence rate of 20-70% in both men and women [45,46]. Sexual side effects attributed to the use of antidepressants are frequently underreported [47]. These side effects can produce psychological distress and impaired quality of life.

Many women experience sexual difficulties after the start of SSRI therapy. These women do not meet the criteria of FSIAD but are diagnosed with SSRI-induced sexual dysfunction (SD) which falls under the diagnosis of substance induced sexual dysfunction. In the DSM-IV-TR (as well as in the DSM-5), the diagnosis of substance induced sexual dysfunction is described as a clinically significant sexual dysfunction that results in marked distress. Furthermore, the sexual dysfunction must be fully explained by use of the substance (symptoms developed during or soon after substance intoxication, or etiologically related to the disturbance). Most importantly, the disturbance is not better accounted for by another diagnosis of sexual dysfunction [2,4]. To date, there is no approved pharmacological treatment for SSRI-induced SD.

As stated in the section regarding the combination of sublingual testosterone and a 5-HT_{1A} receptor agonist in women with high inhibition, serotonin is involved in inhibitory effects on sexual behavior. In explaining sexual functioning, 5-HT_{1A} and 5-HT_2 receptors are particularly involved [48,49]. Synaptic serotonin levels are increased by SSRIs and are thought to inhibit the sexual excitatory effects of dopamine and norepinephrine in the mesolimbic reward system via tonic increased serotonergic activity in the PFC. High serotonin activity increases the threshold for the processing of negative and positive emotional information by the brain. Buspirone, a 5-HT_{1A} agonist, lowers serotonergic neuron firing

activity for a short time after a single administration via activation of somatodendritic 5-HT_{1A} autoreceptors [42-44]. The inhibition of serotonin release from the presynaptic terminal reduces extracellular serotonin levels in the PFC [44]. This decrease of serotonin in the PFC might lead to a decreased inhibitory control, and as a result in an increased activity in the mesolimbic system involved in sexual motivation. It must be noted that this effect is only accomplished if a 5-HT_{1A} receptor agonist is given as an acute treatment; chronic treatment has the opposite effect [44]. Because of the pharmacodynamic properties of buspirone and other 5-HT_{1A} receptor agonists, we hypothesized that the combination of sublingual testosterone and buspirone could be part of an 'on-demand' treatment for women with SSRI-induced SD. However, the subdivision we used in the FSIAD population could also be of influence in women with SSRI-induced SD. For example, a woman can have a low sensitive brain system for sexual stimuli or an dysfunctional activation of inhibitory mechanisms in response to sexual stimuli before the start of the antidepressant therapy. Her sensitivity for sexual cues before the start of an antidepressant can influence her response on our two treatments (sublingual testosterone in combination with a PDE5 inhibitor and sublingual testosterone in combination with a 5-HT_{1A} receptor agonist). Therefore, we investigated in a preliminary explorative study the effects of the combination of sublingual testosterone and a PDE5-inhibitor (sildenafil, 50 mg), as well as the combination of sublingual testosterone and a 5-HT_{1A} receptor agonist (buspirone, 10 mg) in women diagnosed with SSRI induced sexual dysfunction. In this study, which is discussed in chapter 6, the effect of both treatments was investigated in a placebo-controlled, randomized, crossover, exploratory study in which 21 women received both study medications. In order to assess sensitivity for testosterone, and investigate a possible subdivision within this population, a genetic polymorphism was measured which is known to influence the function of the androgen receptor. This genetic polymorphism is located on the androgen receptor (AR) gene. The behavioral effects of sublingual testosterone are mediated, at least in part, by binding to the AR. The polymorphic polyglutamine stretch in the aminoterminal domain of the AR, encoded by the nucleotides cysteine, adenine, and guanine (CAG), is known to influence the function of the receptor as a transcription factor, so that relatively long CAG repeat lengths are associated with a decreased level of androgen receptor function [50-52]. We hypothesized that

women with relatively long CAG repeat lengths are less sensitive to sexual cues because their AR has a lower level of receptor functioning compared to women with relatively short CAG repeat lengths. Therefore, it was hypothesized that the women with relatively long CAG repeat lengths would benefit more from sublingual testosterone in increasing their brain's sensitivity compared to women with relatively shorter CAG repeats. The results of this preliminary study show that in these women treatment with sublingual testosterone and a PDE5-inhibitor or with a 5-HT_{1A} receptor agonist might be a promising treatment. See chapter 6 for the results of this study.

The pharmacokinetic profile of the combination of sublingual testosterone and the 5-HT_{1A} receptor agonist buspirone

In the studies presented in the chapters 4, 5, and 6 of this thesis, the two components of the investigated drugs (the combination of sublingual testosterone with sildenafil or the combination of sublingual testosterone with buspirone) were administered separately in such a timeframe that the peak plasma concentration of sildenafil or buspirone coincides with the behavioral window of testosterone (approximately 3-6 hours after administration). In our earlier studies, the women were instructed to take a testosterone solution under the tongue for 1 minute, and take a capsule of sildenafil or buspirone 2.5 hours later to create overlapping peaks in effect of testosterone and sildenafil or buspirone. This method of administration is not suitable for daily practice. Therefore, a single oral combination tablet was developed which delivers testosterone sublingually, and releases sildenafil or buspirone approximately 2.5 hours later in the gastro-intestinal tract. With this combination tablet, women with FSIAD can take just one single tablet 3-6 hours before anticipated sexual activity and is therefore more suitable for clinical practice. The pharmacokinetic properties of the new combination tablet (consisting of a combination of sublingual testosterone and buspirone) and the previous administration method are described in chapter 7. The comparison between the pharmacokinetics of these administration methods showed that the new combination tablet fulfills its design criteria and was therefore used in our recent clinical trials.

GENERAL OUTLINE

In this thesis pharmacokinetic and clinical studies are presented on the treatment of sublingual testosterone in combination with either sildenafil or buspirone in women diagnosed with FSIAD.

In chapter 2 an investigator-blind, randomized, cross-over placebo-controlled study is presented in which the pharmacokinetics of three different doses (0.25, 0.50 and 0.75mg) of sublingual testosterone are characterized in premenopausal women without sexual complaints. Furthermore, the SHBG saturation threshold that determines the free levels and free fraction of testosterone was investigated. The results show that the three doses are rapidly absorbed and quickly metabolized. Area under the curves (AUCs) and the maximum concentration (Cmax) differed significantly between the three doses and increased dose-dependently. Furthermore, these data suggests that a SHBG threshold exists which influences the increase in free fraction levels of testosterone.

Chapter 3 addresses the theoretical basis for the hypothesis that different causal mechanisms are responsible for the sexual complaints in FSIAD: low sexual desire could be due to an insensitive brain system for sexual cues or to dysfunctional activity of sexual inhibitory mechanisms. It describes, inter alia, the role of androgens and serotonin in sexual functioning. Furthermore, this chapter provides a rationale for the use of an on-demand combined administration of sublingual testosterone and a PDE5 inhibitor for women with low sensitivity for sexual cues, and for the use of on-demand combined administration of sublingual testosterone and a 5-HT_{1A} receptor agonist in women with dysfunctional activity of sexual inhibitory mechanisms.

Chapter 4 presents the results of a randomized, double blind, placebo-controlled cross-over study regarding the efficacy and safety of on-demand administration of the combination of sublingual testosterone and a PDE5-inhibitor (sildenafil) in women with FSIAD and low sensitivity for sexual cues is described. This study investigates the effects of this treatment on sexual functioning measured during ambulatory psychophysiological lab conditions as well as during sexual events at

home. The data shows that, compared to placebo, sublingual testosterone in combination with a PDE5-inhibitor improves physiological and subjective measured of sexual functioning in women with low sensitivity for sexual cues and is a safe potentially promising treatment for this indication.

Chapter 5 presents the results of a randomized, double blind, placebo-controlled cross-over study regarding the efficacy and safety of on-demand administration of the combination of sublingual testosterone and a 5-HT_{1A} receptor agonist in women with FSIAD and prone to sexual inhibition. This study investigates the effects of this treatment on sexual functioning measured during ambulatory psychophysiological lab conditions as well as during sexual events at home. The data shows that, compared to placebo, sublingual testosterone in combination with a 5-HT_{1A} receptor agonist improves physiological and subjective measured of sexual functioning in women with high inhibition and is a safe potentially promising treatment for this indication.

Chapter 6 reports a preliminary, placebo-controlled, randomized, crossover, exploratory study in which 21 women with SSRI induced sexual dysfunction received the combination of sublingual testosterone and a PDE5-inhibitor and the combination of sublingual testosterone and a 5-HT_{1A} receptor agonist. Besides subjective measures of sexual functioning, the role of the CAG repeat polymorphism of the AR was investigated. The results show that there was a significant interaction effect between SSRI dose, CAG repeat length and treatment response on sexual satisfaction. Women with relatively long CAG repeat lengths and who use a low dose of SSRI reported an increase in sexual satisfaction after both drugs. These findings should be further explored in future studies.

Chapter 7 describes the comparison of the pharmacokinetics of our first clinical prototype of the combination of sublingual testosterone and buspirone versus an innovative fixed-combination tablet in premenopausal women without sexual complaints. The clinical prototype consisted of sublingual testosterone cyclodextrine solution in combination with buspirone orally that had to be administered separately (the testosterone solution was followed by a capsule of buspirone 2.5 hours later) in such a manner that there were two overlapping

peaks in effect of both components. The objective of this study was to investigate whether the fixed-combination tablet, which is more suitable for daily practice, mimics the pharmacokinetics of the prototype. The results showed an immediate testosterone absorption from both formulations. It is also demonstrated that there is adequate absorption of buspirone after administration of the combination tablet. This newly developed combination tablet fulfills its design criteria and will be used for further clinical studies in FSIAD.

Chapter 8 contains a general discussion and presents future perspectives.

REFERENCES

1. Davison SL, Bell RJ, LaChina M, Holden SL, Davis SR. The relationship between self-reported sexual satisfaction and general well-being in women. J Sex Med 2009 Oct;6(10):2690-2697.

2. American Psychiatric Association. Diagnostic and statistical manual of mental disorders. 4th ed. Washington, DC; 2000.

3. Brotto LA. The DSM diagnostic criteria for hypoactive sexual desire disorder in women. Arch Sex Behav 2010 Apr;39(2):221-239.

4. American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders. 5th ed. Arlington, VA: American Psychiatric Publishing; 2013.

5. Mitchell KR, Mercer CH, Wellings K, Johnson AM. Prevalence of low sexual desire among women in Britain: associated factors. J Sex Med 2009 Sep;6(9):2434-2444.

6. Segraves R, Woodard T. Female hypoactive sexual desire disorder: History and current status. J Sex Med 2006 May;3(3):408-418.

7. Shifren JL, Monz BU, Russo PA, Segreti A, Johannes CB. Sexual problems and distress in United States women: prevalence and correlates. Obstet Gynecol 2008 Nov;112(5):970-978.

8. Leiblum SR, Koochaki PE, Rodenberg CA, Barton IP, Rosen RC. Hypoactive sexual desire disorder in postmenopausal women: US results from the Women's International Study of Health and Sexuality (WISHeS). Menopause (New York, N Y 2006 Jan-Feb;13(1):46-56.

9. Laumann EO, Paik A, Rosen RC. Sexual dysfunction in the United States: prevalence and predictors. JAMA 1999 Feb 10;281(6):537-44.

10. Janssen E, Bancroft J. The dual control model: the role of sexual inhibition and excitation in sexual arousal and behaviour. In: Janssen E, editor. The psychophysiology of sex Bloomington: Indiana University Press; 2007. p. 197.

11. Bancroft J, Graham CA, Janssen E, Sanders SA. The dual control model: current status and future directions. J Sex Res 2009 Mar-Jun;46(2-3):121-142.

12. Chambers CD, Garavan H, Bellgrove MA. Insights into the neural basis of response inhibition from cognitive and clinical neuroscience. Neurosci Biobehav Rev 2009 May;33(5):631-646.

13. Beauregard M, Levesque J, Bourgouin P. Neural correlates of conscious self-regulation of emotion. J Neurosci 2001 Sep 15;21(18):RC165.

14. Stoleru S, Redoute J, Costes N, Lavenne F, Bars DL, Dechaud H, et al. Brain processing of visual sexual stimuli in men with hypoactive sexual desire disorder. Psychiatry Res 2003 Oct 30;124(2):67-86.

15. Bloemers J, van Rooij K, Poels S, Goldstein I, Everaerd W, Koppeschaar H, et al. Toward personalized sexual medicine (part 1): integrating the "dual control model" into differential drug treatments for Hypoactive Sexual Desire Disorder and female sexual arousal disorder. J Sex Med 2013 Mar;10(3):791-809.

16. Bos PA, Panksepp J, Bluthe RM, Honk JV. Acute effects of steroid hormones and neuropeptides on human social-emotional behavior: A review of single administration studies. Front Neuroendocrinol 2011 Jan 21.

17. Eisenegger C, Haushofer J, Fehr E. The role of testosterone in social interaction. Trends Cogn Sci 2011 Jun;15(6):263-271.

18. Auger AP. Steroid receptor control of reproductive behavior. Hormones and behavior 2004 Mar;45(3):168-72.

19. Hull EM, Dominguez JM. Sexual behavior in male rodents. Hormones and behavior 2007 Jun;52(1):45-55.

20. Shifren JL, Braunstein GD, Simon JA, Casson PR, Buster JE, Redmond GP, et al. Transdermal testosterone treatment in women with impaired sexual function after oophorectomy. N Engl J Med 2000 Sep;343(10):682-688.

21. Sherwin BB. Randomized clinical trials of combined estrogen-androgen preparations: effects on sexual functioning. Fertil Steril 2002 Apr;77(4):S49-S54.

22. Simon J, Braunstein G, Nachtigall L, Utian W, Katz M, Miller S, et al. Testosterone patch increases sexual activity and desire in surgically menopausal women with hypoactive sexual desire disorder. J Clin Endocrinol Metab 2005 Sep;90(9):5226-5233.

23. Mendel CM. The free hormone hypothesis: a physiologically based mathematical model. Endocr Rev 1989 Aug;10(3):232-274.

24. Tuiten A, Van Honk J, Koppeschaar H, Bernaards C, Thijssen J, Verbaten R. Time course of effects of testosterone administration on sexual arousal in women. Arch Gen Psychiatry 2000 Feb;57(2):149-53; discussion 155-6.

25. Postma A, Meyer G, Tuiten A, van Honk J, Kessels RP, Thijssen J. Effects of testosterone administration on selective aspects of object-location memory in healthy young women. Psychoneuroendocrinology 2000 Aug;25(6):563-75.

26. Aleman A, Bronk E, Kessels RP, Koppeschaar HP, van Honk J. A single administration of testosterone improves visuospatial ability in young women. Psychoneuroendocrinology 2004 Jun;29(5):612-7.

27. Schutter DJ, van Honk J. Decoupling of midfrontal delta-beta oscillations after testosterone administration. Int J Psychophysiol 2004 Jun;53(1):71-3.

28. van Honk J, Schutter DJ, Hermans EJ, Putman P, Tuiten A, Koppeschaar H. Testosterone shifts the balance between sensitivity for punishment and reward in healthy young women. Psychoneuroendocrinology 2004 Aug;29(7):937-43.

29. van Honk J, Peper JS, Schutter DJ. Testosterone reduces unconscious fear but not consciously experienced anxiety: implications for the disorders of fear and anxiety. Biological psychiatry 2005 Aug 1;58(3):218-25.

30. van Honk J, Schutter DJ. Testosterone reduces conscious detection of signals serving social correction: implications for antisocial behavior. Psychol Sci 2007 Aug;18(8):663-7.

31. van Honk J, Tuiten A, Hermans E, Putman P, Koppeschaar H, Thijssen J, et al. A single administration of testosterone induces cardiac accelerative responses to angry faces in healthy young women. Behavioral neuroscience 2001 Feb;115(1):238-42.

32. Hermans EJ, Putman P, van Honk J. Testosterone administration reduces empathetic behavior: a facial mimicry study. Psychoneuroendocrinology 2006 Aug;31(7):859-66.

33. Hermans EJ, Putman P, Baas JM, Gecks NM, Kenemans JL, van Honk J. Exogenous testosterone attenuates the integrated central stress response in healthy young women. Psychoneuroendocrinology 2007 Sep-Nov;32(8-10):1052-61.

34. Hermans EJ, Ramsey NF, van Honk J. Exogenous testosterone enhances responsiveness to social threat in the neural circuitry of social aggression in humans. Biological psychiatry 2008 Feb 1;63(3):263-70.

35. Bos PA, Terburg D, van Honk J. Testosterone decreases trust in socially naive humans. Proceedings of the National Academy of Sciences of the United States of America 2010 Jun 1;107(22):9991-5.

36. Bos PA, van Honk J, Ramsey NF, Stein DJ, Hermans EJ. Testosterone administration in women increases amygdala responses to fearful and happy faces. Psychoneuroendocrinology 2013 Jun;38(6):808-817.

37. Eisenegger C, Naef M, Snozzi R, Heinrichs M, Fehr E. Prejudice and truth about the effect of testosterone on human bargaining behaviour. Nature 2010 Jan 21;463(7279):356-9.

38. van der Made F, Bloemers J, Yassem WE, Kleiverda G, Everaerd W, van Ham D, et al. The influence of testosterone combined with a PDE5-inhibitor on cognitive, affective, and physiological sexual functioning in women suffering from sexual dysfunction. The journal of sexual medicine 2009 Mar;6(3):777-90.

39. Van der Made F, Bloemers J, Van Ham D, El Yassem W, Kleiverda G, Everaerd W, et al. Childhood sexual abuse, selective attention for sexual cues and the effects of testosterone with or without vardenafil on physiological sexual arousal in women with sexual dysfunction: A pilot study. J Sex Med 2009;6:429-439; 429.

40. Eagle DM, Bari A, Robbins TW. The neuropsychopharmacology of action inhibition: cross-species translation of the stop-signal and go/no-go tasks. Psychopharmacology 2008 Aug;199(3):439-56.

41. Evers EA, Cools R, Clark L, van der Veen FM, Jolles J, Sahakian BJ, et al. Serotonergic modulation of prefrontal cortex during negative feedback in probabilistic reversal learning. Neuropsychopharmacology 2005 Jun;30(6):1138-1147.

42. Sprouse JS, Aghajanian GK. Electrophysiological responses of serotoninergic dorsal raphe neurons to 5-HT1A and 5-HT1B agonists. Synapse 1987;1(1):3-9.

43. Hamon M, Fattaccini CM, Adrien J, Gallissot MC, Martin P, Gozlan H. Alterations of central serotonin and dopamine turnover in rats treated with ipsapirone and other 5-hydroxytryptamine1A agonists with potential anxiolytic properties. J Pharmacol Exp Ther 1988 Aug;246(2):745-752.

44. Liu YP, Wilkinson LS, Robbins TW. Effects of acute and chronic buspirone on impulsive choice and efflux of 5-HT and dopamine in hippocampus, nucleus accumbens and prefrontal cortex. Psychopharmacology (Berl) 2004 Apr;173(1-2):175-185.

45. Serretti A, Chiesa A. Treatment-emergent sexual dysfunction related to antidepressants: a meta-analysis. Journal of clinical psychopharmacology 2009 Jun;29(3):259-66.

46. Montejo AL, Llorca G, Izquierdo JA, Rico-Villademoros F. Incidence of sexual dysfunction associated with antidepressant agents: a prospective multicenter study of 1022 outpatients. Spanish Working Group for the Study of Psychotropic-Related Sexual Dysfunction. The Journal of clinical psychiatry 2001;62 Suppl 3:10-21.

47. Bonierbale M, Lancon C, Tignol J. The ELIXIR study: evaluation of sexual dysfunction in 4557 depressed patients in France. Current medical research and opinion 2003;19(2):114-24.

48. Clayton AH, Hamilton DV. Female sexual dysfunction. Obstetrics and gynecology clinics of North America 2009 Dec;36(4):861-76, xi.

49. Berger M, Gray JA, Roth BL. The expanded biology of serotonin. Annual review of medicine 2009;60:355-66.

50. Chamberlain NL, Driver ED, Miesfeld RL. The length and location of CAG trinucleotide repeats in the androgen receptor N-terminal domain affect transactivation function. Nucleic Acids Res 1994 Aug 11;22(15):3181-3186.

51. Kazemi-Esfarjani P, Trifiro MA, Pinsky L. Evidence for a repressive function of the long polyglutamine tract in the human androgen receptor: possible pathogenetic relevance for the (CAG)n-expanded neuronopathies. Hum Mol Genet 1995 Apr;4(4):523-527.

52. Tut TG, Ghadessy FJ, Trifiro MA, Pinsky L, Yong EL. Long polyglutamine tracts in the androgen receptor are associated with reduced trans-activation, impaired sperm production, and male infertility. J Clin Endocrinol Metab 1997 Nov;82(11):3777-3782.

Chapter 2

Pharmacokinetics of three doses of sublingual testosterone in healthy premenopausal women

Kim van Rooij, Jos Bloemers, Leo de Leede, Irwin Goldstein, Eef Lentjes, Hans Koppeschaar, Berend Olivier and Adriaan Tuiten

Psychoneuroendocrinology 2012;37:773-781

ABSTRACT

Context: Sublingual testosterone is a single-dose treatment often used in studies regarding social, cognitive and sexual behavior. It is hypothesized that an increase in the ratio of free to total testosterone (free fraction) is indirectly, via genomic effects, responsible for the behavioral effects after sublingual testosterone administration.

Objective: To characterize the pharmacokinetics of three doses sublingual testosterone in premenopausal women. Also, to investigate the SHBG saturation threshold influencing the free level and free fraction of testosterone.

Design: We conducted an investigator-blind, randomized, cross-over placebo-controlled study.

Setting: This study was undertaken at the research and development department of a scientific company for research regarding female sexual dysfunction.

Participants: 16 healthy premenopausal women (mean age 27.3 ± 5.3 yr).

Interventions: Sublingual testosterone solution; 0.25, 0.50 and 0.75 mg.

Main Outcomes Measure: The pharmacokinetics of three single doses sublingual testosterone solution; the influence of SHBG levels on free and total levels of testosterone.

Results: After sublingual testosterone administration, serum free and total testosterone levels peaked at 15 min. and reached baseline levels within 150 min. The AUCs and C_{max} of free and total testosterone differed significantly between the three doses (P<0.0001) and increased dose-dependently.

A dose-dependent increase in free fraction of testosterone was found in women with low SHBG levels, but not in women with high SHBG levels.

Conclusions: The three doses sublingual testosterone are rapidly absorbed and quickly metabolized in premenopausal women. These data demonstrate the influence of SHBG levels on the treatment induced alterations in plasma free testosterone.

INTRODUCTION

Results of scientific research indicate that testosterone is involved in social behavior [1,2] including sexual behavior [3,4]. Sexual behavior is influenced by endogenous testosterone levels as well as to exogenously administered testosterone. For exogenous testosterone administration, two different methods of treatment can be distinguished: chronic treatment versus single dose administration. Each method of treatment has its own pharmacokinetic profile, which may affect the influence of testosterone on behavior. Chronic testosterone administration is utilized as the delivery option in the majority of studies regarding the influence of testosterone on women's sexual behavior, including hormonal replacement therapy in naturally or surgically (bilateral oophorectomy) menopausal women [5-7].

More recently however, several studies have investigated the effects of single dose testosterone administration on women's sexual behavior [8-10]. Tuiten et al. reported that a single sublingual dose of 0.50 mg testosterone significantly increased vaginal vasocongestion and experiences of sexual lust and genital sensation in premenopausal women without sexual complaints [8]. These effects occurred 3 to 4 h after the induced testosterone peak and about 2 h after testosterone returned to baseline levels. This delay in behavioral effects after sublingual testosterone administration has been replicated in several other studies regarding social behavior and cognitive functions [11-22].

There are very few studies that have defined the pharmacokinetic profile of sublingual testosterone. Salehian et al. [23], compared the pharmacokinetic profiles of 2 doses of sublingual testosterone (2.5 and 5.0 mg) with the pharmacokinetic profile of a long-acting testosterone ester, testosterone enanthate (TE) (in oil, im. 200 mg) in hypogonadal men. Compared to sublingual testosterone, the total and the free testosterone levels peaked days later in the male subjects studied who received TE. In the sublingual conditions the rise of free testosterone levels occurred within 1 h after administration, in the TE group this occurred 7 days after administration. Furthermore, it was shown that the free testosterone levels in the TE condition did not increase until the sex

hormone binding globulin (SHBG) levels were suppressed after administration by day 7. The suppression of SHBG levels was significantly greater in the TE group than in either sublingual group [23].

It is widely accepted that free testosterone is the biologically active testosterone [24]. Pharmacodynamic effects (measures of sexual functioning) would thus be expected to increase much later in the TE administered group compared to the sublingual administered group. Unfortunately, in the Salehian et al. study, post-dose sexual motivation was measured for the first time in the week before the first visit on day 20, when the free testosterone rise had already been passed in both groups. Notably, in the study by Tuiten and Van der Made et al., measures of sexual arousal increased 3 to 4 h after the peak of circulating testosterone [8,10] and 2 hours after testosterone levels returned to baseline [8], indicating that sublingual testosterone administration produces a pharmacodynamic effect after 4 h. Van der Made et al. suggested a SHBG saturation threshold hypothesis; i.e., when available binding sites of SHBG are occupied with testosterone after a sufficient single sublingual dose of testosterone, free fraction-, and thus free testosterone levels increase thereby inducing behavioral effects [10]. The exact mechanism responsible for this delay in behavioral effect is not fully understood but it could be that testosterone exerts its behavioral effect via androgenic metabolites, genomic mechanisms [1] or a combination of these factors.

The main purpose of the present study was to establish an extensive pharmacokinetic profile of three different single doses of sublingual testosterone administered as a solution with cyclodextrin. The primary pharmacokinetic endpoints were levels of total and free testosterone. Secondary endpoints included the pharmacokinetics of 5α-dihydrotestosterone (DHT), and 3α-androstanediol glucuronide (3α-diol-G). Additionally serum albumin, 17β-estradiol (E_2) and SHBG were measured.

Moreover, we compared the data of the present study with those of the Tuiten et al.'s pharmacokinetic study [8] with regard to the effect of single dose sublingual testosterone on circulating free and total testosterone levels. Furthermore we sought to determine at which level serum testosterone occupies the available binding sites of SHBG and serum free testosterone

increases, i.e., the postulated SHBG saturation threshold mechanism by van der Made et al. [10].

SUBJECTS AND METHODS

Study subjects

Eligible women were between 21 and 40 years, premenopausal and had a body mass index (BMI) between 18 and 30 kg/m^2. Exclusion criteria included a history of a hormone-dependent malignancy, endocrine disease, neurological problems, psychiatric disorder, cardiovascular condition, hypertension, abnormal liver or renal function. Women taking medications that interfere with metabolism of sex steroids or had used testosterone therapy within 6 months before study entry were excluded also.

Women were recruited and enrolled from referrals, newspaper advertisements, the internet, and an internal database of our lab. To determine eligibility, participants were screened 2 weeks prior to study entry. In addition to an assessment of medical history, all subjects received a physical examination including a 12-lead electrocardiogram, standard biochemistry and hematological laboratory tests. Blood samples for determination of testosterone, SHBG, TSH, Thyroxine, FSH and estrogen were collected at baseline. A urine pregnancy test was applied to all women of child bearing potential.

16 healthy young women participated after providing written informed consent and received reimbursement for expenses for their participation. This study was approved by the local ethics committee (Stichting Therapeutische Evaluatie Geneesmiddelen Medisch Ethische Toetsingscommissie, Almere, The Netherlands) and carried out in agreement with ICH-GCP (International Conference on Harmonization – Good Clinical Practice).

Study design

This was a single-center, investigator-blind, randomized, cross-over, placebo-controlled study with three doses of a testosterone solution containing

cyclodextrin administered sublingually. This solution consists of authentic nonmodified testosterone forming a soluble complex by a cyclodextrin carbohydrate ring. Due to increased solubility the absorption of testosterone through the oral mucosa is facilitated, thereby avoiding the hepatic first-pass metabolism [23,25-27].

All 16 subjects received each investigational drug dose once in random order. Wash-out between treatments was at least 48 h. Subjects had serial blood samples drawn via an intravenous catheter. Pharmacokinetic parameters were monitored at baseline and (at 2, 4, 6, 8, 10, 20, 30, 60, 90, 120, 180, 230 min) after dosing.

Measurement of total testosterone, free testosterone, and DHT were performed at each sampling time; E_2 at -5, 60 and 230 min; 3α-diol-G at -5, 60, 120, and 230 min; SHBG and albumin prior to dosing and at 230 min. Blood samples in the placebo condition were only measured at -5, 10, 60 and 230 min.

Vital signs were measured at regular intervals and an electrocardiogram was performed prior to dosing and at the end of the experimental day. For each experimental day, subjects were asked to attend the visit in fasting state and they received a strict diet (low fat, no caffeine) during the experimental day to minimize the influence of pharmacokinetic parameters. Drug, alcohol and pregnancy screens were performed prior to experimental sessions.

Medication and dosing

Testosterone and placebo were administered sublingually in 4 separate experimental phases with either a 0.25, 0.50, 0.75 mg dose and placebo as a solution using a micropipette (Gilson Pipetman P1000) from a 1 mg/mL solution. The 0.25 mg, 0.50 mg, and 0.75 mg testosterone were dosed from different volumes of the 1 mg/mL solution. For the placebo solution 0.50 mL was administered.

The different doses were prepared by an unblinded research associate and administered by blinded research associates. The blinded research associate administered the solution into the subjects mouth under the tongue, the

subjects were instructed to keep the solution sublingually for 1 minute while moving the tongue slightly to optimize absorption. After 1 minute the blinded research associate instructed the subject to swallow the solution.

Hormone assays

The assay used for the determination of total testosterone, free testosterone (after ultrafiltration), and DHT was High Performance Liquid Chromatography with Mass Spectrometric detection (LC/MSMS) (API 4000, AB Sciex). The method was validated with a lower limit of quantification (LLOQ) of 0.02 ng/mL for testosterone and DHT, and 0.001 ng/mL for free testosterone. The LC/MSMS assay is a reliable method for analysis of free testosterone and overcomes the known limitations of direct immunoassays in measurement of testosterone values in the lower range [28,29].

E_2 was analyzed by a chemiluminescence immunoassay (Siemens), the LLOQ was 0.25 pmol/L. 3α-diol-G was measured by ELISA (BioVendor), the LLOQ was 0.25 ng/mL. SHBG was measured by an electrochemiluminescent assay (ECLIA, Roche). Albumin was measured by Roche Bromocresol Green (BCG) analysis (Roche).

Statistical analysis

The pharmacokinetic parameters were analyzed using the WinNonlin software (version 5.1). Pharmacokinetic parameters including area under the curve, t = 0 till t = 230 min ($AUC_{0\text{-}230}$), maximum concentration ($C_{max)}$ and time to maximum concentration (t_{max}) were calculated based on actual and baseline corrected individual concentration time curves. AUCs were estimated using the linear trapezoidal rule. Individual pharmacokinetic parameters $AUC_{0\text{-}230}$ and C_{max} and corresponding dose normalized parameters were log transformed and analyzed using a mixed maximum likelihood analysis (PROC MIXED in SAS, version 9.1) including subject as a random factor and drug as a fixed effect factor. Contrasts were made of the least square means to compare the different doses. T_{max} was analyzed using a Wilcoxon rank sum test. This was based on the planned times

corresponding to the actual t_{max} to prevent bias in analysis results based on differences in sampling times.

The baseline levels of total and free testosterone, DHT, E_2, 3α-diol-G, SHBG and albumin were calculated by taking the mean of the placebo, 0.25, 0.50 and 0.75 mg predose levels.

Overall analysis of the free fraction (free testosterone levels divided by total testosterone levels at each time point) was analyzed in a 3 Drug (0.25 mg vs 0.50 mg vs 0.75mg) x 6 Time (*t* = 4, 6, 8, 10, 20, 30 min.) repeated measures ANOVA, with Drug and Time as within subjects factors.

In order to meet normality assumptions, baseline SHBG values were log-transformed and Pearson's correlation coefficients were calculated to further investigate relationships between SHBG levels, total testosterone, free testosterone and free fraction percentage of testosterone.

Subsequently, we divided the subjects into two subgroups, on the basis of their baseline SHBG levels (mean of placebo, 0.25, 0.50, 0.75 mg predose levels). This subdivision was based on a median split of the baseline SHBG levels. One group (*N* = 8) with low SHBG levels (≤63 nmol/L) and the other group (*N* = 8) with relatively high SHBG levels (>63 nmol/L). Independent samples *t*-test were used to assess free testosterone levels with SHBG as grouping variable (low vs. high SHBG) for each post-dose measurement.

The dependent variable free fraction was analyzed in a 3 Drug (0.25 mg vs. 0.50 mg vs. 0.75mg) x 6 Time (*t* = 4, 6, 8, 10, 20, 30 min) x 2 Group (SHBG low vs. SHBG high) repeated measures ANOVA, with Drug and Time as within subjects factor and Group as between subjects factor. To analyze the effects of the within subject factors within each group separately, paired-samples *t*-test were used for each SHBG group for each post-dose measurement between the three doses. For all ANOVAs sphericity was not violated. For all analyses a (two-sided) p-value less than 0.05 was considered statistically significant. SPSS 16.0 was used for all statistical analyses.

RESULTS

The baseline characteristics and hormone levels of the 16 study participants are outlined in table 1. One subject was excluded from the 0.50 mg analysis due to an incorrect administration procedure of the testosterone solution.

Table 1. Baseline and clinical characteristics of the participants

Characteristic	Value (*N* = 16)
Age (years)	27.3 ± 5.3
Race (no. (%))	
Caucasian	11 (69)
Black	2 (13)
Asian	1 (6)
Other	2 (13)[a]
BMI (kg/m²)	23.5 ± 3.4
Contraceptive (no. (%))	
Hormonal	11 (69)
-Combined oral contraceptive pill	8 (50)
-IUD (levonorgestrel)	2 (13)
-Vaginal ring (progestin and estrogen)	1 (6)
Non-hormonal	1 (6)
None	4 (25.0)
Total testosterone (ng/mL)	0.2 ± 0.1
Free testosterone (pg/mL)	1.9 ± 0.7[b]
DHT (ng/mL)	0.1 ± 0.03
3α-diol-G (ng/mL)	2.0 ± 1.9
E2 (pmol/L)	207 ± 147[c]
SHBG (nmol/L)	114 ± 120
Albumin (g/L)	44.7 ± 1.5

Plus–minus values are means ± SD. To convert total testosterone to nanomoles per liter, multiply by 3.467; to convert free testosterone to picomoles per liter, multiply by 3467. To convert total DHT to nanomoles per liter, multiply by 3.44. To convert 3α-diol-G to nanomoles per liter, multiply by 2.13. All baseline levels are means of placebo, 0.25, 0.50, 0.75 mg predose levels.

[a] The percentages do not sum up to 100% due to rounding of the numbers

[b] Only measured in 11 subjects; 5 subjects had values below the LLOQ

[c] Only measured in 15 subjects; 1 subject had a value below the LLOQ

Primary pharmacokinetic endpoints

The pharmacokinetic parameters of total and free testosterone are summarized in table 2.

Table 2. Baseline corrected pharmacokinetic parameters of total- and free testosterone following administration of 0.25 to 0.75 mg sublingual testosterone

	Dose (mg)	**$t_{1/2}$ * (min)**	**T_{max} * (min)**	**Baseline corrected AUC $_{0-230}$ ** (ng*min/mL)**	**C_{max} ** (ng/mL)**	**MRT * (min)**
Testosterone	0.25	49.8 ± 16.0	15.6 ± 5.4	194 (37.2)	3.79 (39.9)	57.7±12.2
(ng/mL) [a]	0.50	49.7 ± 22.4	15.1 ± 5.5	266 (37.6)	5.31 (37.8)	55.6±13.9
	0.75	58.5 ± 24.6	14.3 ± 5.3	337 (34.7)	6.73 (39.6)	59.5±16.4
Free	0.25	42.3 ± 14.6	15.6 ± 5.1	0.95 (51.8)	0.021(39.7)	52.6±11.6
testosterone	0.50	55.7 ± 27.5	14.4 ± 5.5	1.51 (40.2)	0.032(37.6)	57.1±15.6
(ng/mL) [b]	0.75	51.1 ± 26.4	12.8 ± 6.3	1.87 (47.8)	0.043(45.7)	51.4±14.5

[a] Total testosterone normal range =0.14 to 0.66 ng/mL [30].

[b] Free testosterone normal range= 0.00072 to 0.0036 ng/mL [30].

To convert total testosterone to nanomoles per liter, multiply by 3.467; to convert free testosterone to picomoles per liter, multiply by 3467.

MRT = mean residence time

* mean ± SD

** geometric mean (%CV)

Total testosterone

The three doses (0.25, 0.50, 0.75 mg) produced maximum levels of total testosterone of 3.79, 5.31 and 6.73 ng/mL, respectively, at means of 15.6, 15.1 and 14.3 min (Figure 1).

The Cmax of total testosterone was significantly different ($P < 0.0001$) among the three doses. We found no statistically significant differences in T_{max} of total testosterone between the three dosages. The AUCs of total testosterone were

also statistically significant different among the three doses ($P < 0.0001$) and showed a dose-dependent increase. The calculated half-life of total testosterone showed a significant difference between the 0.50 mg and 0.75 mg dose ($P = 0.0125$).

Free testosterone

Peak levels for free testosterone during the three dosages were 0.021, 0.032 and 0.043 ng/mL at means of 15.6, 14.4 and 12.8 min respectively (Figure 2). There was a statistically significant difference between the three doses with respect to Cmax of free testosterone ($p < 0.0001$). There were no statistically significant differences for free testosterone T_{max} between the three dosages. Free testosterone AUCs were statistically significant different between the three doses and increased dose-dependently. The differences between the free testosterone AUCs of the 0.25 mg vs 0.50 mg and 0.25 mg vs 0.75 mg have p values < 0.0001, while the difference between the 0.50 and 0.75 mg was significant at $P < 0.01$. There were no statistically significant differences between the three doses for the calculated half-life of free testosterone.

For all doses, baseline levels for total- and free testosterone were reached by 150 min.

Bioavailability

To determine the absolute percentage of the sublingual testosterone dose which is absorbed in the systemic circulation, the fraction of absorbed testosterone needs to be calculated from the formula used also for the AUC calculation after intravenous dosing. Since we did not have an intravenous standard, we took the 0.25 mg dosage as reference value. Thus the bioavailability of the 0.25 mg was set at 100%, and for 0.50 and 0.75 mg were calculated as 69% (or 0.34 mg), and 58% (or 0.43 mg), respectively. The bioavailability of sublingual testosterone administration decreases with increasing doses.

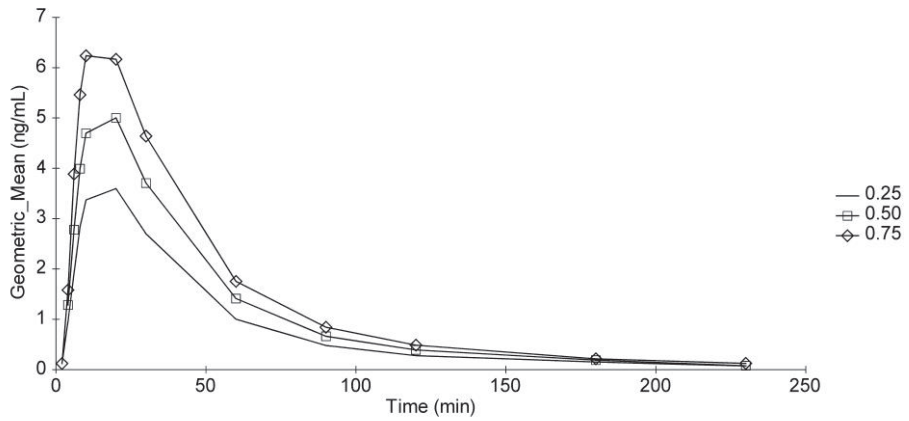

Figure 1. Geometric mean total testosterone levels in serum after administration of 0.25, 0.50 and 0.75 mg sublingual testosterone. Total testosterone normal range = 0.14—0.66 ng/mL (0.5—2.3 nmol/L) [30]. To convert total testosterone to nanomoles per liter, multiply by 3.467.

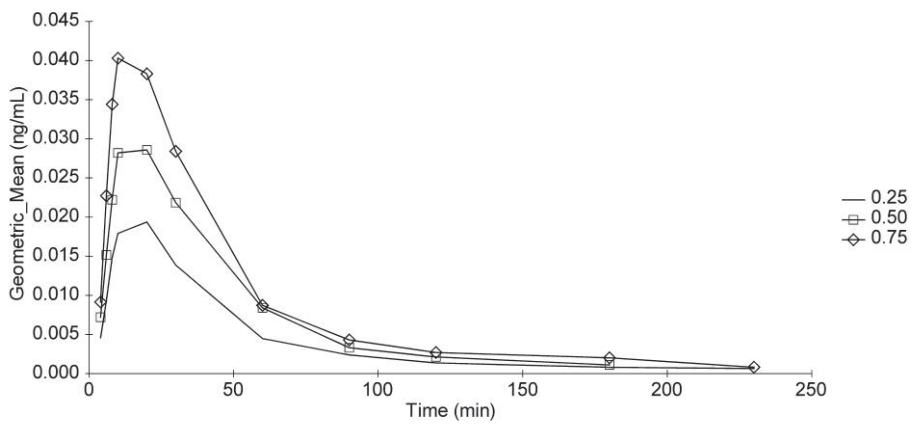

Figure 2. Geometric mean free testosterone levels in serum after administration of 0.25, 0.50 and 0.75 mg sublingual testosterone. Free testosterone normal range = 0.00072—0.0036 ng/mL (2.5—12.5 pmol/L) [30]. To convert free testosterone to picomoles per liter, multiply by 3467.

Free fraction

Our analyses showed a statistically significant effect of drug dose on the free fraction of testosterone (i.e. the ratio of free to total testosterone) during the *t* = 4 through *t* = 30 min measurements (*P* = 0.002). We also found a statistically significant difference for the C_{max} during *t* = 4 through *t* = 30 min between the 0.25 mg and 0.50 mg (*P* = 0.003) and between 0.25 mg and 0.75 mg doses (*P* = 0.010), but not between the 0.50 and 0.75 mg dose (*P* = 0.381) (Figure 3).

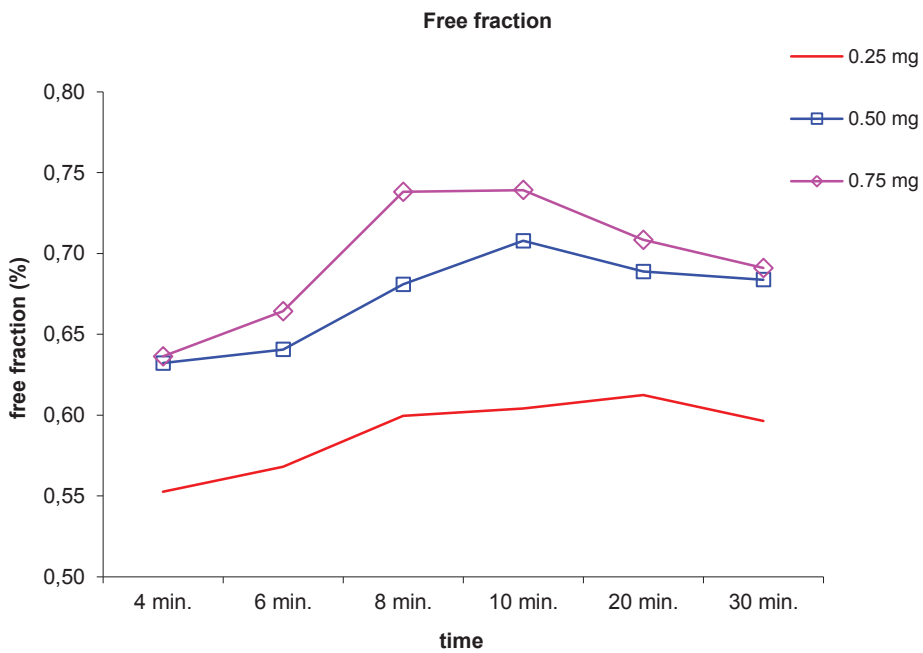

Figure 3. Free fraction of testosterone for 0.25 mg, 0.50 mg and 0.75 mg measured from t = 4 min to 30 min.

As stated above, we expected to find a relationship between circulating SHBG and the increases in the free levels and the free fraction of testosterone induced by the different dosages of sublingual testosterone. Moreover, our experimental manipulations produced no statistically significant changes in SHBG and albumin levels between and on test days (data not shown).

In our study population we found a large between-subject variation in circulating SHBG levels. Baseline SHBG levels (log transformed) were correlated with total testosterone levels (t = 20 min): r =.732, P < 0.0002; r =.930, P < 0.001 and r =.894, P < 0.001 for the 0.25 mg, 0.50 mg and 0.75 mg dose respectively. Baseline SHBG levels (log transformed) were inversely correlated with free testosterone levels (t = 20 min): r = -.702, P < 0.003; r = -.849, P < 0.001 and r = -.798, P < 0.001 for the 0.25 mg, 0.50 mg and 0.75 mg dose respectively. For the free fraction levels and SHBG levels, we observed stronger correlations; r = -.947, P < 0.001; r = -.938, P < 0.001 and r = -.944, P < 0.001 for the 0.25 mg, 0.50 mg and 0.75 mg dose respectively on t = 20.

Because of this large between-subject variation we subdivided the subjects in two group based on a median split of the baseline SHBG levels. The low SHBG group had a mean SHBG baseline level of 44 nmol/L (±11), while the high SHBG group had a mean level of 183 nmol/L (±141).

Total testosterone and SHBG

In subjects with low SHBG, the three doses produced maximum levels of total testosterone of 3.18, 3.93 and 4.73 ng/mL, respectively, at 20 min after dosing. In subjects with high SHBG, the maximum levels of total testosterone were 5.00, 7.08 and 9.04 ng/mL after administration of the three doses sublingual testosterone. Between groups, total testosterone levels were statistically different for t = 10 till t = 30 min in the 0.25 and 0.50 mg dose, and in the 0.75 mg dose 6 till 30 min after dosing.

Free testosterone and SHBG

In subjects with low SHBG, the three doses produced maximum levels of free testosterone of 0.026, 0.039 and 0.048 ng/mL, respectively, at 20 min after dosing. In subjects with high SHBG, the maximum levels of free testosterone were 0.018, 0.026 and 0.034 ng/mL after administration of the three doses sublingual testosterone. Between groups, all differences were statistically different, except for the levels of free testosterone in the 0.25 mg dose 4 and 20 min after dosing and in the 0.75 mg dose 4 and 10 min after dosing.

Our analyses showed that the low SHBG group had overall significantly higher levels of the free fraction compared to the high SHBG group (P = 0.007). Analyses revealed a statistically significant Group x Drug effect for the difference between 0.25 mg and 0.75 mg (P = 0.012) and between 0.25 mg and 0.50 mg (P = 0.031) (see Figure 4). As shown in Figure 4, statistically significant differences between the different doses sublingual testosterone were found in the low SHBG group.

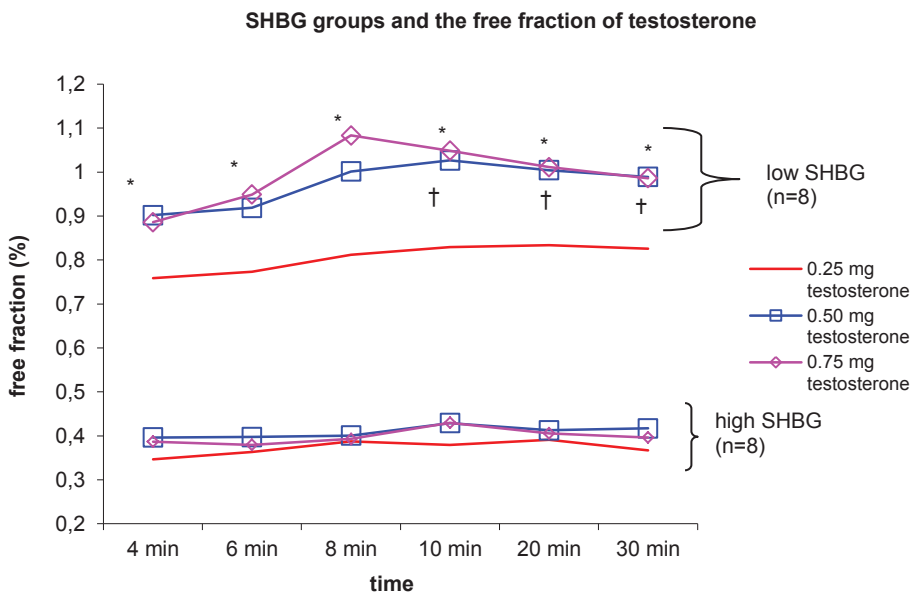

Figure 4. Free fraction of testosterone for 0.25 mg, 0.50 mg and 0.75 mg measured from t = 4 min to t = 30 min for the low and high SHBG groups.
* significant difference between 0.25 mg vs. 0.75 mg (P = <0.05)
† significant difference between 0.25 mg vs. 0.50 mg (P = <0.05)

Secondary pharmacokinetic endpoints

DHT peak levels of 0.285, 0.404 and 0.465 ng/mL were reached at means of 27.5, 28.0 and 27.5 min respectively (Table 3).

Table 3. Baseline corrected pharmacokinetic parameters of DHT following administration of 0.25 to 0.75 mg sublingual testosterone

	Dose (mg)	$t_{1/2}$ * (min)	T_{max}* (min)	AUC $_{0-230}$ ** (ng* min/mL)	C_{max} ** (ng/mL)	MRT* (min)
Dihydro-testosterone (ng/mL)	0.25	45.1 ± 10.5	27.5 ± 4.5	20.6 (44.9)	0.285 (42.5)	75.7 ± 14.4
	0.50	44.5 ± 16.8	28.0 ± 4.1	28.8 (37.9)	0.404 (37.6)	73.4 ± 14.8
	0.75	50.5 ± 30.4	27.5 ± 4.5	34.4 (41.3)	0.465 (43.5)	81.5 ± 36.3

DHT reference range= < 0.29 ng/mL [30]. To convert total DHT to nanomoles per liter, multiply by 3.44. * mean ± SD, ** geometric mean (%CV)

T max differences between the three doses were not significant. The difference between the C_{max} of the 0.25 mg vs. 0.50 mg and 0.25 mg vs. 0.75 mg was significant ($P < 0.0001$), and the difference between the Cmax of 0.50 mg and 0.75 mg was statistically significant ($P = 0.031$). Mean residence time of the dihydrotestosterone levels were not different between the three sublingual doses. AUCs were statistically significant different between the three doses and increased dose-dependently.

The difference between the AUCs of the 0.25 mg vs 0.50 mg and 0.25 mg vs 0.75 mg was statistically significant ($P < 0.0001$), while the difference between the 0.50 and 0.75 mg was significant at $P = 0.021$. There were no statistically significant differences between the three doses, for the calculated half-life of DHT. For all doses, return to DHT baseline levels occurred within 180 min (Figure 5).

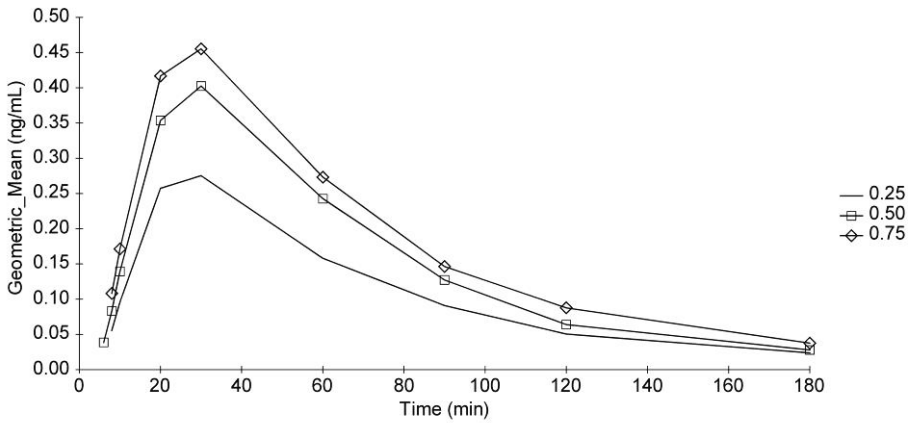

Figure 5. Geometric mean DHT levels in serum after administration of 0.25, 0.50 and 0.75 mg sublingual testosterone. DHT reference range= < 0.29 ng/mL [30]. To convert total DHT to nanomoles per liter, multiply by 3.44.

Increasing doses of sublingual testosterone did not have a statistically significant influence on the 3α-diol-G concentrations as measured at *t* = 0, *t* = 60, *t* = 120, and *t* = 230. Cmax and AUCs differences were not statistically significant between the three doses (data not shown).

E_2 levels did not change between the three doses of sublingual testosterone and did not increase significantly compared to baseline on *t* = 60 and *t* = 230 min (data not shown).

The three doses sublingual testosterone were well tolerated.

DISCUSSION

Our results demonstrate that sublingual administration of each of the three doses testosterone was followed by a quick and steep increase of total and free testosterone levels; with peak levels reached at 15 min. Serum levels of total and free testosterone rapidly declined to reach baseline levels by 2.5 h, which is in line with our previous study [8,30], and with the reported pharmacokinetic profile following inhalation of testosterone [30].

The total testosterone C_{max} following administration of 0.50 mg sublingual testosterone showed consistency with the reported C_{max} of Tuiten et al [8]. Also, the time to reach C_{max} of total testosterone in this study showed uniformity with the data of Tuiten et al. and the study of Salehian et al., who administered 2.5 mg and 5.0 mg sublingual testosterone [23].

DHT levels showed a significant dose-dependent increase, peak levels were reached within 30 min and levels returned to baseline levels within 3 h. DHT is metabolized to 3α-diol-G, so an elevation of 3α-diol-G levels was expected after administration of sublingual testosterone. However, no dose-dependent effect of sublingual testosterone on the concentration of 3α-diol-G was found.

According to the SHBG saturation threshold hypothesis by van der Made et al.[10], an increased influx of testosterone into the body will occupy binding sites of SHBG. When most binding sites are occupied, free (non-SHBG bound) testosterone and consequently the free fraction will increase and thereby inducing, probably via genomic mechanisms [1], behavioral effects after approximately 4 h.

The results of the present study show that free and total testosterone levels significantly increase dose-dependently, which is reflected by an increase in the free fraction of testosterone. However, the difference in free fraction of testosterone between the 0.50 and 0.75 mg condition did not reach statistical significance. It is interesting that around T_{max} of free and total testosterone, six women have lower free fraction levels in the 0.75 mg condition compared to the 0.50 mg condition. Whether this is the result of variation in drug absorption, or

the large between-subject variation in SHBG levels which could have influenced the results, is not clear. Furthermore, it is also possible that the number of subjects was probably too small to detect a significant increase in free fraction levels between these two doses.

Testosterone has a high affinity to SHBG and slowly dissociates from SHBG. Free testosterone is rapidly metabolized ($T_{1/2}$ 10 min.) which demonstrates the importance of SHBG binding and dissociation capacity, indicating that SHBG is the major determinant of the free fraction equilibrium. Fig 4 shows the free fraction levels for subjects with low and high SHBG levels. In the low SHBG group we observed an increase of the free fraction of testosterone levels induced by increasing dosages of sublingual testosterone, while this pattern was not found in the women with high SHBG. These results corroborate the hypothesis of van der Made et al. [10], namely: absorbed testosterone is bound to SHBG which has a limited capacity and only when this binding capacity is saturated, free testosterone and the free fraction increase.

According to van der Made, the increase in the free fraction might be responsible for behavioral effects observed 3.5 to 4 h later. However, in this study we measured free testosterone levels directly (with LC/MSMS) and we found these to be dose-dependently increased in both SHBG groups, in contrast to the free fraction which did not show a dose-dependent increase. Therefore we propose an adjustment to the SHBG saturation threshold hypothesis as postulated by van der Made et al [10]; it is confirmed that SHBG levels influence the percentage of free fraction of testosterone (and the maximum concentration of free testosterone), however, an increase in free testosterone levels seems to be relatively less dependent of circulating SHBG levels after administration of the used dosages of sublingual testosterone. Further studies are necessary to investigate if free testosterone levels or free fraction levels are responsible to the observed behavioral effects as described by van der Made et al.

The data of the bioavailability show that sublingual testosterone absorption decreases with increasing doses and is 69% and 58% for the 0.50 and 0.75 dose respectively when the 0.25 mg condition is used as the reference value (100%).These data suggest a limitation of the total amount of testosterone

absorbed. The volumes of the sublingual testosterone solution in the higher dose conditions were larger compared to the lower dosages. These increasing volumes could possibly influence the absorption at the limited surface area in the mouth.

In this study we did not take into account the cyclical and diurnal variation of testosterone. It is well known that testosterone levels are highest during the ovulatory and midluteal phase of the menstrual cycle and lowest in the early follicular phase and late luteal phase [31-33]. In this study, blood samples were taken irrespective of menstrual cycle phase. However, almost 60% of the women in this study used some form of hormonal contraceptive (combined oral contraceptive pill, combined-contraceptive vaginal ring) which is known to suppress ovulation [34,35]. Moreover, we assumed that the used dosages used in the present study overruled considerably the natural occurring relatively subtle cyclical and diurnal variation of testosterone. Furthermore, in a recent study by Braunstein et al. it was shown that SHBG levels of 161 women remained relatively stable across the menstrual cycle. They found a relatively small increase in testosterone levels in the mid-cycle period compared to the overall variability and suggest that the reference ranges described can be applied irrespective of the day in the menstrual cycle [36]. So it is therefore unlikely that the dose-dependent increase in total and free testosterone levels are biased by the cyclical and diurnal variation of testosterone.

Next to the sublingual route of testosterone administration other routes could be investigated as well. However for the desired immediate uptake and rapid return of testosterone to baseline levels the intramuscular and transdermal route are not suitable since both will result in gradual systemic uptake and prolonged higher plasma levels after drug administration via these routes. Oral administration is impossible at all, due to the very large first-pass effect no unmodified testosterone will reach the systemic circulation. For alternative routes next to sublingual with a very fast uptake and quick return to baseline of testosterone, the pulmonal and nasal delivery could be used for which in that case suitable and convenient dosage forms need to be developed.

In conclusion, the three doses testosterone are rapidly absorbed by the sublingual route and quickly metabolized without sustained elevations of DHT and E_2. These data suggest that a SHBG threshold exists which influences the increase in free fraction levels.

REFERENCES

1. Bos PA, Panksepp J, Bluthe RM, Honk JV. Acute effects of steroid hormones and neuropeptides on human social-emotional behavior: A review of single administration studies. Front Neuroendocrinol 2011 Jan 21.

2. Eisenegger C, Haushofer J, Fehr E. The role of testosterone in social interaction. Trends Cogn Sci 2011 Jun;15(6):263-271.

3. Auger AP. Steroid receptor control of reproductive behavior. Hormones and behavior 2004 Mar;45(3):168-72.

4. Hull EM, Dominguez JM. Sexual behavior in male rodents. Hormones and behavior 2007 Jun;52(1):45-55.

5. Sherwin BB. Randomized clinical trials of combined estrogen-androgen preparations: effects on sexual functioning. Fertil Steril 2002 Apr;77(4):S49-S54.

6. Shifren JL, Braunstein GD, Simon JA, Casson PR, Buster JE, Redmond GP, et al. Transdermal testosterone treatment in women with impaired sexual function after oophorectomy. N Engl J Med 2000 Sep;343(10):682-688.

7. Simon J, Braunstein G, Nachtigall L, Utian W, Katz M, Miller S, et al. Testosterone patch increases sexual activity and desire in surgically menopausal women with hypoactive sexual desire disorder. J Clin Endocrinol Metab 2005 Sep;90(9):5226-5233.

8. Tuiten A, Van Honk J, Koppeschaar H, Bernaards C, Thijssen J, Verbaten R. Time course of effects of testosterone administration on sexual arousal in women. Arch Gen Psychiatry 2000 Feb;57(2):149-53; discussion 155-6.

9. Tuiten A, van Honk J, Verbaten R, Laan E, Everaerd W, Stam H. Can sublingual testosterone increase subjective and physiological measures of laboratory-induced sexual arousal? Archives of general psychiatry 2002 May;59(5):465-6.

10. van der Made F, Bloemers J, Yassem WE, Kleiverda G, Everaerd W, van Ham D, et al. The influence of testosterone combined with a PDE5-inhibitor on cognitive, affective, and physiological sexual functioning in women suffering from sexual dysfunction. The journal of sexual medicine 2009 Mar;6(3):777-90.

11. Aleman A, Bronk E, Kessels RP, Koppeschaar HP, van Honk J. A single administration of testosterone improves visuospatial ability in young women. Psychoneuroendocrinology 2004 Jun;29(5):612-7.

12. Bos PA, Terburg D, van Honk J. Testosterone decreases trust in socially naive humans. Proceedings of the National Academy of Sciences of the United States of America 2010 Jun 1;107(22):9991-5.

13. Eisenegger C, Naef M, Snozzi R, Heinrichs M, Fehr E. Prejudice and truth about the effect of testosterone on human bargaining behaviour. Nature 2010 Jan 21;463(7279):356-9.

14. Hermans EJ, Putman P, van Honk J. Testosterone administration reduces empathetic behavior: a facial mimicry study. Psychoneuroendocrinology 2006 Aug;31(7):859-66.

15. Hermans EJ, Putman P, Baas JM, Gecks NM, Kenemans JL, van Honk J. Exogenous testosterone attenuates the integrated central stress response in healthy young women. Psychoneuroendocrinology 2007 Sep-Nov;32(8-10):1052-61.

16. Hermans EJ, Ramsey NF, van Honk J. Exogenous testosterone enhances responsiveness to social threat in the neural circuitry of social aggression in humans. Biological psychiatry 2008 Feb 1;63(3):263-70.

17. Postma A, Meyer G, Tuiten A, van Honk J, Kessels RP, Thijssen J. Effects of testosterone administration on selective aspects of object-location memory in healthy young women. Psychoneuroendocrinology 2000 Aug;25(6):563-75.

18. Schutter DJ, van Honk J. Decoupling of midfrontal delta-beta oscillations after testosterone administration. Int J Psychophysiol 2004 Jun;53(1):71-3.

19. van Honk J, Peper JS, Schutter DJ. Testosterone reduces unconscious fear but not consciously experienced anxiety: implications for the disorders of fear and anxiety. Biological psychiatry 2005 Aug 1;58(3):218-25.

20. van Honk J, Tuiten A, Hermans E, Putman P, Koppeschaar H, Thijssen J, et al. A single administration of testosterone induces cardiac accelerative responses to angry faces in healthy young women. Behavioral neuroscience 2001 Feb;115(1):238-42.

21 van Honk J, Schutter DJ, Hermans EJ, Putman P, Tuiten A, Koppeschaar H. Testosterone shifts the balance between sensitivity for punishment and reward in healthy young women. Psychoneuroendocrinology 2004 Aug;29(7):937-43.

22. van Honk J, Schutter DJ. Testosterone reduces conscious detection of signals serving social correction: implications for antisocial behavior. Psychol Sci 2007 Aug;18(8):663-7.

23. Salehian B, Wang C, Alexander G, Davidson T, McDonald V, Berman N, et al. Pharmacokinetics, bioefficacy, and safety of sublingual testosterone cyclodextrin in hypogonadal men: comparison to testosterone enanthate--a clinical research center study. The Journal of clinical endocrinology and metabolism 1995 Dec;80(12):3567-75.

24. Mendel CM. The free hormone hypothesis: a physiologically based mathematical model. Endocr Rev 1989 Aug;10(3):232-274.

25. Brewster ME, Estes KS, Loftsson T, Perchalski R, Derendorf H, Mullersman G, et al. Improved delivery through biological membranes. XXXL: Solubilization and stabilization of an estradiol chemical delivery system by modified beta-cyclodextrins. Journal of pharmaceutical sciences 1988 Nov;77(11):981-5.

26. Stuenkel CA, Dudley RE, Yen SS. Sublingual administration of testosterone-hydroxypropyl-beta-cyclodextrin inclusion complex simulates episodic androgen release in hypogonadal men. The Journal of clinical endocrinology and metabolism 1991 May;72(5):1054-9.

27. Zhang H, Zhang J, Streisand JB. Oral mucosal drug delivery: clinical pharmacokinetics and therapeutic applications. Clinical pharmacokinetics 2002;41(9):661-80.

28. Labrie F, Belanger A, Belanger P, Berube R, Martel C, Cusan L, et al. Androgen glucuronides, instead of testosterone, as the new markers of androgenic activity in women. The Journal of steroid biochemistry and molecular biology 2006 Jun;99(4-5):182-8.

29. Miller KK, Rosner W, Lee H, Hier J, Sesmilo G, Schoenfeld D, et al. Measurement of free testosterone in normal women and women with androgen deficiency: comparison of methods. The Journal of clinical endocrinology and metabolism 2004 Feb;89(2):525-33.

30. Davison S, Thipphawong J, Blanchard J, Liu K, Morishige R, Gonda I, et al. Pharmacokinetics and acute safety of inhaled testosterone in postmenopausal women. Journal of clinical pharmacology 2005 Feb;45(2):177-84.

31. Judd HL, Yen SSC. Serum Androstenedione and Testosterone Levels During Menstrual-Cycle. J Clin Endocrinol Metab 1973;36(3):475-481.

32. Rothman MS, Carlson NE, Xu M, Wang C, Swerdloff R, Lee P, et al. Reexamination of testosterone, dihydrotestosterone, estradiol and estrone levels across the menstrual cycle and in postmenopausal women measured by liquid chromatography-tandem mass spectrometry. Steroids 2011 Jan;76(1-2):177-82.

33. Salonia A, Pontillo M, Nappi RE, Zanni G, Fabbri F, Scavini M, et al. Menstrual cycle-related changes in circulating androgens in healthy women with self-reported normal sexual function. The journal of sexual medicine 2008 Apr;5(4):854-63.

34. Bancroft J, Sherwin BB, Alexander GM, Davidson DW, Walker A. Oral contraceptives, androgens, and the sexuality of young women: II. The role of androgens. Archives of sexual behavior 1991 Apr;20(2):121-35.

35. Mulders TMT, Dieben TOM. Use of the novel combined contraceptive vaginal ring NuvaRing for ovulation inhibition. Fertil Steril 2001 May;75(5):865-870.

36. Braunstein GD, Reitz RE, Buch A, Schnell D, Caulfield MP. Testosterone Reference Ranges in Normally Cycling Healthy Premenopausal Women. J Sex Med 2011 Jul 19.

Chapter 3

Toward Personalized Sexual Medicine (Part 1): Integrating the "Dual Control Model" into Differential Drug Treatments for Hypoactive Sexual Desire Disorder and Female Sexual Arousal Disorder

Jos Bloemers, Kim van Rooij, Saskia Poels, Irwin Goldstein, Walter Everaerd, Hans Koppeschaar, Meredith Chivers, Jeroen Gerritsen, Diana van Ham, Berend Olivier and Adriaan Tuiten

Journal of Sexual Medicine 2013;10:791-809

ABSTRACT

In three related manuscripts we describe our drug development program for the treatment of Hypoactive Sexual Desire Disorder (HSDD). In this first theoretical article we will defend the hypothesis that different causal mechanisms are responsible for the emergence of HSDD: low sexual desire in women (with HSDD) could be due to either a relative insensitive brain system for sexual cues *or* to enhanced activity of sexual inhibitory mechanisms. This distinction in etiological background was taken into account when designing and developing new pharmacotherapies for this disorder.

Irrespective of circulating plasma levels of testosterone, administration of sublingual 0.5 mg testosterone increases the sensitivity of the brain to sexual cues. The effects of an increase in sexual sensitivity of the brain depend on the motivational state of an individual. It might activate sexual excitatory mechanisms in low sensitive women, while it could evoke (or strengthen) sexual inhibitory mechanisms in women prone to sexual inhibition. Sexual stimulation in the brain is necessary for phosphodiesterase type 5 inhibitor (PDE5i)-mediated increase in genital sexual response. Accordingly, a single dose of T+PDE5i might enhance sexual responsiveness, especially in women with low sensitivity to sexual cues. In other women sexual stimulation might elicit a prefrontal cortex (PFC)-mediated phasic increase in sexual inhibition, in which activity of 5-hydroxytryptamine (5-HT, serotonin) is involved. We hypothesize that a single dose of 5-hydroxytryptamine$_{1A}$ receptor agonist (5-HT$_{1A}$ra) will reduce the sexual-stimulation-induced PFC-mediated sexual inhibition during a short period after administration. Consequently, treatment with T+5-HT$_{1A}$ra will be more effective, in particular in women exhibiting sexual inhibition.

Based on the results of our efficacy studies described in parts 2 and 3 of the series, we conclude that tailoring on-demand therapeutics to different underlying etiologies might be a useful approach to treat common symptoms in subgroups of women with HSDD.

INTRODUCTION

Human sexual behavior has been extensively studied in biology and psychology, but so far there is very limited success in the development of drugs for treatment of sexual dysfunction in women. Low sexual desire, with or without sexual arousal problems, is the most common sex-related complaint reported by women [1–3]. As a result, many women suffer from sexual dissatisfaction, which often negatively interferes with psychological well-being [4]. This has been classified as a clinical condition, referred to as Hypoactive Sexual Desire Disorder (HSDD) [5].

To date, no medication for this condition has been approved by the U.S. Food and Drug Administration (U.S. FDA). In the present article we will describe our hypothesis that different causal mechanisms are responsible for the lack of sexual desire in women, which was taken into account when designing and developing two new medicines for HSDD. In particular, in some women with HSDD, sexual dysfunction results from a relative insensitivity for sexual cues, whereas in others, sexual complaints result from dysfunctional automatic activation of sexual inhibitory mechanisms during exposure to sexual stimulation. One drug has been developed for a subgroup of patients in which sexual dysfunction occurs as the result of a relatively insensitive system for sexual cues. The other drug has been developed for women in which the sexual complaints result from (dysfunctional) activation of sexual inhibitory mechanisms.

The efficacy of both drugs is based on a delay in effect of sublingual testosterone. That does not mean that low sexual desire can be attributed to an absolute deficiency of testosterone. There are other intervening biological and psychological variables influencing the sensitivity of the brain for sexual stimuli and mediating the effects of treatment with testosterone. In this first theoretical part we will try to clarify these assertions and our assumptions. The second and third parts of this trilogy are empirical studies, in which we will describe the results on the efficacy of both proposed treatments in two subgroups of women with HSDD, in which the complaints have a different etiological origin.

Administration of one single dose of 0.5 mg sublingual testosterone increases, with a delay in effect of about 4 hours, the sensitivity of the brain to cues associated with behavior driven by social interaction, including sexual behavior [6–8]. This effect on the brain's sensitivity to sexual cues affects mechanisms involved in sexual motivation, and has divergent effects in the proposed subgroups of women. A single-dose sublingual testosterone might increase central sexual stimulation, a necessary condition for the efficacy of a phosphodiesterase type-5 (PDE5) inhibitor on physiological sexual responding. Sublingual testosterone combined with a PDE5 inhibitor (dosed in such a manner that the time of the peak plasma concentration of the PDE5 inhibitor coincides with the 4-hour delay in behavioral effects of the testosterone) will enhance sexual responsiveness, especially in women with low sensitivity for sex [9,10]. On the other hand, in women already prone to sexual inhibition, an increase in central sexual stimulation might elicit a phasic prefrontal cortex (PFC)-mediated serotonin-dependent increased response in sexual inhibition. A single dose of a 5-hydroxytryptamine$_{1A}$ (5-HT$_{1A}$) receptor agonist will reduce the sexual stimulation-induced PFC-mediated sexual inhibition during a short period after intake. Consequently, treatment with sublingual testosterone and 5-HT$_{1A}$ receptor agonist (dosed in such a manner that the pharmacological effects of the 5-HT$_{1A}$ receptor agonist coincide with the behavioral window induced by the testosterone administration) will be more effective, in particular in women prone to sexual inhibition.

Multifactorial Determination of Human Sexual Behavior

A range of biological and psychological regulatory mechanisms control human social and sexual functions. However, the degree of activation of these mechanisms (i.e., their relative strength) varies within and between subjects. One possible reason for the limited success in developing drug treatments for HSDD could be the lack of consideration of multiple interacting biological *and* psychological mechanisms in the existence of individual differences in sexual (dys)function. In humans, a range of complex social, cognitive, and affective processes mediate the transition from a preparatory sexual motivational state to actual sexual behavior. These conscious and subconscious processes are partly the result of present and previous beneficial and/or adverse

experiences and significantly influence the extent to which an individual is willing to engage in sexual activities and the extent to which those activities are enjoyable. Therefore, the delicate and relative balance between activation of central mechanisms governing sexual excitation and sexual inhibition controls sexual activities in humans, including fantasizing, masturbation, and sexual intercourse.

Human sexual behavior occurs in the context of social relationships. These social relationships themselves are influenced by (or can influence) biological (e.g., testosterone) and psychological variables. The relationship between endogenous or exogenous induced testosterone levels and social behavior is subject of extensive research. Here we will start to discuss some of the established relationships between this steroid and social behavior and highlight the reciprocal nature of this relationship and the implications this has on sexual behavior. In other words, we will discuss the influence of testosterone on social behavior in the context of the interrelationship between social and sexual behavior.

On the Physiological Control of Social and Sexual Behavior

In many mammalian species, female sex steroids are essential for the expression of female sexual behavior. As a result, the capability for copulation in these animals is limited to the period of ovulation [11,12]. In higher primates, like humans, sexual intercourse is not limited to the peri-ovulatory period, and it has been suggested that testosterone plays an important role in sociosexual behavior of women [13]. This is supported by the fact that the depletion of testosterone in women following ovariectomy and adrenalectomy is accompanied by a complete loss of libido [14], while substitution of testosterone restores sexual desire and fantasies after surgical menopause [15]. Because testosterone plays a central role in the steroid-responsive neural network of human (males and) females, testosterone will influence the sensitivity of the brain for social and sexual cues in both sexes.

Although the testosterone production in adult men is about 10 to 15 times higher than in women, it is believed that women are more sensitive to this steroid

[16]. Despite the large differences in circulating testosterone levels between the sexes, it has been shown that testosterone has similar effects on behavior in men and women [17]. Thus, conclusions about the influence of testosterone on behavior in men are generalizable to women. Testosterone has been associated with behavior driven by social interaction, in particular competitive and sexual behavior. Competitive behaviors are linked to the personality trait "dominance." This trait refers to the motivated behavior through which high status within a social hierarchy will be attained and maintained, and is partly determined by the production of endogenous testosterone [17,18].

For a long time it has been assumed that testosterone also encourages competition between males to attract attention of sexually attractive females. Dominance is often used by males in competition with others to get reproductive advantage [19,20]. They do so by using direct (e.g., self-promotion) and indirect (e.g., derogation of the competitors) dominance strategies. In animal studies it is often shown that testosterone is involved in various behaviors related to competition to get access to the opposite sex, including increased sexual motivation, territorial demarcation, mate guarding, increased aggression, and display behavior [21–23]. In human males it has recently been shown that precompetitive testosterone levels were positively associated with dominance behavior during a competition experiment, in which men had to compete with other men to attract attention of an attractive female confederate. High testosterone levels were associated with higher assertiveness and the degree of control these men took during an interview setting with these women, and the extent to which women had "felt a click" with these men. These effects of testosterone on the behavior of dominant men were especially pronounced in those who also reported a strong need for social dominance. Moreover, men with more testosterone and a high need for social dominance were found to be able to suppress the competitor's ability to also attract potential partners. In contrast, among the men who had indicated that they had little or no need for social dominance, there was neither a correlation between their testosterone levels and their dominant behaviors, nor in their perception of the behaviors of their competitors [21]. These findings emphasize an important difference between humans and animals. In humans—in contrast to animals—explicit and conscious motives influence the effects of

testosterone on behavior.

Thus testosterone promotes approach behavior focused on dominance and status especially to get access to sexual rewards. Further evidence for this comes from a functional magnetic resonance imaging (fMRI) study, in which it was demonstrated that in anticipation of a reward, treatment with testosterone enhanced the activity of neural pathways involved in reward seeking [24].

However, not only does testosterone influence social behavior, but a reciprocal relationship also exists between social behavior and testosterone levels. In rhesus monkeys it has been demonstrated that an increase in social status induces an increase in testosterone [25,26], whereas a loss of social status is accompanied by a decrease in testosterone [25,26], which was also found in men [27,28]. Moreover, increases and decreases in testosterone levels in response to mating cues also influence men's mating behavior in a reciprocal fashion. Testosterone levels in men rise when they interact with female confederates in the lab, and the degree of increase of these testosterone levels correlates with the confederates' experience of how much they felt the men tried to impress them [29]. Furthermore, testosterone levels of men also increase during exposure to sexually explicit movies as compared with neutral ones [30]. In women it has also been demonstrated that salivary testosterone levels rise in anticipation of sexual intercourse compared with anticipation of nonsexual activities. These levels stayed higher up until 15 minutes after the intercourse [31]. Also, Hamilton and Meston [32] studied women in long-distance relationship and found that testosterone increases the day before women were reunited with their partners after a separation. These results seem to support the hypothesis that testosterone increases in anticipation of sexual activity. A recent study of Goldey and Van Anders [33] showed that sexual thoughts in women can also influence testosterone levels. These studies employed salivary testosterone measures, and it should be noted that salivary testosterone measurements as compared with serum testosterone levels may underestimate testosterone-behavior correlations in women [34].

Concluding, testosterone not only influences social behavior, but socially

challenging stimuli and social behavior can also induce increases and decreases of testosterone levels, which can be interpreted as a functional adaptation of the action of this hormone to changing circumstances. Although many factors and circumstances elicit fluctuations in testosterone levels, the basal level of this steroid appears to be relatively constant over longer periods [35]. As a result, many researchers consider these basal levels as a trait-level factor. The increases and decreases elicited in these testosterone levels may be considered as transient states, which are also trait-dependent in size, and which are superimposed on the baseline trait levels.

Sex Steroids in Animals and Humans: Sensitivity for Sexual Cues

The idea of the existence of individual differences in sensitivity of the brain for sexual cues is an important concept for our hypothesis that different causal mechanisms are responsible for HSDD, and that sublingual testosterone can increase this sensitivity with different effects in the differentiated subgroups [10].

In animal experiments it has been shown that sex hormones affect sexual behavior, through alterations in functions and activity of the brain [36]. In the development of animal models, scientists have focused on the relationship between neurophysiological brain mechanisms and several indices of sexual behavior. Based on these experiments, a steroid-responsive neural network has been postulated, a highly interconnected group of sex hormone receptor-containing neurons in the brain [36]. According to the authors this network is not a closed circuit, but serves reproductive aims by functioning as an integrating and activating center between external sensory cues, hormonal processes, and reproductive behavior. This is partly accomplished by selective filtering of sensory input and amplification of signals that may facilitate sexual behavior. In human sex research ethical and practical barriers exist for observing sexual behavior directly, and of assessing the underlying neurophysiological brain processes in a similar fashion as in animal research. Although models of human and animal sexuality are to a large extent incomparable, they might bear in common that the influence of sex hormones operate through a steroid-responsive network which can vary in sensitivity.

Two Paradigms in Testosterone Treatment and Sex Research

There are two main research paradigms used for investigating the effects of treatments with testosterone on sexual behavior: (i) chronic increases of testosterone levels through continuous administration; and (ii) short transient increases of testosterone through single-dose sublingual administration. We will give a short description of the differences between both approaches.

Chronic Testosterone Treatment and Sexual Behavior in Women

Chronic testosterone preparations affecting sexual functioning of women have been prescribed by physicians for surgically and naturally postmenopausal women for decades. Clinical trials documenting positive effects of androgens on sexual desire and sexual responsiveness indicated a significant positive effect of testosterone on women's sexual function [15,37–41].

It is well known that during the natural menopause, testosterone levels decrease very gradually. However, this is more likely a result of declining ovarian and adrenal function due to aging than a function of natural menopause. In contrast, women who undergo bilateral oophorectomy (surgical menopause) experience a more rapid dramatic decline in testosterone production with levels that are 50% lower than they are for natural menopause. Since aging and menopause have been linked to low libido or HSDD, the decline in androgen levels in women with a natural menopause or following oophorectomy is supportive for the hypothesis that decreased testosterone is related to reduced sexual desire and/or satisfaction.

In the 1980s, the first experiments were conducted to support this hypothesis. Sherwin et al. examined the effect of intramuscular testosterone on sexual function in 53 surgically postmenopausal women. The subjects were randomized to testosterone alone, estrogen alone, testosterone (T) combined with estrogen (E) or no treatment. Participants in the T + E group showed significant higher rates of sexual desire, arousal, and sexual fantasy compared with E alone or the untreated group [15]. Since then, others reported increased desire and other parameters of sexual well-being in postmenopausal women receiving oral methyltestosterone in combination with esterified estrogens [42],

and subcutaneous testosterone implants [43]. Considering the significant effect of testosterone therapy on sexual functioning in postmenopausal women, further studies explored the most efficacious route of administration for testosterone. Transdermal administration has the advantage of avoiding first-pass hepatic metabolism, of delivering consistent and reliable doses of testosterone, and it is less invasive than intramuscular administration. Shifren et al. conducted the first randomized, double blind, placebo-controlled trial of a transdermal testosterone patch in 75 women who had undergone surgical menopause. The participants were randomized to receive a 150 mg/day testosterone patch, 300 mg/day testosterone patch or placebo for 12 weeks. Only the highest dose group showed significant higher scores for frequency of sexual activity, sexual fantasies, masturbation, and orgasm [39]. Since then, several studies reported beneficial effects of the transdermal testosterone patch in surgically menopausal women [38,40] and in naturally menopausal women [44]. Also, others studies have demonstrated that administration of transdermal testosterone gel in surgical and natural menopausal women increases sexual desire and frequency of sex [45,46].

In premenopausal women only a few studies have been performed on chronic testosterone treatment for HSDD as compared with the large database of studies for postmenopausal women. Davis et al. conducted a study in 261 premenopausal women with decreased sexual satisfaction who received one of three different daily doses of transdermal testosterone spray (50 µg testosterone per mL: one 56-µL spray, one 90-µL spray, and two 90-µL sprays) or placebo for 16 weeks. Only the treatment group on the single 90-µL dose showed a significant increase in satisfactory sexual events as compared with placebo [47].

The above described studies show promising results for testosterone as treatment for women with HSDD. However, chronic testosterone treatment is accompanied with adverse events due to a longlasting higher androgen status. Transdermal testosterone preparations result in physiologic serum testosterone levels and the most common side effects reported are increased hair growth and acne [47,48]. When supraphysiological testosterone levels are reached, cardiovascular adverse events have been described [49]. Low-dose testosterone is safe in postmenopausal women with regard to endometrial

effects and breast cancer risk [50–53]. However, safety studies in premenopausal women are sparse, and long-term safety studies regarding exogenous testosterone preparations are necessary. At this moment there is no complete long-term safety profile for a testosterone-based product in premenopausal women and therefore the U.S. FDA has not yet approved any for treatment in women with HSDD.

Sublingual Single Dose Administration of Testosterone

An important aspect of sexual motivation is physiological sexual responding. An increase in vaginal vasocongestion (as measured by vaginal pulse amplitude [VPA]) elicited by sexual stimuli is considered to be preparatory for copulatory behavior [54]. Increases in VPA are consistently observed during exposure to erotic stimuli. In eight hypogonadotropic hypogonadal females we found that substitution with testosterone undecanoate 40 mg orally per day during an 8-week period enhanced the VPA during exposure to erotic visual stimuli [54]. This effect was not found in another group of eight women with panhypopituitarism (also hypogonadotropic hypogonadal) (unpublished data). In both studies subjects received testosterone each morning, but patients in the first experiment were tested during the afternoon and patients in the second experiment during the morning. The different outcomes on physiological responding between these experiments may be caused by the difference in patient population, but also by a delay in effect of testosterone on physiological sexual responding. In a third experiment, we examined if administration of a single dosage of sublingual testosterone (0.5 mg), as compared with a placebo, increases the VPA during exposure to visual erotic stimuli as compared with neutral stimuli [6]. On the 2 treatment days, eight sexually functional women viewed a neutral and erotic film clip 25 minutes before drug intake, and at five time points after drug intake (at $t = 0$, 90, 180, 270, and 360 minutes). The intake of testosterone caused a sharp increase in plasma levels of testosterone of short duration. About 3.5 hours after this testosterone peak, we found a striking increase in VPA and subjective sexual experiences (self-report ratings of sexual lust and genital sensations) when the subjects were exposed to the visual sexual stimuli. These findings indeed demonstrated a time lag in the effect of sublingually administered testosterone on genital arousal in sexually functional

women. A subsequent study was designed to investigate the influence of repeated measurements on the physiological and subjective sexual responses. The same experimental design was used as in the former study, but with only two measurement points: at *t* = -25 minutes and *t* = 270 minutes. The same results were obtained for the physiological sexual response in 10 sexually functional women. However, the subjective indices of sexual arousal showed no significant increase. Repeated exposure to sexual stimuli seemed to be an important condition for the subjective arousal to also increase under sublingual testosterone [7]. This delay in effect of testosterone on sexual arousal was replicated in 10 sexually functional postmenopausal women [8], and for other cognitive and affective functions [8,55–64].

It is not fully understood which exact mechanism is responsible for this delay in behavioral effect, it could be that testosterone exerts its effect via an increase in free levels of T, androgenic metabolites (e.g., 5α-dihydrotestosterone [DHT], androsterone glucoronide), via genomic or non-genomic mechanisms or a combination of these factors [65]. There is some debate as to the importance of testosterone aromatization to estradiol in determining testosterone effects in human sexual behavior [66–68]. However, the delay in effect of about 4 hours of sublingual testosterone on behavior cannot be explained by the process of aromatization of testosterone to estradiol. In animal experiments it is well documented that the time course of aromatization of testosterone to estradiol starts about 16 hours after the increase in testosterone and reaches its maximum level after about 48 hours. In these experiments it has been demonstrated that activation of copulatory behavior follows a similar time course, and occurs with a delay of 24–48 hours after the induced increase of testosterone [69,70]. So, it is not plausible that aromatization to estradiol accounts for the at least 20 replications of the 4-hour delay in effect of sublingual testosterone. Direct evidence that testosterone's effect on sexual motivation is not due to aromatization to estradiol comes from Davis et al. [71]. In this study, it was evaluated if the effects of transdermal testosterone in postmenopausal women is the direct effect of testosterone itself or indirectly influenced by the aromatization of testosterone by aromatase. Sixty postmenopausal women using transdermal estrogen therapy with low sexual satisfaction were treated with transdermal testosterone gel and

randomly assigned to receive treatment with the aromatase inhibitor letrozole, or placebo. It was demonstrated that treatment with testosterone, which induced an increase in the total and free testosterone levels, was associated with improved sexual satisfaction, mood, and well-being in these women. These increases were not due to aromatization to estradiol because testosterone administration with simultaneous treatment with an aromatase inhibitor did not have any effect on these outcomes. Further studies are necessary to investigate what then causes this delay in effect of testosterone.

During chronic administration of testosterone the behavioral effects occur often only after several weeks of treatment. This time-effect interval is in stark contrast to the time lag of about 3 to 4 hours induced by the sublingual dosage form. This difference in time on effects may be partially caused by the differences in the pharmacokinetic profile of both treatment forms.

Chronic vs. Sublingual Administration of Testosterone

Only few pharmacokinetic studies have been performed with sublingual testosterone administration, and mostly in hypogonadal men [72,73]. Besides the different influence of chronic and single-dose administration of testosterone on sexual behavior, pharmacokinetic differences exist between these administration methods. According to Salehian et al. [73], investigating the pharmacokinetic profiles of testosterone enanthate (intramuscular 200 mg) and two doses of sublingual testosterone (2.5 and 5.0 mg) in hypogonadal men, free and total levels of testosterone increased much faster in the sublingual group compared with the group receiving the testosterone enanthate. Only two pharmacokinetic studies of sublingual testosterone administration in women have been described [6,74]. These two studies demonstrated that after administration of 0.5 mg sublingual testosterone, peak concentrations of serum testosterone were reached at 15 minutes post dose, and that baseline levels were reached within 150 minutes. The time to reach maximum concentrations is consistent with that found in men [73].

It is widely accepted that the unbound fraction of testosterone is the most bioactive and therefore responsible for the effects on behavior. If free fraction

testosterone levels increase much later in the chronic administration method it is expected that pharmacodynamic effect, e.g., on sexual behavior, will also occur in such a time-dependent manner. This is consistent with several studies describing the effects of a testosterone patch on sexual behavior. Women who started with the testosterone patch treatment reported improvements in sexual functioning over placebo after 4–8 weeks [75]. This is in sharp contrast with the series of sublingual testosterone (0.5 mg) experiments, demonstrating a delay in effect of about 4 hours after the peak in circulating testosterone [6–9,55–64].

Free Fraction and Sex Hormone Binding Globulin (SHBG) Saturation Threshold Mechanism

As mentioned earlier, chronic administration of testosterone causes a slow increase of total and free testosterone levels, and therefore a slow increase of the free fraction, in contrast to the rapid increase of these parameters after sublingual administration. The free levels and free fraction of testosterone is partly dependent upon circulating SHBG levels. In the study of Salehian et al. [73], it was shown that free testosterone levels in the testosterone enanthate condition only increased when SHBG levels were suppressed after administration by day 7. Apparently, chronic treatment of testosterone induces a rise in free fraction levels, after the installment of a new equilibrium between testosterone and SHBG. Moreover, there is some evidence that a rise in free fraction levels following sublingual testosterone administration depends on circulating SHBG levels [74]. In the latter study it was demonstrated that the free fraction levels increased in a dose-dependent fashion (after 0.25 mg, 0.50 mg, and 0.75 mg of sublingual testosterone), but only in subjects with relatively low SHBG levels. In the group of women with high SHBG levels, no increase of free fraction levels was observed after sublingual testosterone administration. This observation is partly consistent with the SHBG saturation threshold hypothesis postulated by van der Made et al. [10]. According to this hypothesis, the free fraction of testosterone only increases after saturation of SHBG. However, van der Made et al. state that when testosterone levels are high enough to pass this saturation threshold, this will result in a short supraphysiological peak of free testosterone, and subsequently induce behavioral

effects after approximately 4 hours. This is not consistent with the results described by van Rooij et al. Besides the fact that free fraction levels only increased in women with low SHBG, which is in line with the saturation part of the hypothesis, increases in the free testosterone levels were also observed in the women with high SHBG. However, maximum concentrations of free testosterone in the women with low SHBG were significantly higher compared with the women with high SHBG. Apparently, rapid influx of testosterone does induce a peak in free and total testosterone irrespective of SHBG level. See also Figure 1.

It is unclear at this point, whether the increase of free testosterone levels or the free fraction is responsible for the induced behavioral effects of the sublingual testosterone dosage form. Future studies specifically designed for this purpose are needed to clarify the influence of free testosterone and free fraction levels on behavior.

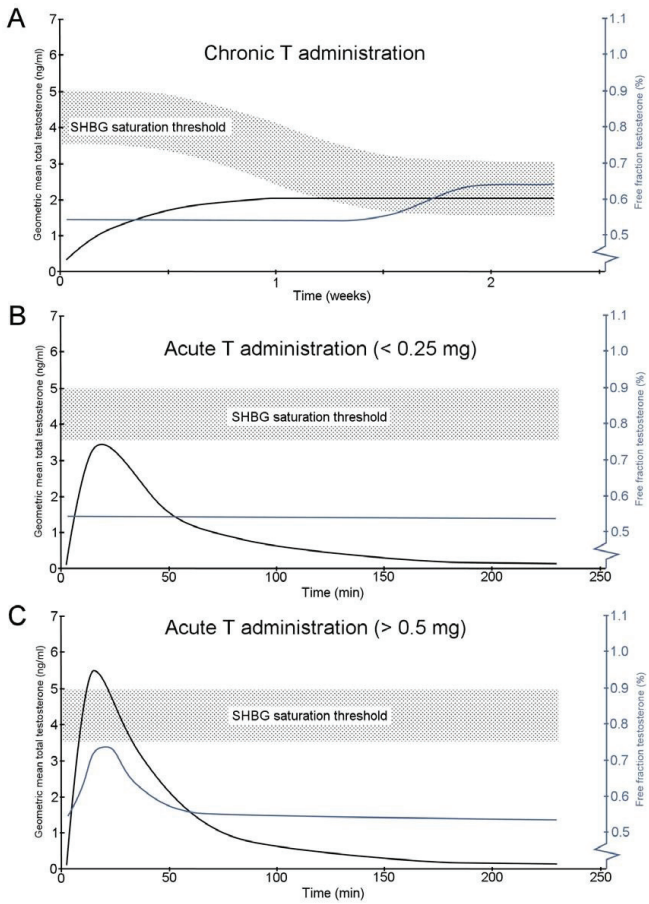

Figure 1. Free fraction testosterone and the Sex Hormone Binding Globulin (SHBG) saturation hypothesis. (A) A slow increase in testosterone levels following chronic administration. This decreases SHBG after several days—weeks, and thereby also the SHBG saturation threshold. Once this threshold is lowered, the free fraction increases and behavioral effects become manifest. (B) A fast transient increase in testosterone levels following acute sublingual administration of doses too low to reach SHBG saturation, thus not influencing the free fraction. (C) A fast transient increase in testosterone levels following acute sublingual administration of doses that are high enough to reach SHBG saturation, thereby also transiently increasing the free fraction. SHBG saturation threshold is depicted in bandwidth to indicate that this threshold is depends on a person's endogenous SHBG level. Testosterone levels are shown as baseline corrected levels. (A) A hypothetical figure adapted from several studies reporting pharmacokinetic and efficacy measures of chronic testosterone administration (see text). (B) and (C) Adapted from van Rooij et al. [74].

Automatic Responses and Cognitive Factors in Sexual Functioning of Women

Models of human sexuality are mainly based on subjective reports, sometimes combined with manifestations of physiological sexual arousal. An increase in vaginal vasocongestion induced by sexual stimuli results from activation of neural pathways involved in reward-seeking and associated areas. Visual exposure to sexual intercourse between members of the species of the onlooker is a potent releasing stimulus for such a preparatory motivational response. Both men and women have a marked capacity to respond to erotic films with a genital response, irrespective of whether they found such exposure pleasant or not [76,77]. Discordance between genital responses and subjective sexual functioning, as monitored in the laboratory, has been frequently reported [54,78]. As a result, an increase in vaginal responding has been considered as an automatic preparatory physiological response for copulatory behavior [54].

As stated above, the capability of sexual cues to evoke an increase or decrease in the sexual motivational brain activity depends on individual biological and psychological characteristics and social circumstances, such as the sensitivity of the steroid receptor system (e.g., polymorphism of the androgen receptor [79]), prenatal testosterone exposure influencing organization of the sexual brain (measured by the second to fourth digit ratio [80]), levels of sex steroids, and environmental factors, e.g., the proximity of an attractive mate. Moreover, the transition of preparatory motivational brain activity to the execution of copulatory behavior is, at least in humans, mediated by higher cortical processes (which can feedback on the motivational systems involved in reward-seeking). Aside from the induction of a preparatory physiological response during conscious perception of erotic stimuli, it has been shown that subliminally presented erotic stimuli can activate the limbic reward circuitry involved in sexual motivation [81]. Thus, even erotic cues which do not enter conscious awareness induce alterations in (sexual) reward- related motivational brain areas. A sexual cue belongs to the domain of stimuli—e.g., an odor, a thought, a memory, a word, a sound, a picture, flirtatious body language, or someone's physical appearance, etc.—which can elicit sexual responses in the brain or body. Individual differences in sexual-cue sensitivity might be reflected in differences in responses induced by exposure to subliminal sexual cues. One

method to investigate this is by the measurement of preconscious attentional bias for sexual cues, using an emotional Stroop task. The emotional Stroop task measures attentional bias for emotional cues [9,10]. In this task, subjects are instructed to name the color in which emotional and neutral stimuli are printed as quickly as possible, while ignoring the meaning of the word. The motivational state of the subject and the emotional content of the stimuli determine the performance on this task, in terms of slowing down or speeding up in color naming. Attentional bias for emotional cues is demonstrated when color naming latencies for emotional stimuli are greater or smaller than color-naming latencies for neutral stimuli. It has been assumed that attention is automatically allocated to the emotional value of the stimuli. In the context of sexual function, with another cognitive task it has been demonstrated that low levels of attention for sexual stimuli is associated with low sexual desire [82].

A masked version of this task turned out to be a more reliable measurement of (preconscious) attentional bias for emotional cues [9,10]. Thus, deceleration in color-naming of masks, preceded by sexual words and as compared with neutral words, indicates increased resource allocation to the processing of sexual stimuli, while acceleration indicates relatively decreased resource allocation to the processing of sexual stimuli as compared with neutral ones. In the masked version of this task measuring preconscious attentional bias for sexual cues words with erotic content ("orgasm") and words without erotic content ("chair") are presented for 26 milliseconds on a computer screen in different print colors. Directly after presentation, the word is masked (i.e., covered) by a scrambled letters in the same print color. This method of presentation ensures that the target word does not enter the subject's conscious awareness. The subject is instructed to name the print color of the word mask as fast as possible. Thus, deceleration in color naming of masks which were preceded by erotic words (as compared with neutral words) indicates increased preconscious attentional resource allocation to the processing of sexual cues. In other words, subconscious attentive processing capacity is automatically allocated to the emotional value of the cues [83]. Differences in this preconscious attentional bias between subjects then indicate differences in sexual cue sensitivity, and thus differences in the strength with which these subjects respond sexually to sexual stimuli as the examples mentioned above. The sexual response can be

anything ranging from facilitation or activation of brain motivational systems to full blown sexual arousal and desire, depending on the sensitivity of the brain for sex and on stimulus intensity.

Sexual behavior in humans depends on a range of interacting biological and psychological regulatory mechanisms, which are influenced by situational cues. The absolute and relative degree of activation of these mechanisms varies within and between subjects. Psychological mechanisms (including conscious and subconscious processes) are partly established by present and previous beneficial and/or adverse experiences, and significantly influence the extent to which an individual is willing to engage in sexual activities and the extent to which those activities are enjoyable. Therefore, the delicate and relative balance between activation of neurobiological and psychological mechanisms governing sexual excitation and sexual inhibition, controls sexual activities in humans, including fantasizing, masturbation, and sexual intercourse.

Sublingual Testosterone and Treatment of Female Sexual Dysfunction

Single administration of sublingual testosterone (0.5 mg) can induce an increase in physiological and subjective indices of sexual responding in sexually functional women [6,7]. This might indicate this dosage form can be part of a potential pharmacotherapy for HSDD.

In a randomized, placebo-controlled, double- blind, cross-over study, we investigated the efficacy of sublingual testosterone (0.5 mg), the PDE5 inhibitor vardenafil and the combination of these drugs on preconscious attentional bias for sexual cues and physiological sexual function in women diagnosed as having HSDD [9]. We assume that the delay in effect of testosterone on physiological sexual arousal in sexually functional women occurred as the result of an increase in activation of central sexual motivational mechanisms. Central sexual stimulation is necessary for a PDE5 inhibitor to induce an increase in the amount of blood in erectile tissue of the genitals. This occurs in the following way: In genital erectile tissue of both men and women, sexual stimulation will induce the release of nitric oxide (NO) from nerves and endothelium. NO induces an increase in production of cyclic guanosine monophosphate (cGMP).

cGMP is a key mechanism in relaxing smooth muscle necessary for the induction of enlargement of the erectile tissue. cGMP is hydrolyzed by the phosphodiesterases in the corpora cavernosa, in which PDE5 is the most abundant PDE. Therefore, during sexual stimulation, the action of NO/cGMP on erectile function will be enhanced by PDE5 inhibitors [84]. Thus, without adequate central stimulation, i.e., activation of central sexual motivational mechanisms, a PDE5 inhibitor cannot be effective, which is likely why the trials investigating PDE5 inhibitor efficacy in FSD failed. Indeed, the PDE5 inhibitor alone had no effect on physiological sexual functioning of our patients, but neither did testosterone alone. In contrast, the combined use of sublingual testosterone and the PDE5 inhibitor produced an increase in physiological sexual responding about 4 hours after the intake of the testosterone. Apparently, in addition to testosterone-mediated facilitation of central sexual stimulation, peripheral facilitation of the physiological sexual response was needed. Interestingly, we found a striking difference in effect between women who had and women who had not reported the experience of childhood sexual abuse (CSA). In women without CSA, testosterone treatment induced an increase in their originally low levels of preconscious attentional bias for sexual cues, while women with CSA showed a decrease in their originally high levels of attention. The effects of the combination of testosterone and the PDE5 inhibitor on the physiological sexual response also differed between these groups. Women without CSA revealed an increase in their physiological response, while the women with CSA showed no alterations in this response.

These results were reproduced in a second randomized, double-blind, crossover, placebo-controlled study [10]. This study was designed to investigate the effects of testosterone, a PDE5 inhibitor, and the combination of both drugs on alterations in preconscious attentional bias for sexual cues in 28 women suffering from HSDD. Moreover, we investigated the influence of these drugs on physiological and subjective indices of sexual function during neutral and erotic visual stimulation. In this study women who reported experience of CSA were excluded. Based on their initial preconscious attentional bias for sexual cues scores, we construed two groups: women with a high and women with a low sensitive brain system for sexual cues. Testosterone treatment produced in the low sensitive group an increase in their attentional bias for sexual cues, while

in the high sensitive group a reversed pattern was found. We examined in both groups the effects of the different treatments on physiological and subjective sexual responding. Again, neither testosterone (0.5 mg) nor the PDE5 inhibitor (vardenafil, 10 mg) alone had an effect on measures of physiological and subjective arousal. The combined administration of testosterone and vardenafil in the low sensitive group produced 4 hours post dose a significant increase in physiological and subjective sexual functioning, while in the latter group no drug induced alterations in these measures were observed. This study had no subjects with a history of CSA, but the latter group did have a higher prevalence of negative sexual experiences (63% vs. 17%), which implies that this group's past negative sexual experiences may have contributed to the development of HSDD as a result of activation of central inhibitory mechanisms. Vardenafil was used as the PDE5 inhibitor in these studies, but other PDE5 inhibitors such as sildenafil can also be used (see Poels et al. this issue) as the mechanism of action is the same.

Testosterone and Sensitivity of the Brain for Socially—Including Sexually—Relevant Cues

As described above, female sexual behavior in many mammalian species depends on female sex steroids. In human females, testosterone appears to be highly involved in sexual motivation and behavior. Consequently, testosterone plays a central role in the steroid-responsive neural network involved in the regulation of social and sexual behavior from both men and women, and might exert its influence on these behaviors by selective filtering and amplification of cues important for behavioral responses. In other words, testosterone influences the sensitivity of the brain for cues (status, challenge, sex) relevant for an individual's interest. The above described relationships between preconscious attentional biases, testosterone treatment, and sexual responsiveness can be conceptualized in terms of testosterone-induced alterations in sensitivity of the brain for sexual cues [9,10]. The low preconscious attention scores before treatment with testosterone reflected a relatively insensitive brain system for sexual cues in women without CSA. Sublingual testosterone increased this sensitivity about 4 hours after dosing, reflected in an increase in subconscious attention for sexual cues. It might be assumed that an increase in the brain's

sensitivity for sexual cues is accompanied by an increase in central sexual stimulation, making it possible for a PDE5 inhibitor to produce an increase in physiological sexual responding. On the other side, the absence of an effect on physiological sexual response in the women with negative sexual experiences (including a history of CSA) can also be interpreted as the result of an increase in sensitivity of the brain for sexual cues. Because of the negative association with sex in these women, this increased sensitivity resulted in an automatic activation of a sexual inhibitory mechanism [9,10]. Thus, testosterone increases sensitivity of the brain for socially relevant cues, which might produce different behavioral outcomes depending on other involved characteristics of an individual.

Testosterone and Sexual Inhibition

Janssen and Bancroft [85] conceptualized that individual differences in sexual responding depend on a delicate interplay of the earlier mentioned excitatory and inhibitory processes. It is widely accepted that the PFC is involved in the inhibitory control of human behavior [86], including sexual behavior [87,88]. A sexual event which is consciously or subconsciously negatively valenced or which induces inappropriate responses in, for that response, inappropriate situation, can be expected to induce a phasic increase in PFC-mediated sexual inhibition. Amplifying negative valence or response sensitivity in an inappropriate setting would be expected to increase inhibition even further. Indeed, in two studies (unpublished data) we found support for this hypothesis, in that testosterone can actually induce an inhibitory sexual response.

In the first experiment we examined whether treatment with testosterone would cause an increase in neural activity in brain areas associated with sexual behavior. In a randomized, placebo-controlled, double-blind, crossover design, an fMRI-VPA study was conducted in 12 sexually functional eugonadal female volunteers. Four hours after the intake of the placebo or 0.5 mg of testosterone sublingually, the blood-oxygen-level-dependent (BOLD) MRI response was measured while subjects viewed neutral and erotic film excerpts. Immediately after the fMRI session, VPA in response to another comparable set of neutral and erotic film excerpts was measured. As expected, we found comparable BOLD

responses to erotic stimuli in the placebo condition, as in other imaging studies [89,90]: the amygdala and temporal pole, hippocampus, hypothalamus, brain stem, orbitofrontal cortex, anterior cingulate cortex, and decreased BOLD response of dorsal prefrontal areas (Figure 2, top row). We expected this pattern to become more pronounced following sublingual testosterone administration, followed by a concomitant increase in VPA relative to placebo.

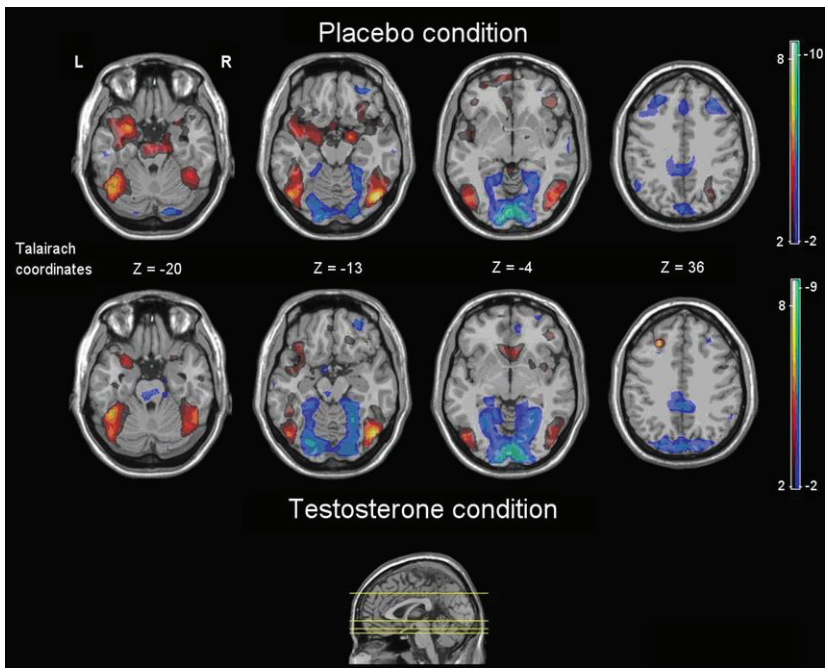

Figure 2. Blood oxygen level dependent responses to erotic stimuli under placebo and testosterone. Transverse images of the brain. Z-coordinates in Talairach space. (Source: unpublished results)

In the testosterone condition, however, women showed decreased BOLD responses in all brain structures implicated in the normal sexual response (Figure 2, bottom row). Additionally, there was an increased BOLD response in the superior part of the middle frontal gyrus, a portion of the dorsolateral PFC (DLPFC), which mediates inhibition of task-irrelevant responses [91–95] and

willful inhibition of the sexual response [87]. Also, there was increased BOLD response of the septal nuclei, which have been postulated to play a constrictive role on subcortical structures, reducing emotional and arousal extremes, thus preventing emotional overshoot [96]. Moreover, and contrary to our former findings [6,7], the VPA showed a relatively blunted response in the testosterone condition as compared with placebo (see Figure 3), as compared with our former studies on the delay effect of testosterone. We hypothesized that exposure of our subjects to the experimental conditions—i.e., a noisy dark narrow cylinder with male technicians and scientists all around—seemed to have demanded induction of an inhibitory mechanism in order to dampen the inappropriate effects of the testosterone-induced increase in sensitivity of the sexual response system.

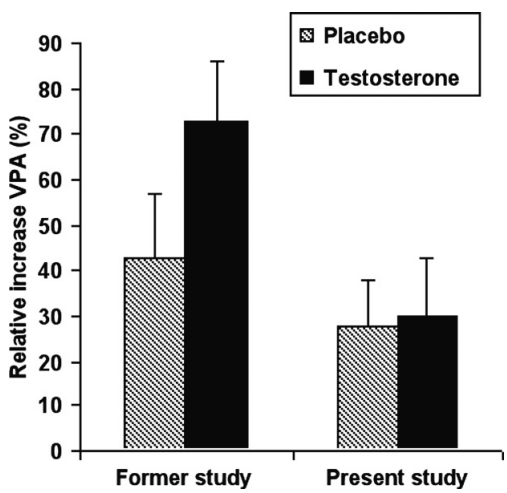

Figure 3. Relative changes in vaginal pulse amplitude (VPA). Comparison between a former [6] and the present experiment for the average relative increases in VPA induced by an erotic film fragment viewed about 4 hours after placebo and testosterone (0.5 mg) intake. A univariate analysis revealed a significant interaction effect [$F(1, 17) = 6.49$; $P < 0.025$] for Drug (Placebo vs. Testosterone) X Study (Former vs. Present), indicating a relatively blunted VPA response in the testosterone condition for the fMRI-VPA experiment as compared with the former VPA-alone experiment. (Source: unpublished results)

The amount of attentional resources directed at sexual stimuli greatly determines the strength of the physiological and subjective sexual response. Focusing on sexual stimuli facilitates the sexual response, while withdrawing attention attenuates it. In subjects with sexual problems, however, the opposite relationship has been observed [97]. Apparently, focusing attention on sexual stimuli can induce inhibition under certain circumstances. Varying attentional resource allocation in the sexually inappropriate MRI measurement setting should then be able to amplify and/or reduce (testosterone-induced) situation-dependent inhibitory BOLD responses.

In a second pilot experiment [98], the hypothesis of testosterone-induced sexual inhibition was further investigated while varying the level of attention for sexual stimuli, in a group of women with HSDD (N=14). In this randomized, double-blind, placebo-controlled, crossover fMRI study, the influence of varying levels of attention (Stroop task superimposed on an erotic film clip, erotic film clip without additional instruction, an erotic film clip and the instruction to monitor bodily sexual response preceded by a neutral film clip, and an erotic film and the instruction to monitor bodily sexual response preceded by an erotic film clip with Stroop task superimposed) for sexual stimuli on BOLD MRI response to erotic vs. neutral film clips were investigated in three treatment conditions: placebo, testosterone (0.5 mg), and the combined administration of testosterone (0.5 mg) and sildenafil (50 mg).

In the placebo condition where subjects viewed erotic film clips without additional instruction, we observed activation patterns comparable to former imaging studies on sexual arousal [87,89,90], but with three noteworthy differences: subjects did not show increased amygdala (stimulus salience perception/attribution) and right frontal insula (interoception) BOLD response, but they did show increased BOLD response in the left DLPFC (Figure 4) indicating that subjects inhibited their sexual (amygdala and insula) response. In the condition when subjects watched an erotic film clip while monitoring their bodily sexual response preceded by a Stroop-superimposed erotic film clip as compared with the condition where a neutral film clip preceded the erotic film clip left DLPFC BOLD response decreased and right insula BOLD response increased under placebo and even more so under testosterone (Figure 5).

However, the combination of testosterone plus sildenafil reversed this pattern; adding the sildenafil apparently increased the sexual response to inappropriate levels, thereby necessitating inhibition.

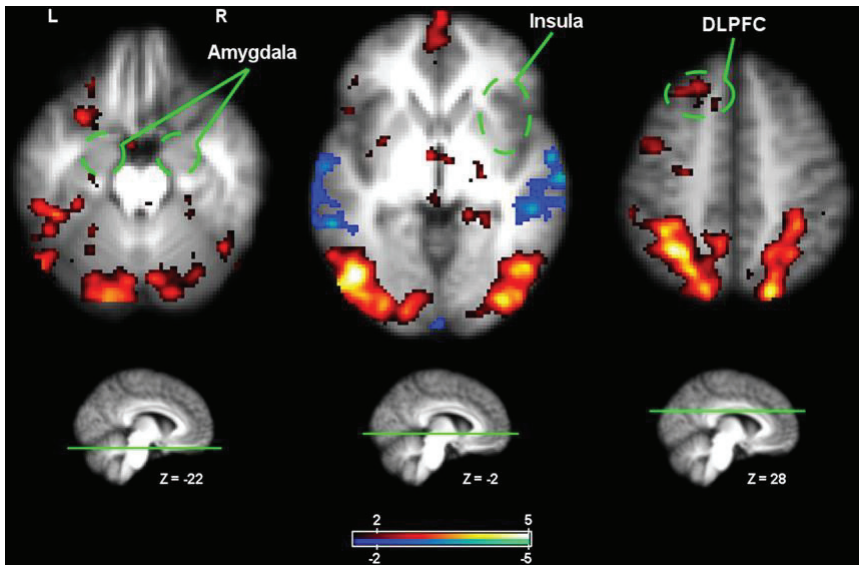

Figure 4. Blood oxygen level dependent responses to passive viewing of erotic minus neutral film clips under placebo. Transverse images of the brain. Z-coordinates in Montreal Neurological Institute space. Source: unpublished results. Presented at the Congress of the International Society for the Study of Women's Sexual Health, 2007 [98]. DLPFC = dorsolateral prefrontal cortex.

In many situations, it is inappropriate to act on a sexual cue, for example, because of social conventions (i.e., there is a threat of consequences that acting on such a cue in the wrong circumstance implies). Under such circumstances, sexual cues can still elicit preparatory sexual responses, but these then have to be inhibited [99]. The need for, or strength of this inhibitory response is dependent on the elicited sexual response. Thus, if a sexual cue is relatively weak (due to the nature of the cue, or to the neurobiological disposition of the individual), no inhibition may be needed. However, if the processing of a relatively weak sexual cue is enhanced through testosterone administration,

inhibition may be needed. Therefore, the net effect of testosterone administration could be an inhibitory response.

In conclusion, depending on circumstances, testosterone can produce effects that deviate from the expectations concerning the functional (i.e., facilitation) role of testosterone in the regulation of sexual behavior.

The Influence of Testosterone and Serotonin on Sexual Inhibition

Given that sublingual testosterone increases the sensitivity of the brain to sexual cues, women prone to sexual inhibition are expected to exhibit stronger inhibitory activity in the PFC during exposure to a sexual stimulus following testosterone administration. An important mediator of inhibitory mechanisms in the brain is the neurotransmitter 5-hydroxytryptamine (5-HT, serotonin) [100], that also exerts abundant inhibitory effects via the PFC [101]. After acute treatment with a 5-HT_{1A} receptor agonist, the agonist binds to somatodendritic autoreceptors of the raphe nuclei in the midbrain. The hyperpolarizing effect of activated 5-HT_{1A} auto receptors decreases serotonergic firing activity [102] and inhibition of serotonin release from the presynaptic terminal [103], and subsequently, reduced extracellular serotonin levels in the PFC [104]. There are differences in effects on serotonergic activity between acute and prolonged treatment with a 5-HT_{1A} receptor agonist, resulting in opposed effects on behavior mediated by 5-HT_{1A}. For example, acute buspirone causes an increase in impulsivity, which effect is reversed following chronic treatment [104]. Accordingly, acute treatment with a 5-HT_{1A} receptor agonist might decrease sexual stimuli induced phasic serotonergic inhibitory control in the PFC, which in turn might prevent or reduce the inhibitory response to sexual cues in women with HSDD as the result of activation of sexual inhibitory mechanisms. Consequently, when exposed to sexual stimuli (whether internally or externally induced), women prone to sexual inhibition might show an increased sexual response when treated with sublingual testosterone and a 5-HT_{1A} receptor agonist.

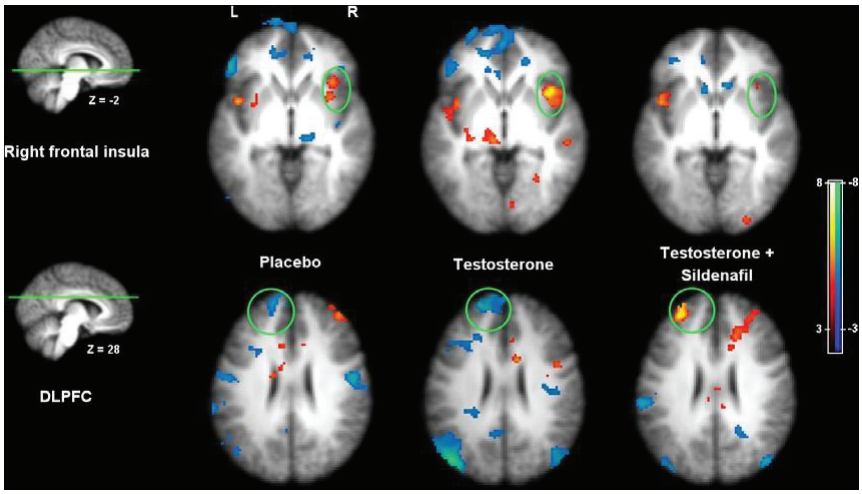

Figure 5. Blood oxygen level dependent responses to attentional engagement under conditions of increased sexual stimulation. BOLD responses to attentional engagement under conditions of increased sexual stimulation, under placebo, testosterone, and the combination of testosterone and sildenafil. Transverse images of the brain. Z-coordinates in Montreal Neurological Institute space. Source: unpublished results. Presented at the Congress of the International Society for the Study of Women's Sexual Health, 2007 [98]. DLPFC = dorsolateral prefrontal cortex

Two studies above [9,10] describe the use of the emotional Stroop task to differentiate between high and low sensitive women. The high sensitive women did not show an increase, or showed decreases in physiological and subjective sexual responding following the combined administration of testosterone and vardenafil. These women also showed a higher incidence of negative sexual experiences, which led to the hypothesis that these women suffered from HSDD as a result of (over)activation of sexual inhibitory systems. Women who have negative sexual experiences are about twice as likely to develop a sexual dysfunction, but it is not a given [105]. A potential risk factor for developing a sexual dysfunction after negative sexual experiences is, according to our hypothesis, a person's sensitivity to sexual cues, which will affect positive and negative sexual experiences. Irrespective of the absolute levels of testosterone, different levels of sensitivity for sexual cues might occur, for example, as the result of a difference in the sensitivity of the receptor system for androgens (i.e.,

different length of CAG repeats [79] and/or different levels in androgenic intracellular activity [106]). Other systems might interfere with functioning of the androgen systems in the brain; for example, differences in polymorphisms of the serotonin transporter gene, and the serotonin$_{1A}$ receptor gene have been linked to a higher likelihood of developing affective disorders [107,108] due to the altered processing of emotional stimuli (i.e., more reactive to negative stimuli) [107]. High sensitive women are more sensitive for positive and negative sexual experiences. It is possible that the high sensitive women can develop HSDD (partly) because of altered serotonergic transmission, making them more sensitive to negative sexual experiences leading to subsequent overactivation of sexual inhibitory mechanisms in response to sexual arousal. This is in contrast to the low sensitive women, who have a higher chance to develop HSDD because their insensitivity causes a lack of adequate activation of sexual excitatory mechanisms (see Figure 6). In the near future we will report on the results of our research program into these relationships between the above mentioned brain variables/mechanisms and the vulnerability to develop HSDD.

CONCLUSIONS

Summarizing, we formulated the hypothesis that different causal mechanism are responsible for HSDD. Based on this hypothesis, we designed and developed two new medicines for HSDD. Sublingual testosterone combined with a PDE5 inhibitor has been developed for women who suffer from low sexual motivation and low sexual desire (HSDD), as the result of a relatively insensitive system for sexual cues. This combination should increase the sensitivity for internal and external sexual cues, activates central sexual motivation mechanisms and subsequently the physiological sexual response. The combination of sublingual testosterone and a 5-HT$_{1A}$ receptor agonist was designed to treat HSDD induced by dysfunctional sexual inhibition mechanisms. This combination should also increase sexual motivation, but inhibits overactive sexual inhibition mechanisms in the prefrontal areas.

To investigate whether the testosterone combined with the PDE5 inhibitor was also efficacious at home in women who suffer from HSDD as the result of an insensitive system for sex, and to test the hypothesis that testosterone plus a

5-HT_{1A} receptor agonist are efficacious in women with HSDD who are prone to sexual inhibition, we conducted a randomized, double-blind, placebo-controlled crossover study in 56 women with HSDD with or without Female Sexual Arousal Disorder. In an ambulatory experiment at home (see also [109]), preconscious attentional bias for sexual cues, physiological and subjective indices of sexual functioning were measured three times during the first week of each treatment. In a subsequent bedroom experiment during a 3-week period under condition of each treatment, we evaluated sexual satisfaction following each sexual event. The results of the efficacy of sublingual testosterone combined with a PDE5 inhibitor in women with HSDD as the result of an insensitive system for sexual cues are described in part 2 of this series. In part 3 we will describe the results of treatment with sublingual testosterone combined with a 5-HT_{1A} receptor agonist in women with HSDD as the result of activation of sexual inhibitory mechanisms.

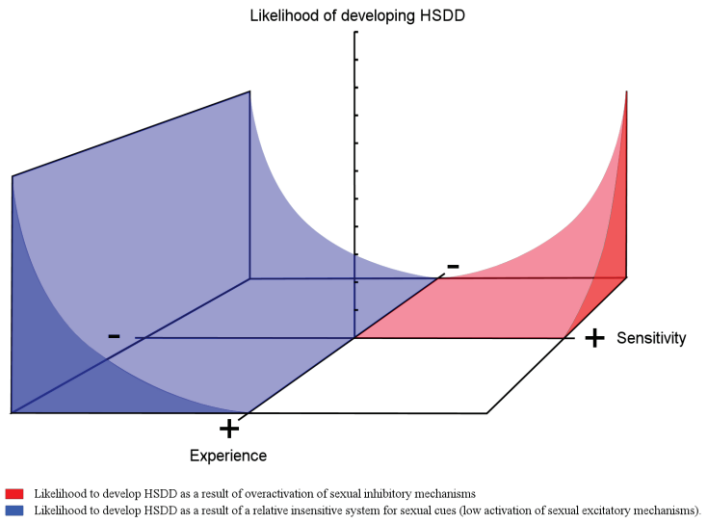

Figure 6. Representation of our hypothetical sexual (low– high) sensitivity-inhibition model. According to the model, HSDD is correlated with low sensitivity to sexual cues (blue area), or by overactivation of sexual inhibitory mechanisms (red area). Subjects with high sensitivity for sexual cues will be more sensitive for positive sexual stimuli and experiences, which can lead to a hedonic sex life. However, high sensitivity subjects are also more vulnerable to negative sexual experiences, and as a result more susceptible for learning a negative association with sex. Exposure to stimuli that make an appeal to the sexual motivational system, can then automatically elicit an inhibitory response (e.g., a phasic increase in serotonin activity in the dorsolateral prefrontal cortex [PFC]) to diminish conscious or subconscious negative affective state induced by the undesired sexual motivational state. The strength of the inhibitory response might be a function of the sensitivity of the sexual brain system, and the duration and severity of negative sexual experiences. Low sensitivity for sexual cues might be the result of a low sensitive androgenic receptor system in the brain, and/or a low level of intracellular androgenic activity, and/or tonic high serotonergic activity in particular areas in the PFC (which may function as a filter for emotional positive and negative stimuli). Subjects with a low sensitive system will have decreased levels of activation of sexual excitatory mechanisms, resulting in low sexual desire. This state of low sensitivity can be interpreted as a biological trait, and is unlikely to be caused by sexual experiences. Different combinations of various levels of sensitivity and inhibition are possible, but high inhibition is more likely to occur in sexually high sensitive subjects (resulting in HSDD). Subjects with HSDD and a low sensitive sexual brain are less likely to suffer from high inhibition because they already have low propensity to respond to sexual stimuli; they have less need to inhibit their sexual response.

REFERENCES

1. Fugl-Meyer K, Fugl-Meyer AR. Sexual disabilities are not singularities. Int J Impot Res 2002;14:487–93.

2. Laumann EO, Paik A, Rosen RC. Sexual dysfunction in the united states: Prevalence and predictors. JAMA 1999;281:537–44.

3. Shifren JL, Monz BU, Russo PA, Segreti A, Johannes CB. Sexual problems and distress in united states women: Prevalence and correlates. Obstet Gynecol 2008;112:970–8.

4. Davison SL, Bell RJ, LaChina M, Holden SL, Davis SR. The relationship between self-reported sexual satisfaction and general well-being in women. J Sex Med 2009;6:2690–7.

5. American Psychiatric Association. Diagnostic and statistical manual of mental disorders. 4th edition. Text Revision. Washington, DC: American Psychiatric Association; 2000.

6. Tuiten A, Van Honk J, Koppeschaar H, Bernaards C, Thijssen J, Verbaten R. Time course of effects of testosterone administration on sexual arousal in women. Arch Gen Psychiatry 2000;57:149–53; discussion 155–6.

7. Tuiten A, van Honk J, Verbaten R, Laan E, Everaerd W, Stam H. Can sublingual testosterone increase subjective and physiological measures of laboratory-induced sexual arousal? Arch Gen Psychiatry 2002;59:465–6.

8. Eisenegger C, Naef M, Snozzi R, Heinrichs M, Fehr E. Prejudice and truth about the effect of testosterone on human bargaining behaviour. Nature 2010;463:356–9.

9. van der Made F, Bloemers J, van Ham D, El Yassem W, Kleiverda G, Everaerd W, Olivier B, Tuiten A. Childhood sexual abuse, selective attention for sexual cues and the effects of testosterone with or without vardenafil on physiological sexual arousal in women with sexual dysfunction: A pilot study. J Sex Med 2009;6:429–39.

10. van der Made F, Bloemers J, Yassem WE, Kleiverda G, Everaerd W, van Ham D, Olivier B, Koppeschaar H, Tuiten A. The influence of testosterone combined with a PDE5-inhibitor on cognitive, affective, and physiological sexual functioning in women suffering from sexual dysfunction. J Sex Med 2009;6:777–90.

11. Pfaff D, Schwartz-Giblin S, McCarthy M, Kow L. Cellular and molecular mechanisms of female reproductive behaviors. New York: Raven Press; 1994.

12. McCarthy MM, Albrecht ED. Steroid regulation of sexual behavior. Trends Endocrinol Metab 1996;7:324–7.

13. Freeman LM, Rissman EF. Neural aromatization and the control of sexual behavior. Trends Endocrinol Metab 1996;7:334–8.

14. Waxenberg SE, Drellich MG, Sutherland AM. The role of hormones in human behavior. I. Changes in female sexuality after adrenalectomy. J Clin Endocrinol Metab 1959;19:193–202.

15. Sherwin BB, Gelfand MM, Brender W. Androgen enhances sexual motivation in females: A prospective, crossover study of sex steroid administration in the surgical menopause. Psychosom Med 1985;47:339–51.

16. Dabbs J, Dabbs M. Heroes, rogues, and lovers: Testosterone and behavior. New York: McGraw-Hill; 2000.

17. Liening SH, Josephs RA. It is not just about testosterone: Physiological mediators and moderators of testosterones behavioral effects. Soc Personal Psychol Compass 2010;4:982–94.

18. Mazur A, Booth A. Testosterone and dominance in men. Behav Brain Sci 1998;21:353–63; discussion 363–97.

19. Geary D. Competing for mates. In: Geary DC, ed. Male, Female: The Evolution of Human Sex Differences. 2nd Ed. Washington, DC: American Psychological Association; 2010:213–45.

20. Schmitt DP, Buss DM. Strategic self-promotion and competitor derogation: Sex and context effects on the perceived effectiveness of mate attraction tactics. J Pers Soc Psychol 1996;70:1185–204.

21. Slatcher RB, Mehta PH, Josephs RA. Testosterone and self-reported dominance interact to influence human mating behavior. Soc Psychol Personal Sci 2011;2:531–9.

22. Wingfield JC, Hegner RE, Dufty AM Jr, Ball GF. The “challenge hypothesis”: Theoretical implications for patterns of testosterone secretion, mating systems, and breeding strategies. Am Nat 1990;136:829–46.

23. Beehner JC, Phillips-Conroy JE, Whitten PL. Female testosterone, dominance rank, and aggression in an ethiopian population of hybrid baboons. Am J Primatol 2005;67:101–19.

24. Hermans EJ, Bos PA, Ossewaarde L, Ramsey NF, Fernandez G, van Honk J. Effects of exogenous testosterone on the ventral striatal BOLD response during reward anticipation in healthy women. Neuroimage 2010;52:277–83.

25. Rose RM, Berstein IS, Gordon TP. Consequences of social conflict on plasma testosterone levels in rhesus monkeys. Psychosom Med 1975;37:50–61.

26. Rose RM, Gordon TP, Bernstein IS. Plasma testosterone levels in the male rhesus: Influences of sexual and social stimuli. Science 1972;178:643–5.

27. Kreuz LE, Rose RM. Assessment of aggressive behavior and plasma testosterone in a young criminal population. Psychosom Med 1972;34:321–32.

28. Thompson WM, Dabbs JM, Frady RL. Changes in saliva testosterone levels during a 90-day shock incarceration program. Crim Justice Behav 1990;17:246–52.

29. Roney JR, Mahler SV, Maestripieri D. Behavioral and hormonal responses of men to brief interactions with women. Evol Hum Behav 2003;24:365–75.

30. Hellhammer DH, Hubert W, Schürmeyer T. Changes in saliva testosterone after psychological stimulation in men. Psychoneuroendocrinology 1985;10:77–81.

31. van Anders SM, Hamilton LD, Schmidt N, Watson NV. Associations between testosterone secretion and sexual activity in women. Horm Behav 2007;51:477–82.

32. Hamilton LD, Meston CM. The effects of partner togetherness on salivary testosterone in women in long distance relationships. Horm Behav 2010;57:198–202.

33. Goldey KL, van Anders SM. Sexual thoughts: Links to testosterone and cortisol in men. Arch Sex Behav 2011. doi: 10.1007/s10508-011-9858-6.

34. Granger DA, Shirtcliff EA, Booth A, Kivlighan KT, Schwartz EB. The “trouble” with salivary testosterone. Psychoneuroendocrinology 2004;29:1229–40.

35. Liening SH, Stanton SJ, Saini EK, Schultheiss OC. Salivary testosterone, cortisol, and progesterone: Two-week stability, interhormone correlations, and effects of time of day, menstrual cycle, and oral contraceptive use on steroid hormone levels. Physiol Behav 2010;99:8–16.

36. Cottingham SL, Pfaff D. Interconnectedness of steroid-binding hormones: Existence and implications. In: Ganten D, Pfaff DW, eds. Current topics in neuroendocrinology. Vol. 7. Berlin: Springer; 1986:223–40.

37. Sherwin BB, Gelfand MM. The role of androgen in the maintenance of sexual functioning in oophorectomized women. Psychosom Med 1987;49:397–409.

38. Simon J, Braunstein G, Nachtigall L, Utian W, Katz M, Miller S, Waldbaum A, Bouchard C, Derzko C, Buch A, Rodenberg C, Lucas J, Davis S. Testosterone patch increases sexual activity and desire in surgically menopausal women with hypoactive sexual desire disorder. J Clin Endocrinol Metab 2005;90:5226–33.

39. Shifren JL, Braunstein GD, Simon JA, Casson PR, Buster JE, Redmond GP, Burki RE, Ginsburg ES, Rosen RC, Leiblum SR, Caramelli KE, Mazer NA, Jones KP, Daugherty CA. Transdermal testosterone treatment in women with impaired sexual function after oophorectomy. N Engl J Med 2000;343:682–8.

40. Braunstein GD, Sundwall DA, Katz M, Shifren JL, Buster JE, Simon JA, Bachman G, Aguirre OA, Lucas JD, Rodenberg C, Buch A, Watts NB. Safety and efficacy of a testosterone patch for the treatment of hypoactive sexual desire disorder in surgically menopausal women: A randomized, placebo-controlled trial. Arch Intern Med 2005;165:1582–9.

41. Buster JE, Kingsberg SA, Aguirre O, Brown C, Breaux JG, Buch A, Rodenberg CA, Wekselman K, Casson P. Testosterone patch for low sexual desire in surgically menopausal women: A randomized trial. Obstet Gynecol 2005;105:944–52.

42. Lobo RA, Rosen RC, Yang HM, Block B, Van Der Hoop RG. Comparative effects of oral esterified estrogens with and without methyltestosterone on endocrine profiles and dimensions of sexual function in postmenopausal women with hypoactive sexual desire. Fertil Steril 2003;79:1341–52.

43. Davis SR, McCloud P, Strauss BJ, Burger H. Testosterone enhances estradiol's effects on postmenopausal bone density and sexuality. Maturitas 1995;21:227–36.

44. Davis SR, Moreau M, Kroll R, Bouchard C, Panay N, Gass M, Braunstein GD, Hirschberg AL, Rodenberg C, Pack S, Koch H, Moufarege A, Studd J, APHRODITE Study Team. Testosterone for low libido in postmenopausal women not taking estrogen. N Engl J Med 2008;359:2005–17.

45. Nathorst-Boos J, Floter A, Jarkander-Rolff M, Carlstrom K, Schoultz B. Treatment with percutanous testosterone gel in postmenopausal women with decreased libido—Effects on sexuality and psychological general well-being. Maturitas 2006;53:11–8.

46. El-Hage G, Eden JA, Manga RZ. A double-blind, randomized, placebo-controlled trial of the effect of testosterone cream on the sexual motivation of menopausal hysterectomized women with hypoactive sexual desire disorder. Climacteric

2007;10:335–43.

47. Davis S, Papalia MA, Norman RJ, O'Neill S, Redelman M, Williamson M, Stuckey BG, Wlodarczyk J, Gard'ner K, Humberstone A. Safety and efficacy of a testosterone metered-dose transdermal spray for treating decreased sexual satisfaction in premenopausal women: A randomized trial. Ann Intern Med 2008;148:569–77.

48. Braunstein GD. Safety of testosterone treatment in postmenopausal women. Fertil Steril 2007;88:1–17.

49. Barrett-Connor E, Young R, Notelovitz M, Sullivan J, Wiita B, Yang HM, Nolan J. A two-year, double-blind comparison of estrogen-androgen and conjugated estrogens in surgically menopausal women. effects on bone mineral density, symptoms and lipid profiles. J Reprod Med 1999;44:1012–20.

50. Tamimi RM, Hankinson SE, Chen WY, Rosner B, Colditz GA. Combined estrogen and testosterone use and risk of breast cancer in postmenopausal women. Arch Intern Med 2006;166:1483–9.

51. Santen RJ, Allred DC, Ardoin SP, Archer DF, Boyd N, Braunstein GD, Burger HG, Colditz GA, Davis SR, Gambacciani M, Gower BA, Henderson VW, Jarjour WN, Karas RH, Kleerekoper M, Lobo RA, Manson JE, Marsden J, Martin KA, Martin L, Pinkerton JV, Rubinow DR, Teede H, Thiboutot DM, Utian WH, Endocrine Society. Postmenopausal hormone therapy: An endocrine society scientific statement. J Clin Endocrinol Metab 2010;95:s1–66.

52. Slagter MH, Gooren LJ, Scorilas A, Petraki CD, Diamandis EP. Effects of long-term androgen administration on breast tissue of female-to-male transsexuals. J Histochem Cytochem 2006;54:905–10.

53. Davis SR, Braunstein GD. Efficacy and safety of testosterone in the management of hypoactive sexual desire disorder in postmenopausal women. J Sex Med 2012;9:1134–48.

54. Tuiten A, Laan E, Panhuysen G, Everaerd W, de Haan E, Koppeschaar H, Vroon P. Discrepancies between genital responses and subjective sexual function during testosterone substitution in women with hypothalamic amenorrhea. Psychosom Med 1996;58:234–41

55. Aleman A, Bronk E, Kessels RP, Koppeschaar HP, van Honk J. A single administration of testosterone improves visuospatial ability in young women. Psychoneuroendocrinology 2004;29:612–7.

56. Bos PA, Terburg D, van Honk J. Testosterone decreases trust in socially naive humans. Proc Natl Acad Sci U S A 2010;107:9991–5.

57. Hermans EJ, Putman P, van Honk J. Testosterone administration reduces empathetic behavior: A facial mimicry study. Psychoneuroendocrinology 2006;31:859–66.

58. Hermans EJ, Putman P, Baas JM, Gecks NM, Kenemans JL, van Honk J. Exogenous testosterone attenuates the integrated central stress response in healthy young women. Psychoneuroendocrinology 2007;32:1052–61.

59. Hermans EJ, Ramsey NF, van Honk J. Exogenous testosterone enhances responsiveness to social threat in the neural circuitry of social aggression in humans. Biol Psychiatry 2008;63:263–70.

60. Schutter DJ, van Honk J. Decoupling of midfrontal delta-beta oscillations after testosterone administration. Int J Psychophysiol 2004;53:71–3.

61. van Honk J, Schutter DJ, Hermans EJ, Putman P, Tuiten A, Koppeschaar H. Testosterone shifts the balance between sensitivity for punishment and reward in healthy young women. Psychoneuroendocrinology 2004;29:937–43.

62. van Honk J, Peper JS, Schutter DJ. Testosterone reduces unconscious fear but not consciously experienced anxiety: Implications for the disorders of fear and anxiety. Biol Psychiatry 2005;58:218–25.

63. van Honk J, Schutter DJ. Testosterone reduces conscious detection of signals serving social correction: Implications for antisocial behavior. Psychol Sci 2007;18:663–7.

64. Postma A, Meyer G, Tuiten A, van Honk J, Kessels RP, Thijssen J. Effects of testosterone administration on selective aspects of object-location memory in healthy young women. Psychoneuroendocrinology 2000;25:563–75.

65. Bos PA, Panksepp J, Bluthe RM, Honk JV. Acute effects of steroid hormones and neuropeptides on human social-emotional behavior: A review of single administration studies. Front Neuroendocrinol 2011;33:17–35.

66. Gooren LJ. Human male sexual functions do not require aromatization of testosterone: A study using tamoxifen, testolactone, and dihydrotestosterone. Arch Sex Behav 1985;14:539–48.

67. Carani C, Qin K, Simoni M, Faustini-Fustini M, Serpente S, Boyd J, Korach KS, Simpson ER. Effect of testosterone and estradiol in a man with aromatase deficiency. N Engl J Med 1997;337:91–5.

68. Carani C, Granata AR, Rochira V, Caffagni G, Aranda C, Antunez P, Maffei LE. Sex steroids and sexual desire in a man with a novel mutation of aromatase gene and hypogonadism. Psychoneuroendocrinology 2005;30:413–7.

69. Roselli CE, Horton LE, Resko JA. Time-course and steroid specificity of aromatase induction in rat hypothalamus-preoptic area. Biol Reprod 1987;37:628–33.

70. Balthazart J, Foidart A, Hendrick JC. The induction by testosterone of aromatase activity in the preoptic area and activation of copulatory behavior. Physiol Behav 1990;47:83–94.

71. Davis SR, Goldstat R, Papalia MA, Shah S, Kulkarni J, Donath S, Bell RJ. Effects of aromatase inhibition on sexual function and well-being in postmenopausal women treated with testosterone: A randomized, placebo-controlled trial. Menopause 2006;13:37–45.

72. Stuenkel CA, Dudley RE, Yen SS. Sublingual administration of testosterone-hydroxypropyl-beta-cyclodextrin inclusion complex simulates episodic androgen release in hypogonadal men. J Clin Endocrinol Metab 1991;72:1054–9.

73. Salehian B, Wang C, Alexander G, Davidson T, McDonald V, Berman N, Dudley RE, Ziel F, Swerdloff RS. Pharmacokinetics, bioefficacy, and safety of sublingual testosterone cyclodextrin in hypogonadal men: Comparison to testosterone enanthate—A clinical research center study. J Clin Endocrinol Metab 1995;80:3567–75.

74. van Rooij K, Bloemers J, de Leede L, Goldstein I, Lentjes E, Koppeschaar H, Olivier B, Tuiten A. Pharmacokinetics of three doses of sublingual testosterone in healthy premenopausal women. Psychoneuroendocrinology 2012;37:773–81.

75. Kingsberg S, Shifren J, Wekselman K, Rodenberg C, Koochaki P, Derogatis L. Evaluation of the clinical relevance of benefits associated with transdermal testosterone treatment in postmenopausal women with hypoactive sexual desire disorder. J Sex Med 2007;4:1001–8.

76. Bancroft J. Human sexuality and its problems. London: Churchill Livingstone; 1989.

77. Bancroft J, Graham CA, Janssen E, Sanders SA. The dual control model: Current status and future directions. J Sex Res 2009;46:121–42.

78. Laan E, Everaerd W, Evers A. Assessment of female sexual arousal: Response specificity and construct validity. Psychophysiology 1995;32:476–85.

79. Quigley CA, De Bellis A, Marschke KB, el-Awady MK, Wilson EM, French FS. Androgen

receptor defects: Historical, clinical, and molecular perspectives. Endocr Rev 1995;16:271–321.

80. Berenbaum SA, Bryk KK, Nowak N, Quigley CA, Moffat S. Fingers as a marker of prenatal androgen exposure. Endocrinology 2009;150:5119–24.

81. Childress AR, Ehrman RN, Wang Z, Li Y, Sciortino N, Hakun J, et al. Prelude to passion: Limbic activation by "unseen" drug and sexual cues. PloS ONE 2008;3:e1506.

82. Prause N, Janssen E, Hetrick WP. Attention and emotional responses to sexual stimuli and their relationship to sexual desire. Arch Sex Behav 2008;37:934–49.

83. Williams JM, Mathews A, MacLeod C. The emotional Stroop task and psychopathology. Psychol Bull 1996;120:3–24.

84. Goldstein I, Lue TF, Padma-Nathan H, Rosen RC, Steers WD, Wicker PA. Oral sildenafil in the treatment of erectile dysfunction. N Engl J Med 1998;338:1397–404.

85. Janssen E, Bancroft J. The dual control model: The role of sexual inhibition and excitation in sexual arousal and behaviour. In: Janssen E, ed. The psychophysiology of sex. Bloomington: Indiana University Press; 2007:197.

86. Chambers CD, Garavan H, Bellgrove MA. Insights into the neural basis of response inhibition from cognitive and clinical neuroscience. Neurosci Biobehav Rev 2009;33:631–46.

87. Beauregard M, Levesque J, Bourgouin P. Neural correlates of conscious self-regulation of emotion. J Neurosci 2001;21:RC165.

88. Stoleru S, Redoute J, Costes N, Lavenne F, Bars DL, Dechaud H, Forest MG, Pugeat M, Cinotti L, Pujol JF. Brain processing of visual sexual stimuli in men with hypoactive sexual desire disorder. Psychiatry Res 2003;124:67–86.

89. Karama S, Lecours AR, Leroux JM, Bourgouin P, Beaudoin G, Joubert S, Beauregard M. Areas of brain activation in males and females during viewing of erotic film excerpts. Hum Brain Mapp 2002;16:1–13.

90. Stoleru S, Gregoire MC, Gerard D, Decety J, Lafarge E, Cinotti L, Lavenne F, Le Bars D, Vernet-Maury E, Rada H, Collet C, Mazoyer B, Forest MG, Magnin F, Spira A, Comar D. Neuroanatomical correlates of visually evoked sexual arousal in human males. Arch Sex Behav 1999;28:1–21.

91. Rubia K, Russell T, Overmeyer S, Brammer MJ, Bullmore ET, Sharma T, Simmons A,

Williams SC, Giampietro V, Andrew CM, Taylor E. Mapping motor inhibition: Conjunctive brain activations across different versions of go/no-go and stop tasks. Neuroimage 2001;13:250–61.

92. Liddle PF, Kiehl KA, Smith AM. Event-related fMRI study of response inhibition. Hum Brain Mapp 2001;12:100–9.

93. Liu X, Banich MT, Jacobson BL, Tanabe JL. Common and distinct neural substrates of attentional control in an integrated simon and spatial Stroop task as assessed by event-related fMRI. Neuroimage 2004;22:1097–106.

94. Milham MP, Banich MT, Barad V. Competition for priority in processing increases prefrontal cortex's involvement in top-down control: An event-related fMRI study of the Stroop task. Brain Res Cogn Brain Res 2003;17:212–22.

95. Konishi S, Jimura K, Asari T, Miyashita Y. Transient activation of superior prefrontal cortex during inhibition of cognitive set. J Neurosci 2003;23:7776–82.

96. Joseph R. Environmental influences on neural plasticity, the limbic system, emotional development and attachment: A review. Child Psychiatry Hum Dev 1999;29:189–208.

97. Janssen E, Everaerd W, Van Lunsen RH, Oerlemans S. Validation of a psychophysiological waking erectile assessment (WEA) for the diagnosis of male erectile disorder. Urology 1994;43:686–95; discussion 695–6.

98. Bloemers J, Van der Made F, Everaerd W, Olivier B, Tuiten A. The neural correlates of female sexual dysfunction. Congress International Society for the Study of Women's Sexual Health. Orlando, FL, USA, 2007.

99. Bancroft J. Central inhibition of sexual response in the male: A theoretical perspective. Neurosci Biobehav Rev 1999;23:763–84.

100. Eagle DM, Bari A, Robbins TW. The neuropsychopharmacology of action inhibition: Cross-species translation of the stop-signal and go/no-go tasks. Psychopharmacology (Berl) 2008;199:439–56.

101. Evers EA, Cools R, Clark L, van der Veen FM, Jolles J, Sahakian BJ, Robbins TW. Serotonergic modulation of prefrontal cortex during negative feedback in probabilistic reversal learning. Neuropsychopharmacology 2005;30:1138–47.

102. Sprouse JS, Aghajanian GK. Electrophysiological responses of serotoninergic dorsal raphe neurons to 5-HT1A and 5-HT1B agonists. Synapse 1987;1:3–9.

103. Hamon M, Fattaccini CM, Adrien J, Gallissot MC, Martin P, Gozlan H. Alterations of central serotonin and dopamine turnover in rats treated with ipsapirone and other 5-hydroxytryptamine$_{1A}$ agonists with potential anxiolytic properties. J Pharmacol Exp Ther 1988;246:745–52.

104. Liu YP, Wilkinson LS, Robbins TW. Effects of acute and chronic buspirone on impulsive choice and efflux of 5-HT and dopamine in hippocampus, nucleus accumbens and prefrontal cortex. Psychopharmacology (Berl) 2004;173:175–85.

105. Lutfey KE, Link CL, Litman HJ, Rosen RC, McKinlay JB. An examination of the association of abuse (physical, sexual, or emotional) and female sexual dysfunction: Results from the Boston area community health survey. Fertil Steril 2008;90:957–64.

106. Labrie F, Belanger A, Simard J, Van L, Labrie C. DHEA and peripheral androgen and estrogen formation: Intracinology. Ann N Y Acad Sci 1995;774:16–28.

107. Beevers CG, Ellis AJ, Wells TT, McGeary JE. Serotonin transporter gene promoter region polymorphism and selective processing of emotional images. Biol Psychol 2010;83:260–5.

108. Lemonde S, Turecki G, Bakish D, Du L, Hrdina PD, Bown CD, Sequeira A, Kushwaha N, Morris SJ, Basak A, Ou XM, Albert PR. Impaired repression at a 5-hydroxytryptamine$_{1A}$ receptor gene polymorphism associated with major depression and suicide. J Neurosci 2003;23:8788–99.

109. Bloemers J, Gerritsen J, Bults R, Koppeschaar H, Everaerd W, Olivier B, Tuiten A. Induction of sexual arousal in women under conditions of institutional and ambulatory laboratory circumstances: A comparative study. J Sex Med 2010;7:1160–76.

Chapter 4

Toward Personalized Sexual Medicine (Part 2): Testosterone combined with a PDE5 inhibitor increases sexual satisfaction in women with HSDD and FSAD, and a low sensitive system for sexual cues

Saskia Poels, Jos Bloemers, Kim van Rooij, Irwin Goldstein, Jeroen Gerritsen, Diana van Ham, Frederiek van Mameren, Meredith Chivers, Walter Everaerd, Hans Koppeschaar, Berend Olivier and Adriaan Tuiten

Journal of Sexual Medicine 2013;10:810-823

ABSTRACT

Introduction. Low sexual desire in women may result from a relative insensitivity of the brain for sexual cues. Administration of sublingual 0.5 mg testosterone (T) increases the sensitivity of the brain to sexual cues. Sexual stimulation in the brain is necessary for phosphodiesterase type 5 inhibitor (PDE5i)-mediated increase in genital sexual response. Accordingly, a single dose of T+PDE5i might enhance sexual responsiveness, especially in women with low sensitivity for sexual cues.

Aim. To assess the hypothesis that treatment with on-demand use of T+PDE5i improves sexual functioning, particularly in women who suffer from Hypoactive Sexual Desire Disorder (HSDD) as the result of a relative insensitivity for sexual cues.

Methods. In a randomized, double-blind, placebo-controlled, cross-over design, 56 women with HSDD underwent three medication treatment regimes (placebo, T+PDE5i, and T with a serotonin $_{1A}$ receptor agonist; see also parts 1 and 3), which lasted 4 weeks each. In a participant-controlled ambulatory psychophysiological experiment at home (the first week of each drug treatment), physiological and subjective indices of sexual functioning were measured. In a bedroom experiment (the subsequent 3 weeks), sexual functioning was evaluated following each sexual event after the self-administration of study medication. Subjective evaluation of sexual functioning was also measured by weekly and monthly reports.

Main Outcome Measures. Subjective: sexual satisfaction, experienced genital arousal, sexual desire. Physiological: vaginal pulse amplitude. Cognitive: preconscious attentional bias.

Results. T+PDE5i, as compared with placebo, significantly improved physiological and subjective measures of sexual functioning during ambulatory psychophysiological lab conditions at home and during the sexual events, in women with low sensitivity for sexual cues.

Conclusions. The present study demonstrated that on-demand T+PDE5i is a potentially promising treatment for women with HSDD, particularly in women with low sensitivity for sexual cues.

INTRODUCTION

Low sexual desire is the most common sexual complaint in women [1]. It has been classified as the clinical condition Hypoactive Sexual Desire Disorder (HSDD), which is characterized by chronic or recurrent loss or decrease in interest in sexual activity, causing sexual dissatisfaction and severely affecting a woman's quality of life [2]. Bancroft and Janssen have described the involvement of dual control systems in the regulation of sexual functioning. They argue that individual differences in sexual responding depend on a delicate interplay of such activating excitatory and inhibitory processes [3,4]. We have hypothesized that in some women with HSDD low sexual desire could be due to a relatively insensitive system for sexual cues, resulting in impaired activity of excitatory mechanisms involved in sexual motivation [5]. In these women, administration of sublingual 0.5 mg testosterone might increase the brain's sensitivity for sexual cues, which might activate excitatory mechanisms in the brain involved in sexual motivation and desire [5–8]. Sufficient sexual stimulation of the brain is necessary for a phosphodiesterase type 5 inhibitor (PDE5i)-mediated increase in the genital sexual response [9,10]. Because central mechanisms (sexual motivation) play a large role in sexual stimulation, PDE5i's will generally have little to no effect when activation of central sexual mechanisms is reduced or absent. In previous studies it was shown that PDE5i administration increases physiological [6] and subjective [5] sexual responding under psychophysiological lab conditions in women with HSDD and low sensitivity for sexual cues, only when the PDE5i administration was preceded by administration of a single dose of sublingual testosterone. It was also shown that testosterone by itself did not increase sexual responding [5,6]. In the present study we measured sexual functioning under the more ecologically valid condition of an ambulatory psychophysiological lab setting at home and sexual satisfaction following sexual events. We hypothesize that treatment with T+PDE5i improves physiological and subjective indices of sexual functioning, particularly in women who suffer from HSDD as the result of an insensitive system for sex.

METHODS

Participants

The 56 heterosexual women who participated in this study signed a written informed consent and received reimbursement for their participation. The local medical ethics committee (Stichting Therapeutische Evaluatie Geneesmiddelen Medisch Ethische Toetsingscommissie, Almere, the Netherlands) approved this study, which was carried out in agreement with International Conference on Harmonization-Good Clinical Practice (ICH-GCP), and monitored by a certified Contract Research Organization (CRO) (PSR Group, Hoofddorp, the Netherlands). Women were eligible if they were healthy, between 21 and 70 years, and had a diagnosis of HSDD or Female Sexual Arousal Disorder (FSAD) according to the Diagnostic and Statistical Manual of Mental Disorders-Fourth Edition (Text Revision) (DSM-IV-TR) criteria [2]. Subjects were diagnosed by an experienced psychologist. If the subject was diagnosed with any psychiatric disorder other than HSDD or FSAD they were excluded; also any treatment for female sexual dysfunction within 7 days before or during the study was excluded. Furthermore women were excluded if they were using oral contraceptives containing anti-androgens or more than 50 µg estrogen, Cytochrome P450 3A4 (CYP3A4) inhibitors, CYP3A4 inducers, nitrates, monoamine oxidase inhibitors, calcium channel blockers, antidepressants, opiates, and medicinal herbs like St. John's wort. Cardiovascular exclusions included a history of myocardial infarction, stroke or life-threatening arrhythmia within the prior 6 months, uncontrolled hypertension, atrial fibrillation/flutter or any other significant abnormality observed on electrocardiogram (ECG). Gynecological exclusions included pelvic inflammatory disease, vaginal infection, previous prolapse and incontinence surgery affecting the vaginal wall, abnormal uterine bleeding patterns, perimenopausal hormonal status, pregnancy and breastfeeding in the past 6 months. Lastly women were excluded with clinically relevant endocrine disease, neurological disease, severe or acute liver disease, history of severe hepatic impairment, body mass index above 35, and vision impairment. Women were recruited and enrolled from referrals, newspaper advertisements, the Internet, and our own database. To determine eligibility, participants were screened 4 weeks prior to

study entry. In addition to an assessment of medical history with detailed sexual, gynecological, and psychological history, all subjects received a physical examination including a 12-lead ECG, a vaginal culture test to exclude infection, standard biochemistry and hematological laboratory tests, and pelvic examination. Biochemical parameters including hematology, liver function tests, electrolytes, creatinine, uric acid, glucose and lipids were collected at baseline, following each treatment period, and at the final follow-up visit. Serum concentrations of total testosterone, sex hormone binding globulin (SHBG), luteinizing hormone (LH), follicle-stimulating hormone (FSH), estradiol, progesterone, and prolactin were measured at baseline. All subjects also had their thyroid status assessed; thyroid stimulating hormone and free thyroxine. A urine pregnancy test was administered to all women of childbearing potential. Investigators monitored and followed up any reports of adverse events. On a pretreatment visit, a trained female experimenter explained the study requirements and procedures to the participants. A practice trial was conducted, including insertion of the vaginal photoplethysmograph and a practice session of the emotional Stroop task.

Study Design

In a randomized, double-blind, placebo-controlled, cross-over design, each participant underwent three different medication treatments: (i) **placebo**: placebo for testosterone (cyclodextrin solution without testosterone) and placebo for the PDE5i (PDE5i = sildenafil) and 5-HT_{1A} receptor agonist (5-HT_{1A}ra = buspirone) (powder-filled gelatin capsule without sildenafil/buspirone); (ii) **T+PDE5i**: the combination of testosterone (0.5 mg) sublingually with cyclodextrin as carrier and sildenafil (50 mg, hidden in a powder-filled gelatin capsule); (iii) **T+5-HT_{1A}ra**: the combination of testosterone (0.5 mg) sublingually with cyclodextrin as carrier and buspirone (10 mg, hidden in a powder-filled gelatin capsule). A single medication regime lasted 4 weeks, and the order of the three medication regimes was randomized. All treatment orders were possible. Between each medication regime there was a washout period of 1 week. Participants received 14 units of medication (throughout the manuscript, 1 medication or 1 unit of medication means the combination of the sublingually administered solution *and* the oral capsule) during each medication

regime; instructions were given to have at least 48 hours between two medication intakes (see Figure 1). Participants were instructed to take the cyclodextrin solution (testosterone or placebo) sublingually 4 hours prior to each measurement session or sexual event and rinse it under the tongue for 1 minute. They were instructed to ingest the capsule (sildenafil, buspirone, or placebo) 2.5 hours later. The sequence of the drugs and time frame were such that the pharmacological effects of the PDE5i and 5-HT_{1A}ra coincide with the window of T-induced behavioral effects, approximately 3–6 hours after T administration. Following the completion of a drug regime, the research physician took in any leftover medication of the previous regime and handed out the next regime's medication box.

Study Procedure

Ambulatory Psychophysiological At-Home Experiment

Participants visited the study site 12 times during a period of 26 weeks for screening and safety control visits. In the first week of each 4-week medication regime, participants underwent three experimental psychophysiological measurement sessions (under condition of study medication) at home using participant-controlled (i.e., no experimenters were present) ambulatory laboratory [11]. In premenopausal women not using oral contraceptives, experimental days were within the first 10 days after their menstruation. Users of oral contraceptives were not measured during their pill-free period. During each experimental session, subjects engaged in 2 minutes each of: (i) self-induced sexual fantasy; (ii) viewing an erotic film clip depicting sexual foreplay; and (iii) viewing an erotic film depicting explicit heterosexual intercourse. The sexual fantasy condition was preceded by a 6-minute baseline establishment period during which subjects viewed a neutral film clip. The foreplay and heterosexual intercourse films were preceded by 2 minutes return to baseline periods (see Stimuli, Apparatus, and Measures below, for a detailed description of the neutral and erotic stimulus material). At the start of the experimental session, and after each exposure to sexual stimuli (the fantasy session and film clips), we measured “experiences of genital arousal” and “sexual desire” by means of the Sexual Arousal Response Self-Assessment Questionnaire

(SARSAQ; see Stimuli, Apparatus, and Measures below, and [5]). During all neutral and sexual stimulus sessions, the vaginal pulse amplitude (VPA) was continuously measured (see Stimuli, Apparatus, and Measures below). Before the baseline establishment period and finally as the last element of the experimental session, we measured biases in preconscious attention for sexual cues by means of an emotional Stroop task (see Figure 1).

Bedroom Experiment

For the remaining 3 weeks, participants were instructed to use at least one medication per week, with an attempt at a sexual event (cuddling, coitus, or masturbation). For the remainder, they were free to take their medication whenever they wanted but not during two consecutive days. When they had experienced a sexual event they were asked to fill out an event diary within 24 hours and a diary at the end of each week.

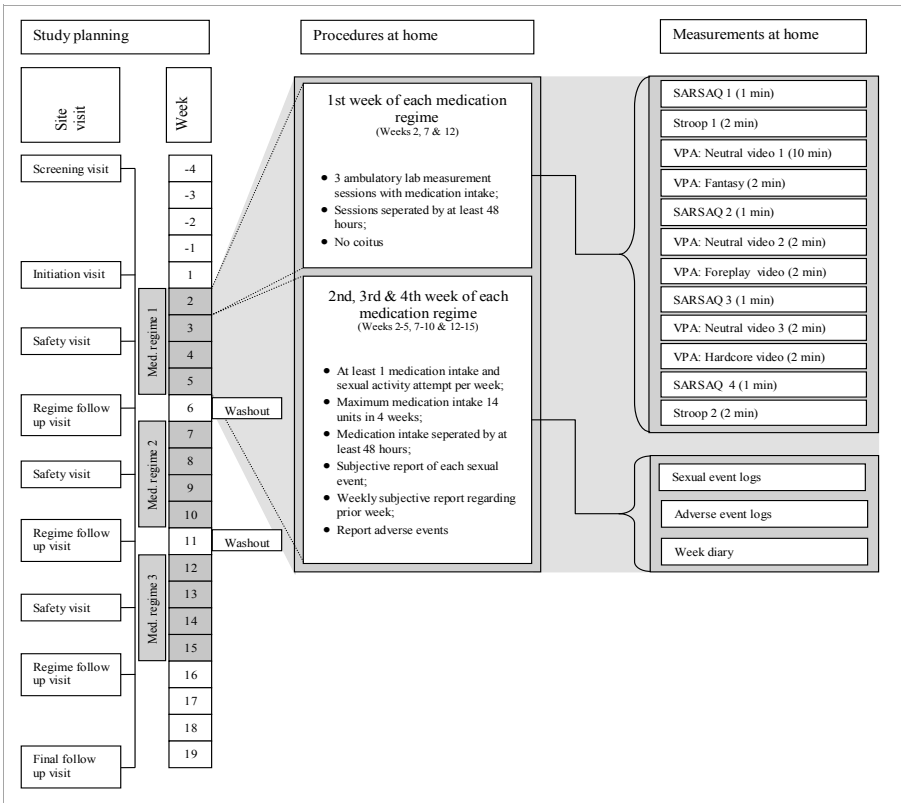

Figure 1. Overview of study design and procedures. Subjects were randomized before the initiation visit and received seven units of medication to take home for the subsequent 2 weeks. At each safety visit, which was planned halfway of each regime, subjects received the other seven units of medication for the last 2 weeks of that regime. Adverse events (AEs) and vital signs were recorded at each visit. Blood safety was also determined at all follow-up visits. During the first week of each regime, subjects received an ambulatory laboratory for three psychophysiological measurements at home. During the subsequent 3 weeks subjects were free to take the medication when they wanted. They were instructed to attempt sexual activity at least once a week with medication during this period. Medication intake was on-demand but separated by at least 48 hours. Subjects reported their subjective experience within 24 hours following each sexual activity via a secure Web-based questionnaire. At the end of each week, a Web-based week diary was completed. AEs experienced at home could also be reported in this manner. AEs which were reported through the Web-based questionnaire were discussed with the subject the following visit. SARSAQ = Sexual Arousal Response Self-Assessment Questionnaire; VPA = vaginal pulse amplitude

Stimuli, Apparatus, and Measures

Audiovisual Stimuli

For this study, neutral, erotic foreplay, and explicit sex film clips were used. Neutral clips were 6-minute and 2-minute clips from Dutch action films. The 6-minute neutral film clips were used to establish a baseline VPA. Erotic foreplay clips were 2-minute clips showing kissing, caressing, and cunnilingus, but no fellatio. Explicit sex clips were 2-minute clips showing cunnilingus and coitus, including visible penetration. The erotic film footage was selected and edited by female research associates and then rated by other female research associates to ensure that all clips were within set specifications. All digitally sampled film clips were presented using Presentation software (Neurobehavioral Systems, Albany, CA, USA). For a more detailed description see Bloemers et al. [11].

Ambulatory Laboratory

The ambulatory laboratory is based on the MobiHealth Mobile remote monitoring system (MobiHealth B.V., Enschede, The Netherlands) [12]. Study-specific functionality of this system enables VPA measurements, stimulus presentation, and execution of emotional Stroop tasks to be performed at an arbitrary time and location (e.g., house of a participant). This laboratory is operated autonomously by the participant. The ambulatory laboratory transmits all measured data to a secure central database server, at which the researcher can obtain the data for further analysis. This method allows for a more ecologically valid psychophysiological measurement of sexual responding. See for a detailed description the study by Bloemers et al. [11].

Main Outcome Measures: Ambulatory Laboratory

VPA

The VPA reflects phasic changes in vaginal engorgement corresponding with each heartbeat. VPA was measured using a vaginal photoplethysmograph, a tampon-shaped device containing an infrared light-emitting diode and a photosensitive light detector (photodiode). VPA was defined as the peak-to-

trough amplitude of the pulse wave and was calculated by acquiring the means of all peaks and troughs and subtracting those. Data from the photoplethysmograph were sampled at 256 Hz and filtered offline (high-pass 1 Hz, 48 dB/oct and low-pass 1.5 Hz, 48 dB/oct), in order to isolate the AC component from the DC coupled amplifier, reduce respiration artifacts and high-frequency oscillations. Large movement artifacts (more than 100% increase for a small number of isolated periodic cycles) were manually removed following visual inspection of the data. The data were divided into 30-second epochs for each 2-minute film clip, thus yielding four discrete values and for the 6-minute film clip 12 values reflecting VPA during different stages of the film clip. Finally, in order to eliminate interpersonal differences and obtain meaningful data, mean VPA scores over the four epochs of the fantasy condition, foreplay clips, and explicit clips were related to activity during the last epoch of the first 6-minute neutral clip, using the following formula:

$$VPA_{rel} = ((VPA_x - VPA_{neu})/VPA_{neu})$$

with VPA_{rel} being the relative change in VPA related to the first neutral clip, VPA_x being the mean of the four 30-second epochs of either the fantasy condition, foreplay clips, or explicit clips, and VPA_{neu} being the last 30-second epoch of the first 6-minute neutral clip.

Emotional Stroop Task

To measure preconscious attentional bias for sexual cues, a masked version of the emotional Stroop task was used [13]. In this task, words were presented for 26 milliseconds in four different colors (red, green, blue, and yellow) on a Dell Latitude D531 laptop (Dell Inc., Round Rock, TX, USA) set at a 75 Hz refresh rate. Words were backwardly masked by randomly cut and reassembled letters in the same color. Backward masking prevents conscious processing of the words. Participants were instructed to name the color of the masks as quickly as possible. A microphone connected to a voice-level detector was placed in front of the participant. Initiation of vocal response was registered by the computer's clock and terminated the target (mask) presentation (with a no-response maximum of 3,000 milliseconds). Accuracy of color naming was not

scored. Thirty-two unambiguous neutral words from one category (furniture; examples are “chair” and “table”) and 32 unambiguous erotic words (examples are “penis,” “coitus,” and “vagina”) were presented in a blocked manner (eight words per block). The same words were used for each test; however, the sequence of words and their colors differed all eight times this task was used. These different versions were randomized over the participants. An extra set of stimuli consisting of meaningless letter strings was used for practice trials directly before each Stroop task. The Stroop reaction times for color naming were visually inspected for outliers. After these outliers were excluded, participants' mean reaction times for erotic and neutral words on each trial were calculated. The differences between the mean reaction times of erotic and neutral words on each trial were used in the analysis.

Sensitivity of the Brain to Sexual Cues

Based on their mean reaction times of erotic and neutral words on the emotional Stroop task taken over the 3 experimental days, we divided patients in two groups: one group with low (negative score; reaction time to neutral words > erotic words) and one group with high (positive score; reaction time to neutral words < erotic words) sensitivity for sexual cues.

SARSAQ

The SARSAQ is a 10-item self-report questionnaire using a seven-point Likert scale (ranging from “not at all” to “extremely”), adapted from Morokoff and Heiman [14] and Heiman and Hatch [15]. It measures current subjective feelings of sexual arousal and sexual desire. Five items concern subjective feelings of genital responding, and five items concern subjective feelings of sexual desire. The SARSAQ was administered via the ambulatory laboratory laptop whereby the number keys (from 1 to 7) were used to complete the questionnaire.

Main Outcome Measures: Bedroom Experiment

Event Diary

In this secure web-based diary, subjects were asked 10 questions (one open-ended, four multiple-choice, five 5-point Likert scale items) concerning the type, duration, pleasantness, and intensity of the sexual event. Participants were instructed to fill out the event diary within 24 hours following each sexual event (e.g., cuddling, coitus, masturbation).

Week Diary

Women were also instructed to fill out the secure Web-based week diary once a week during each medication regime. Subjects were asked their experiences during the past week regarding: (i) sexual desire (six-point Likert scale: 1 = nothing to 6 = much); (ii) vaginal arousal (six-point Likert scale: 1 = nothing to 6 = much); (iii) sexual improvement/deterioration (four-point Likert scale; 1 = nothing to 4 = much); and (iv) if they could attribute the improvement/deterioration to the medicines (yes/no).

Subjective Evaluation of Improvement (SEI)

The SEI questionnaire was used to determine, after each medication regime, if the subject felt there had been an overall improvement in sexual desire, sexual arousal or both, and if they attribute this improvement to the medication. Subjects answered yes or no to both questions. The SEI was administered as a pen and paper test.

Subjective Evaluation of Gain (SEG)

The SEG questionnaire was used to determine, after each medication regime, if the subject felt that she had any meaningful benefit from the study medication, and if she would use it if it were available by prescription. Subjects answered yes or no to both questions. The SEG was administered as a pen and paper test.

Measures: On Site

Hormonal Measures

Serum total testosterone, serum estradiol, serum progesterone, serum prolactin, serum LH, serum FSH, and serum SHBG were measured through electrochemiluminescence radioimmunoassay with COBAS kits of Roche Diagnostics (Mannheim, Germany), using a Modular E170 at OLVG Hospital (Amsterdam, the Netherlands). The measuring range for total testosterone was 0.087–52.0 nmol/L. The coefficient of variation was 8%.

Data Transformation and Reduction

Ambulatory Experiment

For each drug treatment condition, we used the mean levels of the 3 experimental days of the experience of genital arousal, sexual desire, preconscious attentional bias for sexual cues and VPA, respectively, for further analyses.

Bedroom Experiment

Based on high Cronbach's alphas of the six items measuring duration, pleasantness, and intensity of the sexual events (measured by the event diary) during the different drug conditions (placebo: $\alpha = 0.95$; T+PDE5i: $\alpha = 0.95$) we calculated the mean of these items, as a measure of "sexual satisfaction." Each question of the week diary, SEG and SEI questionnaire were analyzed separately. To analyze sexual satisfaction and the week diary items, we calculated the average of each dependent variable over the 3 weeks of drug treatment.

Statistical Analysis

Baseline characteristics and demographics of both subgroups were compared using the Student's *t*-test, Mann–Whitney *U*-test or chi-square test when appropriate.

For each of the dependent variables, separate repeated measures analyses of variance (ANOVAs) were carried out. The within-subject factor had two levels (placebo and T+PDE5i). The between-subject factor also had two levels (low and high sensitivity for sexual cues). For all analyses menopausal status (premenopausal vs. postmenopausal) and hormonal contraception (hormonal contraception vs. non-hormonal contraception) were analyzed in a repeated measures ANOVA for each dependent variable.

We have chosen to report an ANOVA for each of these dependent variables separately, instead of analyzing the variables together in a multivariate analysis (MANOVA). The reasons for this choice are twofold: first, our interest is into effects on each of the dependent variables separately as they are quite different in nature (e.g., we have a physiological measure such as VPA as well as self-report measures such as diaries under different experimental conditions); second, when missing data are scattered over all dependent variables (e.g., sometimes women did not have sexual activities, at other times a VPA measurement failed), participants are eliminated completely from MANOVA leading to unnecessary loss of power.

An assumption of ANOVA is that the means of the variables are normally distributed. Tabachnick and Fidell indicate that the F-test is robust against violations of normality of variables if there are at least 20 degrees of freedom for error in a univariate ANOVA, provided that there are no outliers [16]. In most of our analyses the number of degrees of freedom is 55 (in some of the analyses the number of degrees of freedom is a bit lower because sometimes women did not have sexual activities). The dependent variables have no outliers, except for the six dependent variables of the VPA, who have a few, so, except for the six dependent variables of the VPA the assumptions of ANOVA are met. For VPA we created log-transformed variables and this removed almost all of the outliers. To investigate the robustness of the results for VPA we carried out an analysis on the original variables as well as on the log-transformed variables. The log-transformed variables yielded an almost identical pattern of significant results as the original analysis. We report the results of the original analysis because the original variables are more easily interpretable.

The alpha level was set at 0.05 for all tests carried out. All tests were two-tailed. We made no adjustment for multiple testing. In this article many tests have been carried out and the application of, e.g., the popular Bonferroni correction would lead to conservative results and extreme loss in power. Although we do not control for chance capitalization, nearly all analyses show significant results in the same direction, showing that these findings are not by chance.

There were no data exclusions. Less than 5% of the data were missing. When data from an experimental day during the ambulatory experiment were missing, the data were estimated by taking the averaged data over the other 2 experimental days of the same drug condition.

RESULTS

This article only reports about the T+PDE5i condition compared with placebo in relation to sensitivity for sexual cues. In part 3 the results of the third drug condition (T+5-HT_{1A}ra) will be described along with the subdivision in low and high inhibition (see chapter 5).

Participants

Fifty-six otherwise healthy patients (Mean age 39.8 [± 10.36]; premenopausal: N = 43, postmenopausal: N = 13) diagnosed as having HSDD or FSAD for at least 6 months prior to study entry completed the study (for a trial profile see Figure 2).

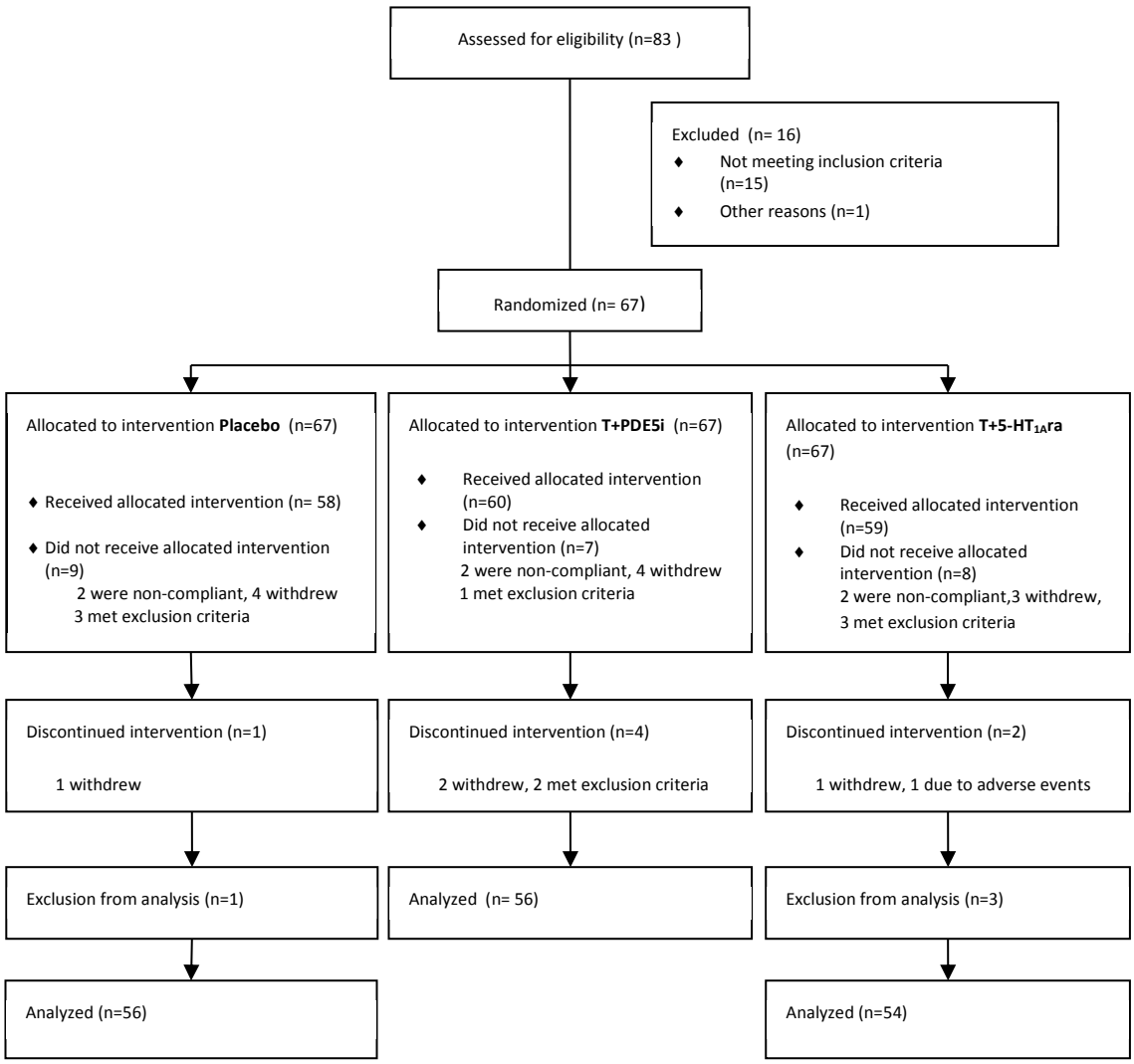

Figure 2. Clinical trial profile and disposition of women. Three women were not analyzed in the T+5-HT_{1A}ra condition. One participant did not receive T+PDE5i, therefore could not be determined as either a high or low inhibitor for analysis in the T+5-HT_{1A}ra condition. The remaining two subjects' data were unreliable; one subject owned a company which went bankrupt during the course of the study and one subject had severe maxillary sinusitis which in her opinion influenced her sexual functioning during one of the treatment regimes (the T+5-HT_{1A}ra regime, as determined after unblinding).

Women diagnosed with primary FSAD (N = 11), reported experiencing low sexual desire. Women rarely perceive desire and arousal as two separate entities of the sexual response cycle, which was also true for the women in our study. This is also one of the reasons for the proposed revision in DSM-V of the combining of the diagnoses HSDD and FSAD into "Sexual Interest/Arousal Disorder." Because of this, these women were included in the study, which had no effect on the results. During the clinical interview all women reported moderate to severe distress from their sexual complaints. Thirty-one women used hormone-containing contraceptives, 15 used other forms of contraceptives, and 10 used no contraceptives. Forty-nine women were Caucasian, three were Black, two were Asian, one was mixed Black/Caucasian, and one was mixed Asian/Caucasian. All baseline hormonal values were in the normal female reproductive and/or postmenopausal range. None of the dependent variables were influenced by menopausal status and hormonal contraception (data not shown). Demographics and baseline characteristics of the subgroups are presented in Table 1.

Effects of T+PDE5i Relative to Placebo: Preconscious Attentional Bias for Sexual Cues

The results on the emotional Stroop task revealed a clear group difference regarding the T+PDE5i induced alterations in preconscious attention for sexual cues [$F(1, 54) = 13.27$, $P < 0.001$]. In women with a low sensitive system for sexual cues, T+PDE5i induced an increase of preconscious attention allocation (Figure 3). This reversed pattern is consistent with the results reported by van der Made et al. [5,6].

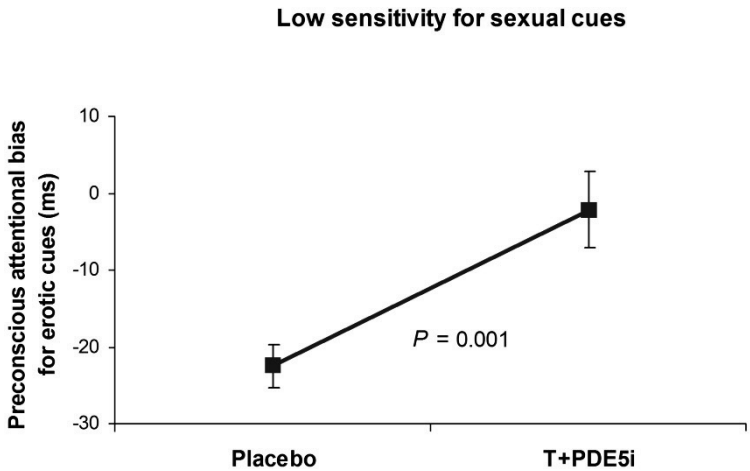

Figure 3. Preconscious attentional bias for sexual cues. Treatment with T+PDE5i relative to placebo produced an increase in the preconscious attentional bias (the differences between the mean reaction times of erotic and neutral words) for sexual cues in women with a relative insensitivity for sexual cues.

Table 1. Baseline characteristics and demographics

	Low sensitive women	High sensitive women	*P*
N	29	27	
Age, years	39.3 ± 10.4	40.3 ± 10.4	n.s
Body mass index, kg/m^2	25.1 ± 4.0	24.0 ± 3.5	n.s
Race, no (%)			n.s.*
Caucasian	25 (86.2)	24 (88.9)	
Black	2 (6.9)	1 (3.7)	
Asian	0 (0)	2 (7.4)	
Other	2 (6.9)	0 (0)	
Parity, no (%)			n.s.*
Para 0	9 (31)	7 (25.9)	
Para 1	5 (17.2)	3 (11.1)	
Para ≥ 2	15 (51.8)	17 (63)	
Menopausal status, no (%)			n.s.*
Premenopausal	22 (75.9)	21 (77.8)	
Postmenopausal	7 (24.1)	6 (22.2)	
Surgical menopause	1 (3.4)	0 (0)	
Contraception, no (%)			n.s.*
Hormonal	18 (62.1)	13 (48.2)	
Combination Pill	8 (44.4)	7 (53.8)	
Progestagen (IUD, implanon)	8 (44.4)	5 (38.5)	
Vaginal ring (progestin and estrogen)	2 (11.1)	1 (7.7)	
Non-hormonal	6 (20.7)	9 (33.3)	
Condoms	1 (16.7)	1 (11.1)	
Sterilization	3 (50.0)	3 (33.3)	
Sterilization partner	2 (33.3)	5 (55.5)	
None	5 (17.2)	5 (18.5)	
FSD diagnosis, no (%)			n.s.*
Primary HSDD	24 (82.8)	21 (77.8)	
Primary FSAD	5 (17.2)	6 (22.2)	
Negative sexual experiences (inappropriate touching, [attempted] rape, other), no (%)			n.s.*
Yes	11 (37.9)	12 (42.9)	
No	18 (62.1)	15 (53.6)	
Duration of current relationship, years	12.5 (38)	18.0 (29)	n.s.†

Age and Body Mass Index are represented as mean ± standard deviation. Duration of current relationship did not fit the normal distribution and therefore is represented as median (range).

*Chi-square test

†Mann–Whitney U-test

IUD = intra uterine device; FSD = female sexual dysfunction; HSDD = Hypoactive Sexual Desire Disorder; FSAD = Female Sexual Arousal Disorder; n.s. = not significant

Effects of T+PDE5i Relative to Placebo: Ambulatory Experiment

During the first part of the study T+PDE5i as compared with placebo produced statistically significant overall increases in the subjective indices of sexual function (experienced genital arousal and sexual desire) in women with a relative insensitivity for sexual cues. There were no treatment-order effects observed.

Low Sensitive Women

The results of the VPA were only significant in the fantasy condition showing higher levels of the VPA in the T+PDE5i treatment (M = 0.39, SE = 0.11) compared with placebo (M = 0.14, SE = 0.05), [F(1,28) = 7.68, *P* = 0.010] in women with low sensitivity for sexual cues (Figure 4).

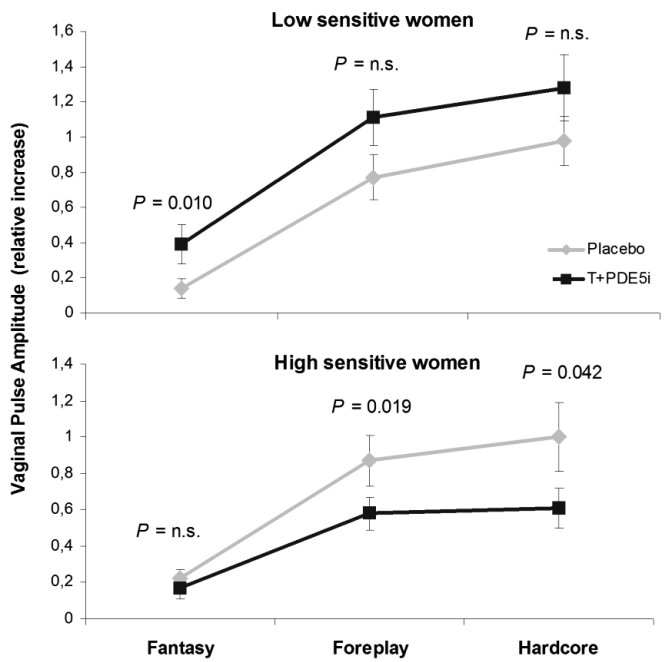

Figure 4. Ambulatory experiment: The effects of T+PDE5i relative to placebo on vaginal pulse amplitude. There was a significant interaction effect between the drug (placebo vs. T+PDE5i) and the group (low vs. high sensitivity for sexual cues), [F(1, 54) = 8.50, *P* = 0.005].

In the subgroup of women with low sensitivity for sexual cues (N = 29) the experience of genital arousal was significantly higher during the T+PDE5i treatment in the fantasy (M = 14.41, SE = 1.21), foreplay (M = 21.00, SE = 1.36) and explicit sex condition (M = 22.34, SE = 1.49) compared to placebo (fantasy; M = 9.59, SE = 1.02, [$F(1,28) = 21.95$, $P < 0.001$], foreplay; M = 14.34, SE = 1.23, [$F(1,28) = 31.92$, $P \leq 0.001$], explicit sex; M = 16.31, SE = 1.44, [$F(1,28) = 21.11$, $P < 0.001$], Figure 5).

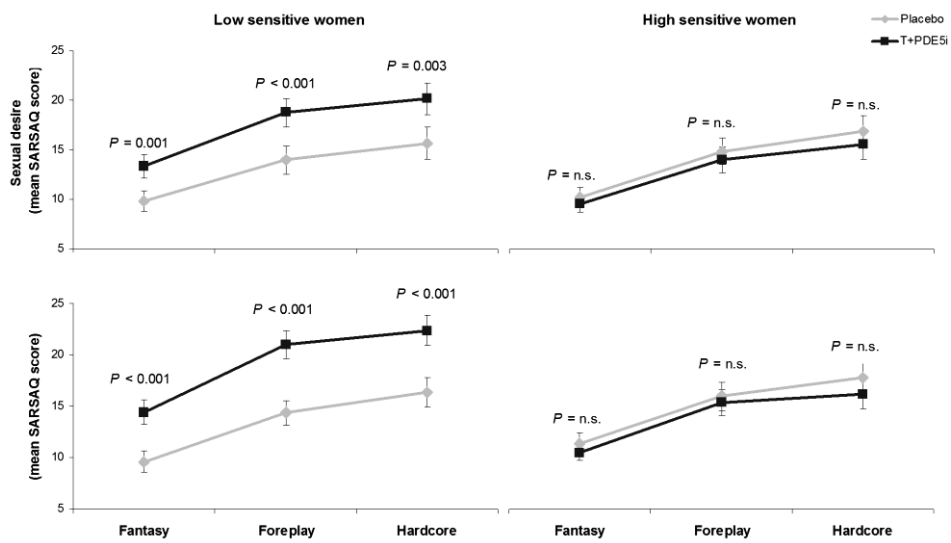

Figure 5. Ambulatory experiment: The effects of T+PDE5i relative to placebo on the experience of genital arousal and sexual desire. T+PDE5i, relative to placebo and under all stimulus conditions, produced higher levels of the experience of genital arousal and sexual desire in women with a relative insensitivity for sexual cues. There was a significant interaction effect between the drug (placebo vs. T+PDE5i) and the group (low vs. high sensitivity for sexual cues) for both measures (experience of genital arousal: [$F(1, 54) = 21.24$, $P < 0.001$], subjective report of sexual desire: [$F(1, 54) = 13.48$, $P < 0.001$]). SARSAQ = Sexual Arousal Response Self-Assessment Questionnaire

The experience of sexual desire was significantly higher during the T+PDE5i treatment in the fantasy (M = 13.34, SE = 1.18), foreplay (M = 18.76, SE = 1.47)

and explicit sex condition (M = 20.14, SE = 1.62) compared with placebo (fantasy; M = 9.97, SE = 1.01, [F(1,28) = 13.39, *P* = 0.001], foreplay; M = 13.97, SE = 1.44, [F(1,28) = 16.65, *P* < 0.001], explicit sex; M = 15.69, SE = 1.58, [F(1,28) = 10.86, *P* = 0.003], Figure 5).

High Sensitive Women

During the T+PDE5i treatment within the high sensitive subgroup (N = 27), the VPA showed lower levels in the foreplay (M = 0.58, SE = 0.09) and explicit sex condition (M = 0.61, SE = 0.11) compared with placebo (foreplay; M = 0.87, SE = 0.14, [F(1,26) = 6.28, *P* = 0.019], explicit sex; M = 1.0, SE = 0.19, [F(1,26) = 4.60, *P* = 0.042], Figure 4).

Effects of T+PDE5i Relative to Placebo: Bedroom Experiment

During the bedroom experiment treatment with T+PDE5i relative to placebo produced overall statistically significant increases in sexual functioning. There were no treatment-order effects observed.

Low Sensitive Women

Sexual Satisfaction During Sexual Events. Treatment with T+PDE5i produced a statistically significant [F(1,23) = 6.34, *P* = 0.019] increase in sexual satisfaction (M = 3.36, SE = 0.16) as compared with placebo (M = 2.96, SE = 0.20), Figure 6.

Weekly Diary. Our analysis of the weekly reports revealed somewhat higher levels (statistically not significant) during treatment with T+PDE5i as compared with placebo for sexual desire (M = 2.72, SE = 0.16 vs. M = 2.54, SE = 0.16), genital arousal (M = 2.80, SE = 0.14 vs. M = 2.59, SE = 0.15), and sexual improvement (M = 1.40, SE = 0.16 vs. M = 1.19, SE = 0.09).

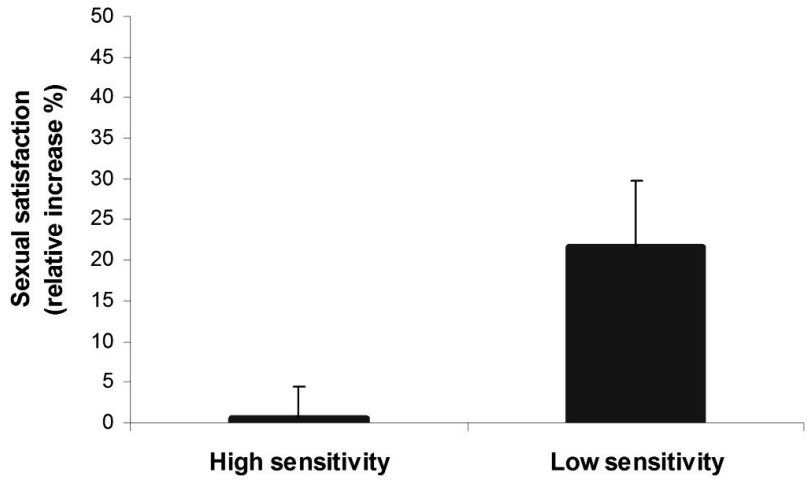

Figure 6. Bedroom experiment: The relative increase in sexual satisfaction following T+PDE5i treatment. In women with a relative insensitivity for sexual cues treatment with T+PDE5i increased sexual satisfaction with 22%, relative to placebo, while in the high sensitive group there was no such relative increase.

Monthly Diary. The results of the SEI questionnaire showed that during the T+PDE5i treatment participants perceived more improvement in sexual arousal/desire (M = 2.07, SE = 0.18) compared with placebo (M = 1.62, SE = 0.14), [F(1,28) = 7.04, *P* = 0.013]. Moreover, this improvement was more frequently attributed to the medication during the T+PDE5i treatment (M = 1.52, SE = 0.09) compared with placebo (M = 1.21, SE = 0.08), [F(1,28) = 7.66, *P* = 0.010], Figure 7.

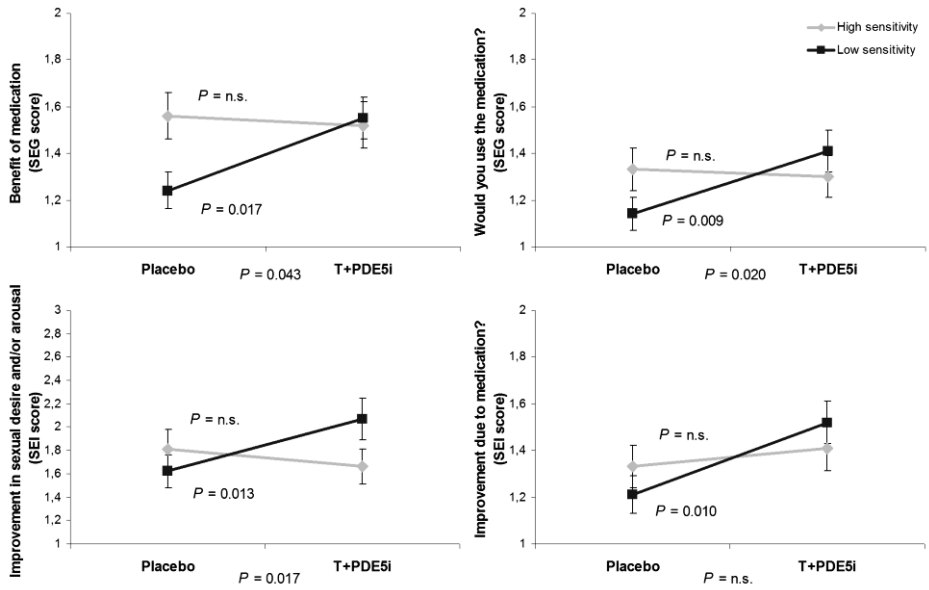

Figure 7. Bedroom experiment: The effects of T+PDE5i relative to placebo on the subjective indices of the Subjective Evaluation of Gain (SEG) and Subjective Evaluation of Improvement (SEI) questionnaire. There was a significant interaction effect between the drug (placebo vs. T+PDE5i) and the group (low vs. high sensitivity for sexual cues) for all dependent measures, with exception of "improvement due to medication". Benefit from medication: [$F(1, 54) = 4.30$, $P = 0.043$], would you use the medication: [$F(1, 54) = 5.80$, $P = 0.020$], and improvement in sexual desire and/or arousal: [$F(1, 54) = 6.03$, $P = 0.017$].

The results of the SEG questionnaire showed that during the T+PDE5i treatment, low sensitive participants experienced more benefit from the study medication (M = 1.55, SE = 0.09) than from the placebo (M = 1.24, SE = 0.08), [$F(1,28) = 6.41$, $P = 0.017$]. Low sensitive women also indicated that when the drugs are available via prescription they would use T+PDE5i (M = 1.41, SE = 0.09) more than placebo, (M = 1.14, SE = 0.07), [$F(1,28) = 7.93$, $P = 0.009$], Figure 7.

High Sensitive Women

The results of the high sensitive subgroup showed no statistically significant drug effects in the bedroom experiment.

Treatment with T+PDE5i was well tolerated. Only "mild" to "moderate" adverse were reported. All adverse events were caused by the PDE5i component (see Table 2). During the study, no serious adverse events were reported.

Table 2. Treatment related adverse events

	T+PDE5i (%)*	Placebo (%)†	Total (%)‡
Flushing	23.0	3.7	9.6
Headache	15.9	2.4	7.4
Lightheadedness	0.9	0.6	3.9
Dizziness	1.1	0.2	4.2

* Percentage T+PDE5i medication = AE T+PDE5i/552 units

† Percentage placebo medication = AE placebo/542 units

‡ Percentage total medication = AE total/1,636 units

T = testosterone; PDE5i = phosphodiesterase type 5 inhibitor; AE = adverse events

DISCUSSION

We suggested different causal mechanisms for the lack of sexual desire in women with HSDD, which was taken into account when designing and developing new medicines for HSDD. In the present article, we have focused on the results of treatment with sublingual testosterone combined with a PDE5i, developed for women with HSDD and a relatively insensitive brain system for sexual cues.

In earlier experiments, we have shown that sublingual testosterone activates central sexual mechanisms thereby increasing salience of sexual stimuli, which affects central sexual stimulation and can thereby increase sexual motivation [7,8]. Central sexual stimulation is a prerequisite for PDE5i to be effective. In the present study, we again showed that sublingual testosterone influences central sexual processing, reflected by the increase in preconscious attentional bias in low sensitive women. Treatment with T+PDE5i affects mechanisms involved in processing of sexual information, and improves physiological and subjective sexual responding under institutional psychophysiological lab conditions, in particular demonstrated for women with HSDD and low sensitivity for sex [5,6]. The present results again show that T+PDE5i influences central sexual information processing, as reflected in an increase in preconscious attentional bias for sex in low sensitive women and that this treatment caused increased physiological sexual responding in the fantasy condition, and more pronounced, significant effects in subjective measures of sexual functioning under all stimulus conditions in an ambulatory psychophysiological lab in the domestic setting. These results demonstrate that the findings of van der Made et al. determined under institutional lab conditions also hold in a psychophysiological measurement setting at home [5,6]. In extension, and more importantly, treatment with T+PDE5i, as compared with placebo, caused a statistically significant higher level of sexual satisfaction during sexual events in women with a relatively low sensitive system for sex. In the active drug condition, these women also reported improvement on other subjective measures of sexual functioning; they indicated that they experienced positive effects, that they attributed these effects to the medication, and that they would use it if available via

prescription. These findings are of importance because they indicate that T+PDE5i's positive effect was also meaningful to the subjects, and not just a statistically significant increase which was not perceived as such by the subjects.

An important difference between our on-demand approach and chronic testosterone treatments is that the effects in our treatment form occur nearly directly (about 3.5 hours post-dose) compared with chronic formulations, which take weeks to take effect. As described in chapter 3, the unbound fraction of testosterone is the most bioactive testosterone and therefore responsible for the effects on behavior. If free fraction testosterone increases much later under chronic treatment, it is expected that the pharmacodynamic effect, i.e., increases in sexual motivation and desire, will occur in the same time-dependent manner [17]. This is consistent with several studies describing the effects of a testosterone patch on sexual behavior as described in chapter 3. As stated in chapter 2 and chapter 3, one decisive factor in the time-dependent difference in behavioral effects between sublingual or chronic treatment with testosterone is the influence of an SHBG saturation threshold. This saturation threshold mechanism will ensure that the use of sublingual testosterone is accompanied by an increase in free levels of testosterone within 15 minutes after administration. These differences between the acute, on-demand dosage forms containing testosterone and the chronic dosage forms are of such importance because the amount of exposure to testosterone is much less in the former. Less exposure means fewer side effects, and in the case of testosterone, this would mean that the chance of adverse events like increased hair growth and acne are much less likely to occur. Indeed, testosterone-related side effects were not observed during this study, and are neither to be expected to occur during long-term treatment. The medication was well tolerated, with only mild to moderate, transient adverse events which were caused by the PDE5i component. The majority of studies investigating chronic testosterone administration in the treatment of women's sexual behavior have been done in naturally or surgically menopausal women [18–20]. The present study demonstrates that T+PDE5i is well tolerated and effective in premenopausal as well as in postmenopausal women. The results revealed no interaction with menopausal status or hormonal contraception.

The medication regimes in the present study were relatively short (3 weeks for the bedroom experiment). However, seeing that drug efficacy in a phase III testosterone gel trial was not statistically significant higher than placebo during 6 months of therapy [21], the current results are promising. More extensive research is needed to establish the effects of T+PDE5i over a longer period of time, but we expect the large placebo effects, which were also encountered in the current study, to decrease over time. Since the present study already shows significant superior efficacy as compared with placebo, this can even be expected to increase when tested during a longer period of time.

In the present study, the ratio between low and high sensitive subjects was approximately 50/50 (N = 29 vs. N = 27). This 50/50 division between low and high sensitivity is not inherent to the calculation which we used to define sensitivity; if a subject is faster in color naming of masked erotic words than of non-erotic words, she is low sensitive. If she is slower in color naming of masked erotic words, she is high sensitive. In our previous studies this division was approximately 40/60 [5,6]. Together, these studies measured 41 women with HSDD. With the present data, we have determined the sensitivity to sexual cues in 97 women. Taken together, our study population revealed a 45/55 division in low and high sensitive subjects. More research in a higher number of women is necessary to determine if this ratio between low and high sensitive subjects with HSDD is generalizable.

The low and high sensitive women only differed from each other on the Stroop task, and on the measures of sexual arousal under condition of T+PDE5i, as compared with placebo. They did not differ in severity of their sexual complaints or any other trait measure. As sexual cue sensitivity is testosterone dependent (see chapter 3), combinations of biological markers reflecting (and influencing) the activity of the androgen and serotonin systems (see chapter 3) may well be able to differentiate between high and low sensitive women. Without such markers, or without the emotional Stroop task, assessing cue sensitivity in the broader HSDD population will be difficult.

The use of an on-demand therapy for HSDD seems counterintuitive. Why would women with low desire bother to take an on-demand sex drug if she is not “in

the mood" for sex? One of the main DSM-IV-TR criteria for HSDD is that the desire problems cause marked distress or interpersonal difficulties. Women seeking help for HSDD do so because of their distress, i.e., they want to want to have sex. These women are thus prepared to take a medicine on-demand, in the hope that several hours later, they will be more receptive to their spouses' advances or more willing to initiate sex.

Based on these results we tentatively conclude that the combination of sublingual testosterone and a PDE5i is a safe and promising potentially effective pharmacotherapy for women with HSDD as the result of an insensitive system for sexual cues. Future studies will have to confirm this.

REFERENCES

1. Laumann EO, Paik A, Rosen RC. Sexual dysfunction in the united states: Prevalence and predictors. JAMA 1999;281:537–44.

2. American Psychiatric Association. Diagnostic and statistical manual of mental disorders. 4th edition. Text revision. Washington, DC: American Psychiatric Association; 2000.

3. Bancroft J. Central inhibition of sexual response in the male: A theoretical perspective. Neurosci Biobehav Rev 1999;23:763–84.

4. Janssen E, Bancroft J. The dual control model: The role of sexual inhibition and excitation in sexual arousal and behaviour. In: Janssen E, ed. The psychophysiology of sex. Bloomington: Indiana University Press; 2007:197.

5. van der Made F, Bloemers J, Yassem WE, Kleiverda G, Everaerd W, van Ham D, Olivier B, Koppeschaar H, Tuiten A. The influence of testosterone combined with a PDE5-inhibitor on cognitive, affective, and physiological sexual functioning in women suffering from sexual dysfunction. J Sex Med 2009;6:777–90.

6. van der Made F, Bloemers J, van Ham D, El Yassem W, Kleiverda G, Everaerd W, Olivier B, Tuiten A. Childhood sexual abuse, selective attention for sexual cues and the effects of testosterone with or without vardenafil on physiological sexual arousal in women with sexual dysfunction: A pilot study. J Sex Med 2009;6:429–39.

7. Tuiten A, van Honk J, Verbaten R, Laan E, Everaerd W, Stam H. Can sublingual testosterone increase subjective and physiological measures of laboratory-induced sexual arousal? Arch Gen Psychiatry 2002;59:465–6.

8. Tuiten A, Van Honk J, Koppeschaar H, Bernaards C, Thijssen J, Verbaten R. Time course of effects of testosterone administration on sexual arousal in women. Arch Gen Psychiatry 2000;57:149–53; discussion 155–6.

9. Rosen RC. Sildenafil: Medical advance or media event? Lancet 1998;351:1599–600.

10. Goldstein I, Lue TF, Padma-Nathan H, Rosen RC, Steers WD, Wicker PA. Oral sildenafil in the treatment of erectile dysfunction. sildenafil study group. N Engl J Med 1998;338:1397–404.

11. Bloemers J, Gerritsen J, Bults R, Koppeschaar H, Everaerd W, Olivier B, Tuiten A. Induction of sexual arousal in women under conditions of institutional and ambulatory

laboratory circumstances: A comparative study. J Sex Med 2010;7:1160–76.

12. Available at: http://www.mobihealth.com/services/en/mh_mobile.php (accessed October 1, 2012).

13. Williams JM, Mathews A, MacLeod C. The emotional stroop task and psychopathology. Psychol Bull 1996;120:3–24.

14. Morokoff PJ, Heiman JR. Effects of erotic stimuli on sexually functional and dysfunctional women: Multiple measures before and after sex therapy. Behav Res Ther 1980;18:127–37.

15. Heiman J, Hatch J. Affective and physiological dimensions of male sexual response to erotica and fantasy. Basic Appl Soc Psych 1980;1:315–27.

16. Tabachnick BG, Fidell LS. Using multivariate statistics. Boston: Allyn and Bacon; 2007.

17. van Rooij K, Bloemers J, de Leede L, Goldstein I, Lentjes E, Koppeschaar H, Olivier B, Tuiten A. Pharmacokinetics of three doses of sublingual testosterone in healthy premenopausal women. Psychoneuroendocrinology 2012;36:773–81.

18. Shifren JL, Braunstein GD, Simon JA, Casson PR, Buster JE, Redmond GP, Burki RE, Ginsburg ES, Rosen RC, Leiblum SR, Caramelli KE, Mazer NA. Transdermal testosterone treatment in women with impaired sexual function after oophorectomy. N Engl J Med 2000;343:682–8.

19. Sherwin BB. Randomized clinical trials of combined estrogen-androgen preparations: Effects on sexual functioning. Fertil Steril 2002;77(suppl 4):S49–54.

20. Simon J, Braunstein G, Nachtigall L, Utian W, Katz M, Miller S, Waldbaum A, Bouchard C, Derzko C, Buch A, Rodenberg C, Lucas J, Davis S. Testosterone patch increases sexual activity and desire in surgically menopausal women with hypoactive sexual desire disorder. J Clin Endocrinol Metab 2005;90:5226–33.

21. Available at: http://www.biosantepharma.com/News-Releases.php?ID=121411 (accessed October 2, 2012).

Chapter 5

Toward Personalized Sexual Medicine (Part 3): Testosterone combined with a serotonin1A receptor agonist increases sexual satisfaction in women with HSDD and FSAD, and dysfunctional activation of sexual inhibitory mechanisms

Kim van Rooij, Saskia Poels, Jos Bloemers, Irwin Goldstein, Jeroen Gerritsen, Diana van Ham, Frederiek van Mameren, Meredith Chivers, Walter Everaerd, Hans Koppeschaar, Berend Olivier and Adriaan Tuiten

Journal of Sexual Medicine 2013;10:824-837

ABSTRACT

Introduction. Among other causes, low sexual desire in women may result from dysfunctional activation of sexual inhibition mechanisms during exposure to sex. Administration of sublingual 0.5 mg testosterone (T) increases the sensitivity of the brain to sexual cues, which might amplify sexual inhibitory mechanisms further in women already prone to sexual inhibition. Sexual stimulation might elicit a prefrontal cortex (PFC)-mediated phasic increase in sexual inhibition, in which activity of 5-hydroxytryptamine (5-HT, serotonin) is involved. A single dose of 5-HT_{1A} receptor agonist (5-HT_{1A}ra) might reduce the sexual stimulation induced PFC-mediated sexual inhibition during a short period after administration. Consequently, treatment with a single dose of T+5-HT_{1A}ra might enhance sexual responsiveness, particularly in women exhibiting sexual inhibition.

Aim. To investigate if treatment with a single dosage of T+5-HT_{1A}ra will produce improvement in sexual functioning in women with Hypoactive Sexual Desire Disorder (HSDD) as the result of dysfunctional high sexual inhibition.

Methods. Fifty-four women were divided on the basis of their excitatory or inhibitory responses during T+phosphodiesterase type 5 inhibitor (PDE5i) in low (N = 26) and high inhibitors (N = 28). Physiological and subjective indices of sexual functioning were measured in a participant-controlled ambulatory psychophysiological experiment at home (the first week of each drug treatment). In a bedroom experiment (the subsequent 3 weeks), sexual functioning was evaluated by event, week, and monthly diaries.

Main Outcome Measures. Subjective: sexual satisfaction, experienced genital arousal, sexual desire. Physiological: vaginal pulse amplitude.

Results. Women with high inhibition show a marked improvement in sexual function in response to treatment with T+5-HT_{1A}ra relative to placebo and relative to T+PDE5i.

Conclusions. The present study demonstrated that on-demand T+5-HT_{1A}ra is a potentially promising treatment for women with HSDD, particularly for those women who are prone to sexual inhibition.

INTRODUCTION

Sexual dissatisfaction is a complaint with a high prevalence which negatively interferes with psychological and social well-being. In women, the most common complaint is low sexual desire, which is classified as Hypoactive Sexual Desire Disorder (HSDD) [1]. As stated in previous chapters, HSDD might result from a relative insensitivity for sexual cues or might be caused by dysfunctional activation of sexual inhibitory mechanisms during sexual stimulation. In chapter 4, it is demonstrated that combined treatment with testosterone and a phosphodiesterase type 5 inhibitor (T+PDE5i) improves sexual functioning in women with HSDD, and who have a relatively insensitive system for sexual cues. As expected, some women deteriorated by treatment with T+PDE5i. This subgroup of women might be prone to sexual inhibition during sexual stimulation (see part 1 of this series, chapter 3). In the present study, our focus is on treatment of these women who did not respond or who had a decreased response to on T+PDE5i, for which we assume, as the result of increased activation of central inhibitory mechanisms.

It is widely accepted that the prefrontal cortex (PFC) is involved in the inhibitory control of human behavior [2], including sexual behavior [3,4] (see also chapter 3). An important mediator of inhibitory mechanisms is the neurotransmitter 5-hydroxytryptamine (5-HT, serotonin) [5], which also exerts abundant inhibitory effects via the PFC [6]. We assume that a sexual event, which is consciously or subconsciously negatively valenced—which is dependent on an individual's trait properties, their experiences, and the particular circumstance in which an event occurs—can induce a phasic increase in serotonergic activity in areas of the PFC involved in sexual inhibition. Acute treatment with a serotonin$_{1A}$ receptor agonist (5-HT$_{1A}$ra) decreases serotonergic activity for a short time after administration. Accordingly, acute treatment with a 5-HT$_{1A}$ra might decrease sexual-stimuli-induced phasic serotonergic inhibitory control in the PFC, which in turn might prevent or reduce the inhibitory response to sexual cues in women with HSDD and prone to sexual inhibition. We hypothesize that for this subgroup of women with HSDD, and enhanced activation of sexual inhibitory mechanisms elicited by sexual stimulation, treatment with T+5-HT$_{1A}$ra will improve their physiological and subjective sexual responses during ambulatory lab conditions, and their sexual satisfaction during sexual events.

METHODS

The 56 heterosexual women who participated in this study signed a written informed consent and received reimbursement for their participation. The local medical ethics committee (Stichting Therapeutische Evaluatie Geneesmiddelen Medisch Ethische Toetsingscommissie, Almere, the Netherlands) approved this study, which was carried out in agreement with International Conference on Harmonization-Good Clinical Practice (ICH-GCP), and monitored by a certified Contract Research Organization (CRO) (PSR Group, Hoofddorp, the Netherlands).

Women were eligible if they were healthy, between 21 and 70 years, and had a diagnosis of HSDD or Female Sexual Arousal Disorder (FSAD) according to the Diagnostic and Statistical Manual of Mental Disorders-Fourth Edition (Text Revision) DSM-IV-TR criteria [1]. Subjects were diagnosed by an experienced psychologist. If the subject was diagnosed with any psychiatric disorder other than HSDD or FSAD they were excluded, also any treatment for female sexual dysfunction within 7 days before or during the study was excluded. Furthermore women were excluded if they were using oral contraceptives containing antiandrogens or more than 50 µg estrogen, Cytochrome P450 3A4 (CYP3A4) inhibitors, CYP3A4 inducers, nitrates, monoamine oxidase inhibitors, calcium channel blockers, antidepressants, opiates, and medicinal herbs like St. John's wort. Cardiovascular exclusions included a history of myocardial infarction, stroke or life-threatening arrhythmia within the prior 6 months, uncontrolled hypertension, atrial fibrillation/flutter or any other significant abnormality observed on electrocardiogram (ECG). Gynecological exclusions included pelvic inflammatory disease, vaginal infection, previous prolapse and incontinence surgery affecting the vaginal wall, abnormal uterine bleeding patterns, perimenopausal hormonal status, pregnancy and breastfeeding in the past 6 months. Lastly women were excluded with clinically relevant endocrine disease, neurological disease, severe or acute liver disease, history of severe hepatic impairment, body mass index above 35, and vision impairment.

Women were recruited and enrolled from referrals, newspaper advertisements, the Internet, and our own database. To determine eligibility, participants were screened 4 weeks prior to study entry. In addition to an assessment of medical history with detailed sexual, gynecological, and psychological history, all subjects received a physical examination including a 12-lead ECG, a vaginal culture test to

exclude infection, standard biochemistry and hematological laboratory tests, and pelvic examination. Biochemical parameters including hematology, liver function tests, electrolytes, creatinine, uric acid, glucose and lipids were collected at baseline, following each treatment period, and at the final follow-up visit. Serum concentrations of total testosterone, sex hormone binding globulin (SHBG), luteinizing hormone (LH), follicle-stimulating hormone (FSH), estradiol, progesterone, and prolactin were measured at baseline. All subjects also had their thyroid status assessed; thyroid stimulating hormone and free thyroxine.

A urine pregnancy test was administered to all women of childbearing potential. Investigators monitored and followed up any reports of adverse events. On a pretreatment visit, a trained female experimenter explained the study requirements and procedures to the participants. A practice trial was conducted, including insertion of the vaginal photoplethysmograph and a practice session of the emotional Stroop task.

Study Design

In a randomized, double-blind, placebo-controlled, cross-over design, each participant underwent three different medication treatments: (i) **placebo**: placebo for testosterone (cyclodextrin solution without testosterone) and placebo for the PDE5i (PDE5i = sildenafil) and 5-HT_{1A}ra (5-HT_{1A}ra = buspirone) (powder-filled gelatin capsule without sildenafil/buspirone); (ii) **T+PDE5i:** the combination of testosterone (0.5 mg) sublingually with cyclodextrin as carrier and sildenafil (50 mg, hidden in a powder-filled gelatin capsule); (iii) **T+5-HT_{1A}ra**; the combination of testosterone (0.5 mg) sublingually with cyclodextrin as carrier and buspirone (10 mg, hidden in a powder-filled gelatin capsule). A single medication regime lasted 4 weeks and the order of the three medication regimes was randomized. Between each medication regime there was a washout period of 1 week. Participants received 14 units of medication (throughout the manuscript, 1 medication or 1 unit of medication means the combination of the sublingually administered solution *and* the oral capsule) during each medication regime; instructions were given to have at least 48 hours between two medication intakes. Participants were instructed to take the cyclodextrin solution (testosterone or placebo) sublingually 4 hours prior to each measurement session or sexual event and rinse it under the tongue for 1 minute.

They were instructed to ingest the capsule (sildenafil, buspirone, or placebo) 2.5 hours later. The sequence of the drugs and time frame were such that the pharmacological effects of the PDE5i and 5-HT_{1A}ra coincide with the window of T-induced behavioral effects, approximately 3–6 hours after T administration. Following the completion of a drug regime, the research physician took in any leftover medication of the previous regime and handed out the next regime's medication box.

Study Procedure

Ambulatory Psychophysiological At-Home Experiment

Participants visited the study site 12 times during a period of 26 weeks for screening and safety control visits. In the first week of each 4-week medication regime, participants underwent three experimental psychophysiological measurement sessions (under condition of study medication) at home using participant-controlled (i.e., no experimenters were present) ambulatory laboratory [7]. See Figure 1 in chapter 4, for an overview of the study design and procedures.

In premenopausal women not using oral contraceptives, experimental days were within the first 10 days after their menstruation. Users of oral contraceptives were not measured during their pill-free period.

During each experimental session, subjects engaged in 2 minutes each of: (i) self-induced sexual fantasy; (ii) viewing an erotic film clip depicting sexual foreplay; and (iii) viewing an erotic film depicting explicit heterosexual intercourse. The sexual fantasy condition was preceded by a 6-minute baseline establishment period during which subjects viewed a neutral film clip. The foreplay and heterosexual intercourse films were preceded by 2 minutes return to baseline periods (see Stimuli, Apparatus, and Measures below, for a detailed description of the neutral and erotic stimulus material). At the start of the experimental session, and after each exposure to sexual stimuli (the fantasy session and film clips), we measured “experiences of genital arousal” and “sexual desire” by means of the Sexual Arousal Response Self-Assessment Questionnaire (SARSAQ; see Stimuli, Apparatus, and Measures below, and [8]). During all neutral and

sexual stimulus sessions, the vaginal pulse amplitude (VPA) was continuously measured (see Stimuli, Apparatus, and Measures below). Before the baseline establishment period and finally as the last element of the experimental session, we measured biases in preconscious attention for sexual cues by means of an emotional Stroop task.

Bedroom Experiment

For the remaining 3 weeks, participants were instructed to use at least one medication per week, with an attempt at a sexual event (cuddling, coitus, or masturbation). For the remainder, they were free to take their medication whenever they wanted but not during 2 consecutive days. When they had experienced a sexual event they were asked to fill out an event diary within 24 hours and a diary at the end of each week.

Stimuli, Apparatus, and Measures

Audiovisual Stimuli

For this study, neutral, erotic foreplay, and explicit sex film clips were used. Neutral clips were 6-minute and 2-minute clips from Dutch action films. The 6-minute neutral film clips were used to establish a baseline VPA. Erotic foreplay clips were 2-minute clips showing kissing, caressing, and cunnilingus, but no fellatio. Explicit sex clips were 2-minute clips showing cunnilingus and coitus, including visible penetration. The erotic film footage was selected and edited by female research associates and then rated by other female research associates to ensure that all clips were within set specifications. All digitally sampled film clips were presented using Presentation software (Neurobehavioral Systems, Albany, CA, USA). For a more detailed description see Bloemers et al. [7].

Ambulatory Laboratory

The ambulatory laboratory is based on the MobiHealth Mobile remote monitoring system (MobiHealth B.V., Enschede, The Netherlands) [9]. Study-specific functionality of this system enables VPA measurements, stimulus presentation, and execution of emotional Stroop tasks to be performed at an

arbitrary time and location (e.g., house of a participant). This laboratory is operated autonomously by the participant. The ambulatory laboratory transmits all measured data to a secure central database server, at which the researcher can obtain the data for further analysis. This method allows for a more ecologically valid psychophysiological measurement of sexual responding. See for a detailed description the study by Bloemers et al. [7].

Main Outcome Measures: Ambulatory Laboratory

VPA

The VPA reflects phasic changes in vaginal engorgement corresponding with each heartbeat. VPA was measured using a vaginal photoplethysmograph, a tampon-shaped device containing an infrared light-emitting diode and a photosensitive light detector (photodiode). For a detailed description of the VPA, see chapter 4.

SARSAQ

The SARSAQ is a 10-item self-report questionnaire using a seven-point Likert scale (ranging from “not at all” to “extremely”), adapted from Morokoff and Heiman [10] and Heiman and Hatch [11]. It measures current subjective feelings of genital arousal and sexual desire. Five items concern subjective feelings of genital responding, and five items concern subjective feelings of sexual desire. The SARSAQ was administered via the ambulatory laboratory laptop whereby the number keys (from 1 to 7) were used to complete the questionnaire.

Main Outcome Measures: Bedroom Experiment

Event Diary

In this secure Web-based diary, subjects were asked 10 questions (one open-ended, four multiple-choice, five 5-point Likert scale items) concerning the type, duration, pleasantness, and intensity of the sexual event. Participants were instructed to fill out the event diary within 24 hours following each sexual event (e.g., cuddling, coitus, masturbation).

Week Diary

Women were also instructed to fill out the secure Web-based week diary once a week during each medication regime. Subjects were asked their experiences during the past week regarding: (i) sexual desire (six-point Likert scale: 1 = nothing to 6 = much); (ii) vaginal arousal (six-point Likert scale: 1 = nothing to 6 = much); (iii) sexual improvement/deterioration (four-point Likert scale: 1 = nothing to 4 = much); and (iv) if they could attribute the improvement/deterioration to the medicines (yes/no).

Subjective Evaluation of Improvement (SEI)

The SEI questionnaire was used to determine, after each medication regime, if the subject felt there had been an overall improvement in sexual desire, sexual arousal or both, and if they attribute this improvement to the medication. Subjects answered yes or no to both questions. The SEI was administered as a pen and paper test.

Subjective Evaluation of Gain (SEG)

The SEG questionnaire was used to determine, after each medication regime, if the subject felt that she had any meaningful benefit from the study medication, and if she would use it if it were available by prescription. Subjects answered yes or no to both questions. The SEG was administered as a pen and paper test.

Measures: On Site

Hormonal Measures

Serum total testosterone, serum estradiol, serum progesterone, serum prolactin, serum LH, serum FSH, and serum SHBG were measured through electrochemiluminescence radioimmunoassay with COBAS kits of Roche Diagnostics (Mannheim, Germany), using a Modular E170 at OLVG Hospital (Roche Diagnostics, Amsterdam, the Netherlands). The measuring range for testosterone was 0.087–52.0 nmol/L. The coefficient of variation was 8%.

Data Transformation and Reduction

Ambulatory Experiment

For each drug treatment condition, we used the mean levels of the 3 experimental days of the experience of genital arousal, sexual desire, preconscious attentional bias for sexual cues and VPA, respectively, for further analyses.

Bedroom Experiment

Based on high Cronbach's alphas of the six items measuring duration, pleasantness, and intensity of the sexual events (measured by the event diary) during the different drug conditions (placebo: $\alpha = 0.95$; T+PDE5i: $\alpha = 0.95$; T+5-HT_{1A}ra: $\alpha = 0.93$) we calculated the mean of these items, as a measure of "sexual satisfaction." Each question of the week diary, SEG and SEI questionnaire were analyzed separately. To analyze sexual satisfaction and the week diary items, we calculated the average of each dependent variable over the 3 weeks of drug treatment.

Different Drug Treatment Responders

The T+PDE5i negative responders were defined as follows. We calculated the reversed scores of items measuring sexual satisfaction and other positive experiences of sexual functioning during the T+PDE5i treatment period (for items of both the event diary and week diary). We used "sexual desire" and "improvement in sexual function" items from the week diaries and "pleasure", "horny," and "genital arousal" of the event diaries. The raw item scores were standardized and summated, representing the variable "Sexual Inhibition." Cronbach's alpha over the five items was sufficiently high ($\alpha = 0.90$) to summate. Subjects with negative summated z-scores were termed "low inhibitors" and subjects with positive summated z-scores were termed "high inhibitors." We used this division of high and low inhibitors as between-subject factor in analyzing the effects of T+5-HT_{1A}ra compared with placebo and to T+PDE5i, respectively, on sexual experiences during the bedroom experiment.

Statistical Analysis

Demographic data were analyzed to investigate possible group differences (low vs. high inhibitors) with an independent *t*-test if the data were normally distributed. For non-normally distributed data, the Mann–Whitney test was used. Categorical data were compared between the groups with a chi-square test.

For each of the dependent variables, separate 2 × 2 repeated measures analyses of variance (repeated measures ANOVAs) were carried out. The within-subject factor had two levels (placebo and T+5-HT_{1A}ra or T+PDE5i and T+5-HT_{1A}ra). The between-subject factor also had two levels (low inhibition vs. high sexual inhibition). For all analyses menopausal status (premenopausal vs. postmenopausal) and hormonal contraception (hormonal contraception vs. nonhormonal contraception) were analyzed in a repeated measures ANOVA for each dependent variable.

We have chosen to report an ANOVA for each of the dependent variables separately, instead of analyzing these variables together in a multivariate analysis (MANOVA). The reasons for this choice are twofold: first, interest goes out into effects on each of the dependent variables separately as they are quite different in nature (e.g., we have a physiological measure such as VPA as well as self-report measures such as diaries under different experimental conditions); second, when missing data are scattered over all dependent variables (e.g., sometimes women did not have sexual activities, other times a VPA measurement failed), participants are eliminated completely from MANOVA leading to unnecessary loss of power.

An assumption of ANOVA is that the means of the variables are normally distributed. Tabachnick and Fidell indicate that the F-test is robust against violations of normality of variables if there are at least 20 degrees of freedom for error in a univariate ANOVA, provided that there are no outliers [12]. In most of our analyses the number of degrees of freedom is 52 (in some of the analyses the number of degrees of freedom is a bit lower because sometimes women did not have sexual activities). The dependent variables have no outliers, except for the nine dependent variables of the VPA (three drug conditions: placebo,

T+PDE5i, and T+5-HT_{1A}ra and three stimuli conditions), who have a few, so, except for the nine dependent variables of the VPA the assumptions of ANOVA are met. For VPA we created log-transformed variables and this removed almost all of the outliers. To investigate the robustness of the results for VPA we carried out an analysis on the original variables as well as on the log-transformed variables. The log-transformed variables yielded an almost identical pattern of significant results as the original analysis. We report the results of the original analysis because the original variables are more easily interpretable.

An alpha level of 0.05 was set for all analyses. We made no adjustment for multiple testing. In parts 2 and 3 of this series, many tests have been carried out and the application of, e.g., the popular Bonferroni correction would lead to an extreme loss in power. Although we do not control for chance capitalization, nearly all analyses show significant results in the same direction, showing that these findings are not by chance.

Less than 5% of the data were missing. When a data for an experimental day during the ambulatory experiment were missing, the data were estimated by taking the averaged data over the other 2 experimental days of the same drug condition.

RESULTS

As described in chapter 4, the analysis in the T+PDE5i condition was done with 56 women, while for the T+5-HT_{1A}ra condition the data of 54 women have been used. There were two data exclusions, both exclusions were caused by factors that retrospectively biased the study results during the T+5-HT_{1A}ra drug regime; one subject lost her own business due to financial problems and another woman had severe maxillary sinusitis, which in her opinion influenced her sexual functioning during the T+5-HT_{1A}ra regime. All baseline hormonal values were in the normal female reproductive and/or postmenopausal range. None of the dependent variables were influenced by menopausal status and hormonal contraception (data not shown). For a detailed description of the study population, see Table 1.

Table 1. Baseline and clinical characteristics of the participants

	Low inhibitors	**High inhibitors**	***P***
N	26	28	
Age, years	39.1 ± 11.3	40.07 ± 9.8	n.s
Body mass index, kg/m^2	25.5 (15.5)	22.2 (15.5)	n.s*
Race, no (%)			
Caucasian	20 (76.9)	27 (96.4)	n.s.†
Black	3 (11.5)	0 (0)	
Asian	2 (7.7)	0 (0)	
Other	1 (3.8)	1 (3.6)	
Parity, no (%)			0.038†
Para 0	12 (46.2)	4 (14.3)	
Para 1	3 (11.5)	5 (17.9)	
Para ≥ 2	11 (42.3)	19 (67.8)	
Menopausal status, no (%)			n.s.†
Premenopausal	18 (69.2)	23 (82.1)	
Postmenopausal	8 (30.8)	5 (17.9)	
Surgical menopause	0 (0)	1 (20)	

	Low inhibitors	High inhibitors	*P*
Contraception, no (%)			n.s.†
Hormonal	16 (61.5)	15 (53.6)	
Combined oral contraceptive pill	6 (37.5)	8 (53.3)	
Progestagen (IUD, implanon)	7 (43.8)	7 (46.7)	
Vaginal ring (progestin and estrogen)	3 (18.8)	0 (0)	
Nonhormonal	5 (19.2)	9 (32.1)	
Condoms	1 (20.0)	1 (11.1)	
Sterilization	3 (60.0)	3 (33.3)	
Sterilization partner	1 (20.0)	5 (55.6)	
None	5 (19.2)	4 (14.3)	
FSD diagnosis, no (%)			n.s.†
Primary HSDD	21 (80.8)	23 (82.1)	
Primary FSAD	5 (19.2)	5 (17.9)	
Duration of current relationship, years	11.56 ± 8.4	16.9 ± 9.2	0.033
Negative sexual experiences (inappropriate touching, [attempted] rape, other), no (%)			0.025†
Yes	7 (26.9)	16 (57.1)	
No	19 (73.1)	12 (42.9)	

Age and relationship duration are in are means ± standard deviation. For the body mass index (BMI) median (range) is described. The BMI is the weight in kilograms divided by the square of the height in meters.

* Mann–Whitney test

† Chi-square test

IUD = intra uterine device; FSD = female sexual dysfunction; HSDD = Hypoactive Sexual Desire Disorder; FSAD = Female Sexual Arousal Disorder; n.s. = not significant

Ambulatory Experiment

When the participants were divided on the basis of their response on T+PDE5i in low (N = 26) and high inhibitors (N = 28), the results showed that treatment with T+5-HT_{1A}ra relative to placebo (or to T+PDE5i) produced statistically significant increases in most dependent variables of the ambulatory experiment in high inhibitors compared with low inhibitors. The interaction effects between drug and group in the ambulatory experiment are described in the caption for each figure.

Effects of T+5-HT1Ara Relative to Placebo

Low Inhibition Group. Our analysis in the low inhibition group (N = 26) revealed no statistically significant effects of the dependent variables in the ambulatory experiment.

High Inhibition Group. The results of the high inhibition group (N = 28) of the VPA during the fantasy condition revealed a statistically significant effect. In the T+5-HT_{1A}ra condition VPA was statistically significant higher (M = 0.33, SE = 0.06) compared with the placebo condition (M = 0.19, SE = 0.05) [F(1,27) = 4.97, *P* = 0.034]. In the foreplay condition this effect was marginally significant (M = 1.17, SE = 0.16) vs. (M = 0.89, SE = 0.13), respectively, [F(1,27) = 4.10, *P* = 0.053]. There were no significant results in the explicit sex condition (see Figure 1).

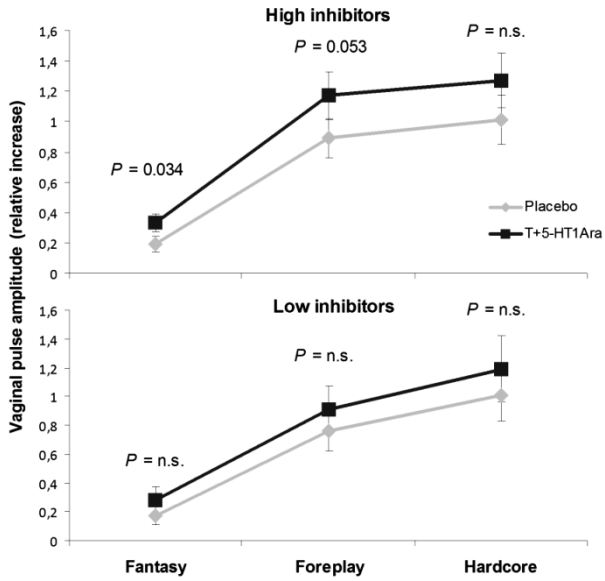

Figure 1. Ambulatory experiment: The effects of T+5-HT_{1A}ra relative to placebo on vaginal pulse amplitude. There was no significant interaction effect between the drug (placebo vs. T+5-HT_{1A}ra) and the group (low vs. high inhibitors), [F(1, 52) = 0.151, *P* = n.s].

The experience of genital arousal was higher after T+5-HT_{1A}ra administration as compared with placebo in the fantasy condition (M = 11.71, SE = 1.01 vs. M = 9.64, SE = 0.94) [F(1,27) = 8.87, *P* = 0.006], the foreplay condition (M = 16.14, SE = 1.26 vs. M = 13.29, SE = 1.08) [F(1,27) = 8.35, *P* = 0.008], and the explicit sex condition (M = 17.86, SE = 1.42 vs. M = 15.18, SE = 1.31) [F(1,27) = 7.03, *P* = 0.013], and see Figure 2.

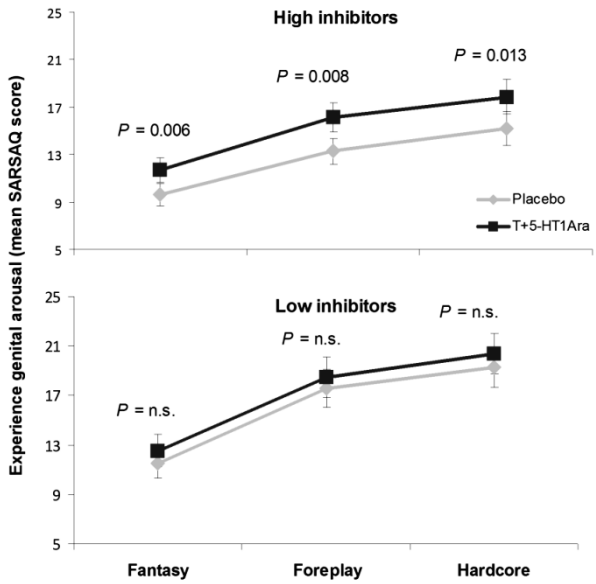

Figure 2. Ambulatory experiment: The effects of T+5-HT_{1A}ra relative to placebo on the experience of genital arousal. T+5-HT_{1A}ra, relative to placebo and under all stimulus conditions, produced higher levels of the experience of genital arousal in women in the high inhibition group. There was no significant interaction effect between the drug (placebo vs. T+5-HT_{1A}ra) and the group (low vs. high inhibitors) for the experience of genital arousal, [F(1, 52) = 1.30, *P* = n.s]. SARSAQ = Sexual Arousal Response Self-Assessment Questionnaire.

Subjective reports of sexual desire were significantly higher during T+5-HT_{1A}ra treatment in the fantasy condition (M = 10.96, SE = 1.03) compared with placebo (M = 8.96, SE = 0.80) [F(1,27) = 6.90, *P* = 0.014]. There were no significant results in the foreplay and explicit sex condition.

Effects of T+5-HT1Ara Relative to T+PDE5i

The results demonstrate that treatment with T+5-HT_{1A}ra (as compared with T+PDE5i) produced no statistically significant differences between levels of physiological (VPA) and subjective indices of sexual functioning (experience of genital arousal and subjective reports of sexual desire) in low and high inhibitors, with the exception of subjective reports of sexual desire in the explicit sex

condition which were higher after T+5-HT_{1A}ra administration (M = 15.61, SE = 1.46) compared with the T+PDE5i condition (M = 13.86, SE = 1.25) [F(1,27) = 4.38, *P* = 0.046].

Bedroom Experiment

The results show that treatment with T+5-HT_{1A}ra relative to placebo produced statistically significant increases in all measures of sexual functioning in high inhibitors compared with low inhibitors. The interaction effects between drug and group in the bedroom experiment are described beneath each figure.

Effects of T+5-HT1Ara Relative to Placebo

Low Inhibition Group

Our analysis in the low inhibition group (N = 26) revealed no statistically significant effects of the dependent variables in the bedroom experiment.

High Inhibition Group

Sexual Satisfaction During Sexual Events. In the T+5-HT_{1A}ra condition "sexual satisfaction" was statistically significant higher (M = 2.98, SE = 0.13) compared with the placebo condition (M = 2.51, SE = 0.14) [F(1,25) = 9.51, *P* = 0.005]. See Figure 3.

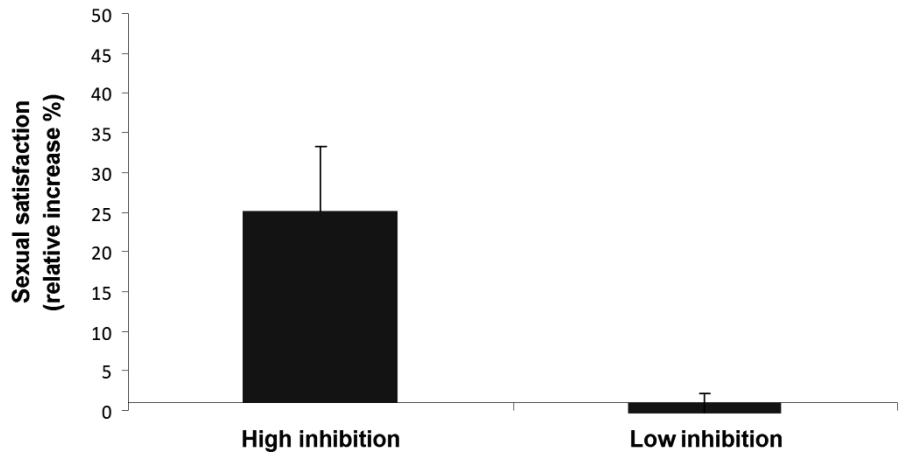

Figure 3. Bedroom experiment: The relative increase in sexual satisfaction following T+5-HT_{1A}ra treatment. In women with high inhibition, treatment with T+5-HT_{1A}ra increased sexual satisfaction with 25%, relative to placebo, while in the low inhibition group there was no such relative increase.

Weekly Diary. According to the weekly diaries, participants in the high inhibitor group reported more sexual desire in the T+5-HT_{1A}ra condition (M = 2.45, SE = 0.11) compared with placebo (M = 2.19, SE = 0.11) [F(1,27) = 6.49, *P* = 0.017]. This effect was also seen for genital arousal (M = 2.60, SE = 0.09 vs. M = 2.29, SE = 0.11) [F(1,27) = 10.08, *P* = 0.004]. In the T+5-HT_{1A}ra condition, more participants reported an improvement of sexual functioning (M = 1.31, SE = 0.09) compared with placebo (M = 1.09, SE = 0.06) [F(1,27) = 4.35, *P* = 0.047]. This improvement was more frequently attributed to T+5-HT_{1A}ra medication (M = 1.48, SE = 0.09) compared with placebo (M = 1.22, SE = 0.07) [F(1,27) = 4.80, *P* = 0.037]. See Figure 4.

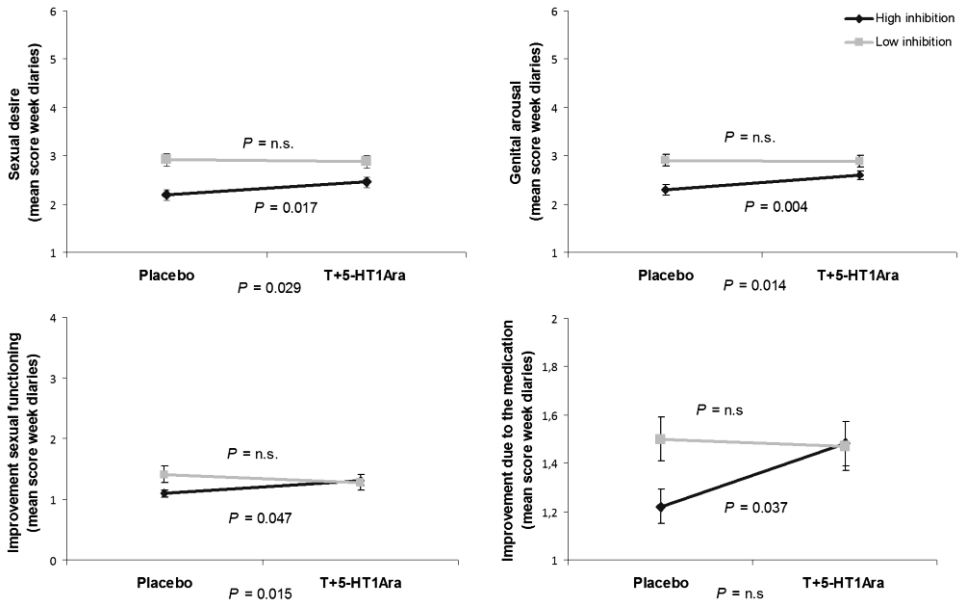

Figure 4. Bedroom experiment: The effects of T+5-HT_{1A}ra relative to placebo on the subjective indices of the week diaries. There was a significant interaction effect between the drug (placebo vs. T+5-HT_{1A}ra) and the group (low vs. high inhibitors) for all dependent measures with exception of "improvement due to medication." Sexual desire: [F(1,52) = 5.05, *P* = 0.029]; genital arousal: [F(1,52) = 6.41, *P* = 0.014]; and improvement of sexual functioning: [F(1, 52) = 6.32, *P* = 0.015].

Monthly Diary. The results of the SEI questionnaire revealed that in the condition high inhibitors reported more sexual improvement in desire and/or arousal during treatment with T+5-HT_{1A}ra (M = 1.93, SE = 0.17) compared with placebo (M = 1.25, SE = 0.10) [F(1,27) = 11.56, *P* = 0.002]. They attributed this improvement to the medication more frequently in the T+5-HT_{1A}ra condition (M = 1.57, SE = 0.10) compared with placebo (M = 1.11, SE = 0.06) [F(1,27) = 18.18, *P* < 0.001].

The results of the SEG questionnaire showed that high inhibitors experienced more benefit from the T+5-HT_{1A}ra medication (M = 1.61, SE = 0.09) as compared with placebo (M = 1.25, SE = 0.83; F(1,27) = 9.25, *P* = 0.005). Regarding the question if they would use the medication if available via prescription, high inhibitors would use T+5-HT_{1A}ra (M = 1.43, SE = 0.10) more compared with placebo, (M = 1.04, SE = 0.36) [F(1,27) = 17.47, *P* < 0.001]. See Figure 5.

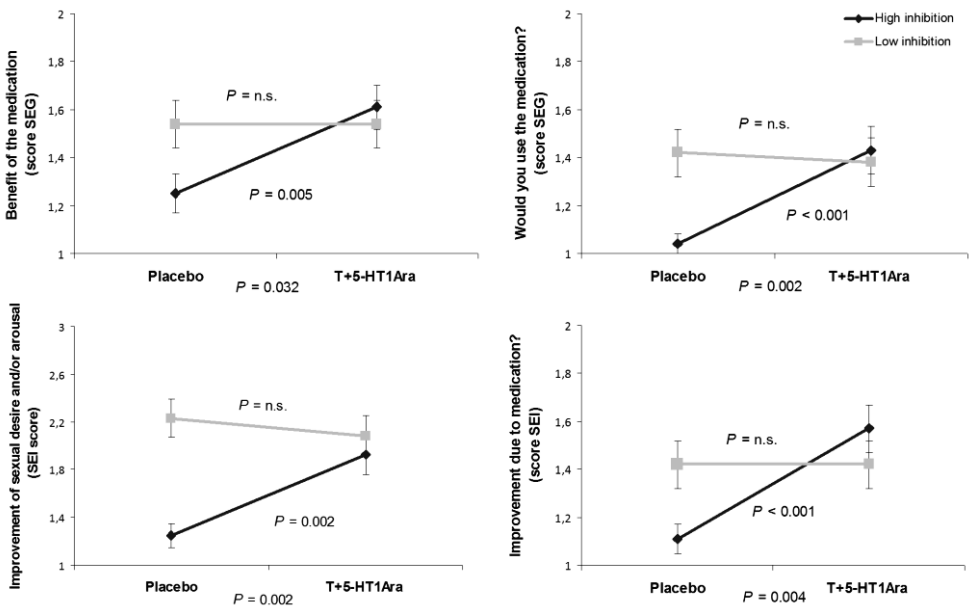

Figure 5. Bedroom experiment: The effects of T+5-HT_{1A}ra relative to placebo on the subjective indices of the Subjective Evaluation of Gain (SEG) and Subjective Evaluation of Improvement (SEI) questionnaire. There was a significant interaction effect between the drug (placebo vs. T+5-HT_{1A}ra) and the group (low vs. high inhibitors) for all dependent measures. Benefit from medication: [$F(1, 52) = 4.85$, $P = 0.032$], would you use the medication: [$F(1, 52) = 11.20$, $P = 0.002$], improvement in sexual desire and/or arousal: [$F(1, 52) = 10.68$, $P = 0.002$], and improvement due to the medication: [$F(1, 52) = 8.91$, $P = 0.004$].

Effects of T+5-HT1Ara Relative to T+PDE5i

The results demonstrate that participants in the low inhibitor group reported more beneficial effects of T+PDE5i treatment, while the high inhibitors have more effect of T+5-HT_{1A}ra administration.

Low Inhibition Group

Sexual Satisfaction During Sexual Events. Our analysis in the low inhibition group (N = 26) revealed that treatment, "sexual satisfaction" was statistically significant higher during T+PDE5i (M = 3.82, SE = 0.11) compared with the T+5-HT_{1A}ra condition (M = 3.37, SE = 0.18) [F(1,22) = 8.84, *P* = 0.007]. See Figure 6.

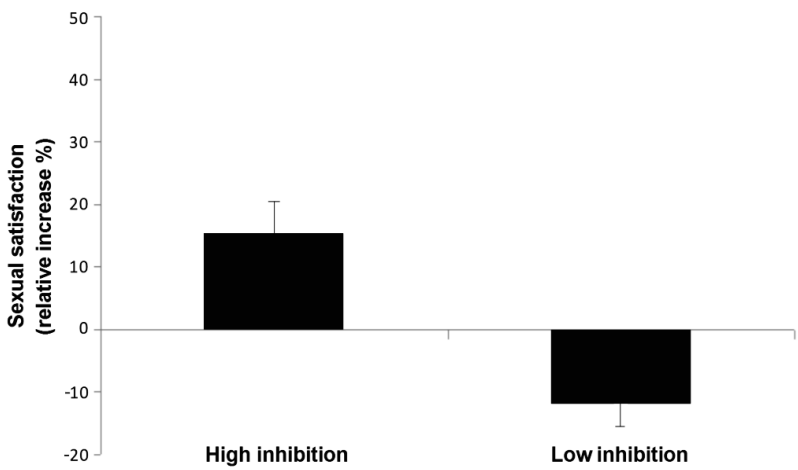

Figure 6. Bedroom experiment: The relative increase in sexual satisfaction following T+5-HT_{1A}ra treatment compared with T+PDE5i treatment (sexual satisfaction T+5-HT_{1A}ra minus satisfaction T+PDE5i). Treatment with T+5-HT_{1A}ra, relative to T+PDE5i increased sexual satisfaction significantly with 15% in women with high inhibition (while women in the low inhibitor group had more effect of T+PDE5i administration).

Weekly Diary. According to the week diaries, no statistically significant differences were observed between the T+PDE5i and T+5-HT_{1A}ra condition for sexual desire or genital arousal. In the T+PDE5i condition, more low inhibition participants reported an improvement of sexual functioning (M = 1.82, SE = 0.16) compared with T+5-HT_{1A}ra (M = 1.28, SE = 0.13) [F(1,25) = 9.94, *P* = 0.004]. This improvement was more frequently attributed to T+PDE5i medication (M = 1.85, SE = 0.13) compared with T+5-HT_{1A}ra (M = 1.47, SE = 0.10) [F(1,25) = 8.58, *P* = 0.007]. See Figure 7.

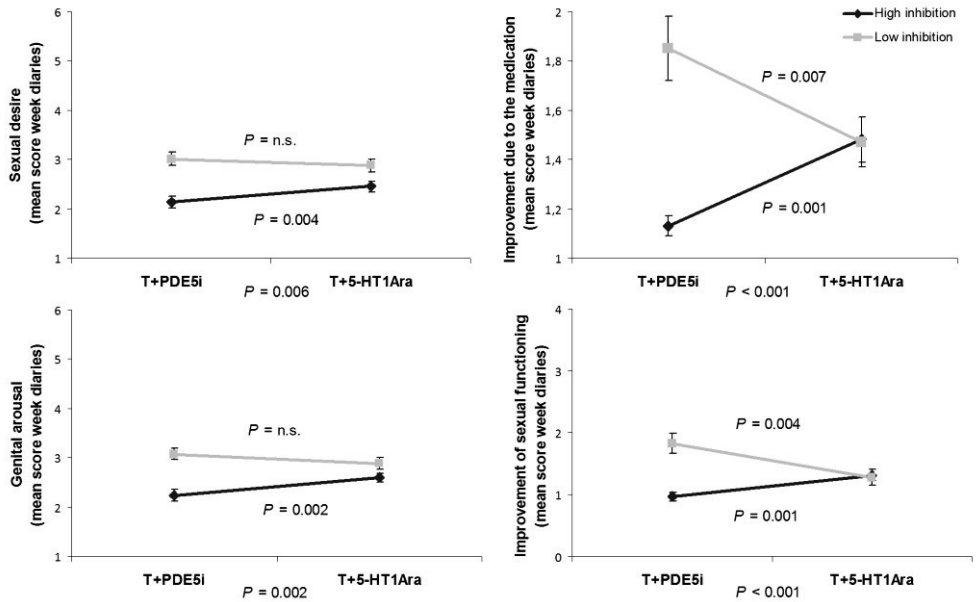

Figure 7. Bedroom experiment: The effects of T+5-HT_{1A}ra relative to T+PDE5i on the subjective indices of the week diaries. There was a significant interaction effect between the drug (T+5-HT_{1A}ra vs. T+PDE5i) and the group (low vs. high inhibitors) for all dependent measures. Sexual desire: [$F(1, 52) = 8.27$, $P = 0.006$], genital arousal: [$F(1, 52) = 11.12$, $P = 0.002$], improvement of sexual functioning: [$F(1, 52) = 21.72$, $P < 0.001$], and improvement due to the medication: [$F(1, 52) = 21.01$, $P < 0.001$].

Monthly Diary. The results of the SEI questionnaire revealed that in the T+5-HT_{1A}ra condition, low inhibitors reported no more sexual improvement in desire and/or arousal compared with T+PDE5i.

The results of the SEG questionnaire showed that low inhibitors experienced more benefit from the T+PDE5i medication (M = 1.81, SE = 0.08) as compared with T+5-HT_{1A}ra (M = 1.54, SE = 0.10) [$F(1,25) = 5.17$, $P = 0.032$]. Regarding the question if they would use the medication if available via prescription, low inhibitors would use T+PDE5i (M = 1.65, SE = 0.10) more compared with T+5-HT_{1A}ra, (M = 1.38, SE = 0.10) [$F(1,25) = 6.62$, $P = 0.016$]. See Figure 8.

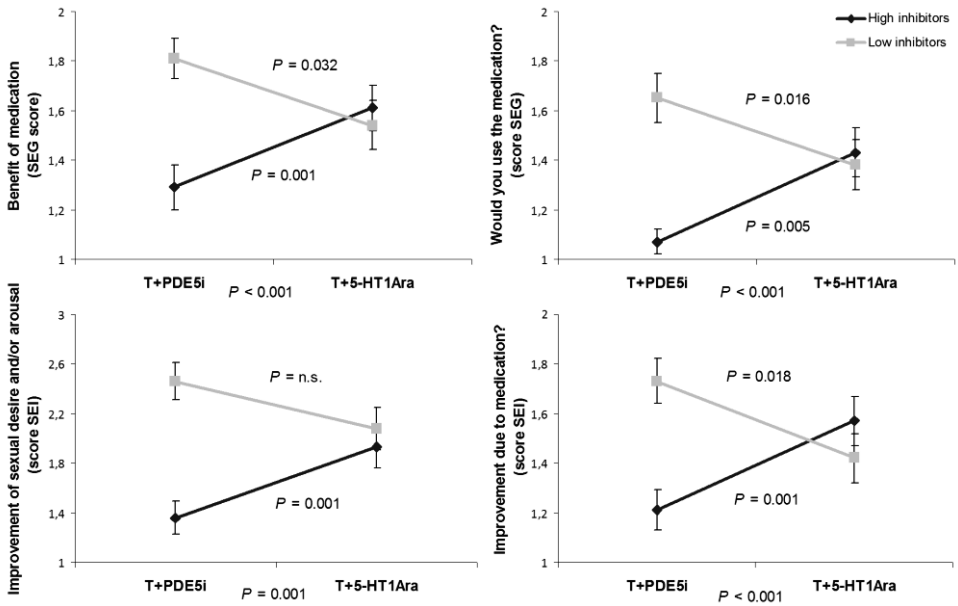

Figure 8. Bedroom experiment: The effects of T+5-HT_{1A}ra relative to T+PDE5i on the subjective indices of the Subjective Evaluation of Gain (SEG) and Subjective Evaluation of Improvement (SEI) questionnaire. There was a significant interaction effect between the drug (T+5-HT_{1A}ra vs. T+PDE5i) and the group (low vs. high inhibitors) for all dependent measures. Benefit from medication: [$F(1, 52) = 16.07$, $P < 0.001$], would you use the medication: [$F(1, 52) = 15.68$, $P < 0.001$], improvement in sexual desire and/or arousal: [$F(1, 52) = 11.24$, $P = 0.001$], and improvement due to the medication: [$F(1, 52) = 19.41$, $P < 0.001$].

High Inhibition Group

Sexual Satisfaction During Sexual Events. "Sexual satisfaction" was statistically significant higher during T+5-HT_{1A}ra treatment (M = 2.98, SE = 0.14) compared with the T+PDE5i condition (M = 2.64, SE = 0.13) [$F(1,23) = 8.88$, $P = 0.007$]. See Figure 6.

Weekly Diary. According to the week diaries, participants in the high inhibitor group reported more sexual desire in the T+5-HT_{1A}ra condition (M = 2.45, SE = 0.11) compared with T+PDE5i (M = 2.13, SE = 0.12) [F(1,27) = 10.08, *P* = 0.004]. This effect was also seen for genital arousal (M = 2.60, SE = 0.09 vs. M = 2.23, SE = 0.12) [F(1,27) = 11.50, *P* = 0.002]. In the T+5-HT_{1A}ra condition, more participants reported an improvement of sexual functioning (M = 1.31, SE = 0.09) compared with T+PDE5i (M = 0.96, SE = 0.07) [F(1,27) = 13.92, *P* = 0.001]. This improvement was more frequently attributed to T+5-HT_{1A}ra medication (M = 1.48, SE = 0.09) compared with T+PDE5i (M = 1.13, SE = 0.04) [F(1,27) = 13.50, *P* = 0.001]. See Figure 7.

Monthly Diary. The results of the SEI questionnaire revealed that in the condition high inhibitors reported more sexual improvement in desire and/or arousal compared during treatment with T+5-HT_{1A}ra (M = 1.93, SE = 0.17) compared with T+PDE5i (M = 1.36, SE = 0.13) [F(1,27) = 13.10, *P* = 0.001]. They attributed this improvement to the medication more frequently in the T+5-HT_{1A}ra treatment condition (M = 1.57, SE = 0.10) compared with T+PDE5i (M = 1.21, SE = 0.08) [F(1,27) = 15.00, *P* = 0.001].

The results of the SEG questionnaire showed that high inhibitors experienced more benefit from the T+5-HT_{1A}ra medication (M = 1.61, SE = 0.09) as compared with T+PDE5i (M = 1.29, SE = 0.87) [F(1,27) = 12.89, *P* = 0.001]. Regarding the question if they would use the medication if available via prescription, high inhibitors would use T+5-HT_{1A}ra (M = 1.43, SE = 0.10) more compared with T+PDE5i, (M = 1.07, SE = 0.05) [F(1,27) = 9.25, *P* = 0.005]. See Figure 8.

Both T+PDE5i and T+5-HT_{1A}ra were tolerated well. Only "mild" to "moderate adverse" were reported. All adverse events were caused by the PDE5i or 5-HT_{1A}ra components. See Table 2. During the study, no serious adverse events were reported.

Table 2. Treatment-related adverse events

	T+PDE5i (%)*	**T+5-HT_{1A}ra (%)†**	**Placebo (%)‡**	**Total (%)§**
Flushing	23.0	1.9	3.7	9.6
Headache	15.9	3.7	2.4	7.4
Lightheadedness	0.9	10.3	0.6	3.9
Dizziness	1.1	11.3	0.2	4.2

* Percentage T+PDE5i medication = AE T+PDE5i/552 units

† Percentage T+5-HT_{1A}ra medication = AE 5-HT_{1A}ra/542 units

‡ Percentage placebo medication = AE placebo/542 units

§ Percentage total medication = AE total/1,636 units

T = testosterone; PDE5i = phosphodiesterase type 5 inhibitor; 5-HT_{1A}ra = 5-HT_{1A} receptor agonist; AE = adverse events

DISCUSSION

We suggested (chapter 3) the existence of different causal mechanisms for the lack of sexual desire in women with HSDD, which were taken into account when designing and developing new medicines for HSDD. In chapter 4 it is demonstrated that women with a relative insensitivity of the brain for sexual cues benefit from T+PDE5i. In this part, women with high inhibition show a marked improvement in sexual function in response to treatment with T+5-HT_{1A}ra relative to placebo and relative to T+PDE5i. These results demonstrate that, in accordance with our hypothesis, the efficacy of the treatments seems to depend on the etiological origin of the complaints.

The majority of studies investigating chronic testosterone administration or other medicines in the treatment of women's sexual behavior have been done in naturally or surgically menopausal women [13–15]. Based on the results of chapter 4 and the present study, T+PDE5i and T+5-HT_{1A}ra are well tolerated and effective in premenopausal as well as in postmenopausal women. The results revealed no interaction with menopausal status or hormonal contraception.

As described in chapter 4, the medication regimes in the present study were relatively short (3 weeks for the bedroom experiment). However, given that in most drug efficacy studies have a much longer treatment period, the current results are promising. More extensive research is needed to establish the effects of T+PDE5i and T+5-HT_{1A}ra over a longer period of time, but we expect the large placebo effects to decrease, while the drug effects are expected to increase over time. Thus, since the present study already shows significant effect of both treatment forms as compared with placebo in the subgroups which these treatments were designed, this may even be expected to increase when tested during a longer period of time.

In the present study, the ratio between low and high inhibitors was approximately 50/50 (N = 26 vs. N = 28). This near 50/50 division between low and high inhibitors is at least in part attributable to the calculation which we used to define inhibition, and thus, we are unable to determine at this time if this ratio reflects the ratio in the general HSDD population. In chapter 4, the ratio between low and high *sensitive* subjects was also approximately 50/50 (N = 29 vs. N = 27). This 50/50 division between low and high sensitivity was *not* inherent to the calculation which we used to define sensitivity and also roughly

corroborated our previous findings of a division in sensitivity in HSDD subjects of approximately 40/60 (low/high sensitivity) [8]. So in the case of sensitivity, there is good reason to believe that approximately 40 to 50% of the HSDD population is low sensitive, although this will have to be confirmed in a larger population. In the present series, we have treated the analysis population as belonging to one of two ends of either continuum (a sensitivity or inhibition continuum). Future studies should take into account the interaction between sensitivity and inhibition, both in terms of prevalence as in terms of this interaction's influence on sexual behavior.

Our former studies showed a relationship between negative sexual experiences and high sensitivity for sexual cues [8,16]. The results of these studies led to the hypothesis that these women suffered from HSDD as a result of (over)activation of sexual inhibitory systems. A potential risk factor for developing a sexual dysfunction after negative sexual experiences is, according to our hypothesis, a person's sensitivity to sexual cues, which will affect positive and negative sexual experiences. High sensitive women are more sensitive for positive and negative sexual experiences. It is possible that the high sensitive women can develop HSDD (partly) because of altered serotonergic transmission, making them more sensitive to negative sexual experiences leading to subsequent over-activation of sexual inhibitory mechanisms in response to sexual arousal. In the present study we observed that women with high inhibition reported negative sexual experiences significantly more often than women with low inhibition, but we observed no such difference between the high and low sensitive women (chapter 4). It has to be noted that negative sexual experiences by themselves do not induce HSDD or high inhibition.

Number of parity was also related to group assignment, where women who were high inhibitors tended to have more children. As stated in chapter 3, women with over-activation of sexual inhibitory mechanisms are more sensitive to emotional stimuli, so any type of negative or emotionally laden experience will impact these people more profoundly. Raising children can be very demanding and can increase the pressures a woman or a couple endures in their lives and is known to affect the sexual relationship between couples [17]. On the other hand, women in the high inhibition group also had a longer relationship duration compared with the women in the low inhibitor group, this could also

influence the parity and might influence induction of inhibitory mechanisms. However, further research on this topic is needed.

As stated in chapter 4, one could think that on-demand therapy for HSDD is counterintuitive. We think that because these women with HSDD experiencing distress or interpersonal difficulties with their sexual complaints, which is one of the main criteria of the DSM-IV-TR, they want to want to have sex because of the fact they seek help. We contend that different causal mechanisms could give rise to common symptoms in HSDD (see also chapter 3). While biological factors play an important role in human social interactions, psychosocial factors should also be taken into account when attempting to understand and explain these phenomena. In the present study (see also chapter 4), a common biological factor (testosterone) had opposing effects, depending on the subject's psychological state and/or psychosocial circumstances. In low sensitive women, sublingual testosterone boosted sexual motivation, while in others it caused sexual inhibition. In this latter group of women, HSDD is most likely caused by dysfunctional activation of sexual inhibitory systems. For a potential pharmacotherapy for women with HSDD and prone to sexual inhibition, the mode of action should thus be directed at alleviating this dysfunctional inhibition. Indeed, as the present study shows, in these subjects, T+5-HT_{1A}ra could be a potentially effective pharmacotherapy. Future studies will have to confirm this.

Considerable effort has been put into developing drugs for HSDD, but with little success to date. A main assumption in these endeavors is to find one generally applicable drug for the improvement of all women with HSDD, irrespective of the different causal mechanisms involved in the sexual suffering. Halford et al. [18] recently described a similar situation with regard to drug development programs for obesity. One of the main reasons for the limited success in the development of antiobesity drugs is that too little emphasis is placed on behavioral analyses of eating behavior, in particular the heterogeneity in the motives for eating. While biological factors play an important role in human behavior, psychosocial factors should also be taken into account when attempting to understand and explain these phenomena (see also Eisenegger et al. [19]) and thus also when developing drugs for psychopathology. Along the same lines, we described in chapter 3 that different causal mechanisms could give rise to common symptoms in HSDD. In order to obtain a comprehensive

understanding of human sexual (dys)function, research should be extended to conceptual analyses and empirical control of the reciprocal influences of biological and psychological mechanisms. Ideally, sexual dysfunction in human subjects should be described in terms of a constellation of interacting mechanisms, both biological and psychological, which at the same time should provide an adequate indication for treatment. Based on such an analyses, we have demonstrated that our approach of tailoring on-demand therapeutics to different underlying etiologies can be used to treat common symptoms in subgroups of women with HSDD.

REFERENCES

1. American Psychiatric Association. Diagnostic and statistical manual of mental disorders. 4th edition. Text revision. Washington, DC: American Psychiatric Association; 2000.

2. Chambers CD, Garavan H, Bellgrove MA. Insights into the neural basis of response inhibition from cognitive and clinical neuroscience. Neurosci Biobehav Rev 2009;33:631–46.

3. Beauregard M, Levesque J, Bourgouin P. Neural correlates of conscious self-regulation of emotion. J Neurosci 2001;21:RC165.

4. Stoléru S, Redouté J, Costes N, Lavenne F, Le Bars D, Dechaud H, Forest MG, Pugeat M, Cinotti L, Pujol J. Brain processing of visual sexual stimuli in men with hypoactive sexual desire disorder. Psychiatry Res 2003;124:67–86.

5. Eagle DM, Bari A, Robbins TW. The neuropsychopharmacology of action inhibition: Cross-species translation of the stop-signal and go/no-go tasks. Psychopharmacology (Berl) 2008;199:439–56.

6. Evers EA, Cools R, Clark L, van der Veen FM, Jolles J, Sahakian BJ, Robbins TW. Serotonergic modulation of prefrontal cortex during negative feedback in probabilistic reversal learning. Neuropsychopharmacology 2005;30:1138–47.

7. Bloemers J, Gerritsen J, Bults R, Koppeschaar H, Everaerd W, Olivier B, Tuiten A. Induction of sexual arousal in women under conditions of institutional and ambulatory laboratory circumstances: A comparative study. J Sex Med 2010;7:1160–76.

8. van der Made F, Bloemers J, Yassem WE, Kleiverda G, Everaerd W, van Ham D, Olivier B, Koppeschaar H, Tuiten A. The influence of testosterone combined with a PDE5-inhibitor on cognitive, affective, and physiological sexual functioning in women suffering from sexual dysfunction. J Sex Med 2009;6:777–90.

9. Available at: http://www.mobihealth.com/services/en/mh_mobile.php (accessed October 1, 2012).

10. Morokoff PJ, Heiman JR. Effects of erotic stimuli on sexually functional and dysfunctional women: Multiple measures before and after sex therapy. Behav Res Ther 1980;18:127–37.

11. Heiman J, Hatch J. Affective and physiological dimensions of male sexual response to erotica and fantasy. Basic Appl Soc Psych 1980;1:315–27.

12. Tabachnick BG, Fidell LS. Using multivariate statistics. Boston: Allyn and Bacon; 2007.

13. Shifren JL, Braunstein GD, Simon JA, Casson PR, Buster JE, Redmond GP, Burki RE, Ginsburg ES, Rosen RC, Leiblum SR, Caramelli KE, Mazer NA. Transdermal testosterone treatment in women with impaired sexual function after oophorectomy. N Engl J Med 2000;343:682–8.

14. Sherwin BB. Randomized clinical trials of combined estrogen-androgen preparations: Effects on sexual functioning. Fertil Steril 2002;77(suppl 4):S49–54.

15. Simon J, Braunstein G, Nachtigall L, Utian W, Katz M, Miller S, Waldbaum A, Bouchard C, Derzko C, Buch A, Rodenberg C, Lucas J, Davis S. Testosterone patch increases sexual activity and desire in surgically menopausal women with hypoactive sexual desire disorder. J Clin Endocrinol Metab 2005;90:5226–33.

16. van der Made F, Bloemers J, van Ham D, El Yassem W, Kleiverda G, Everaerd W, Olivier B, Tuiten A. Childhood sexual abuse, selective attention for sexual cues and the effects of testosterone with or without vardenafil on physiological sexual arousal in women with sexual dysfunction: A pilot study. J Sex Med 2009;6:429–39.

17. Leeman LM, Rogers RG. Sex after childbirth: Postpartum sexual function. Obstet Gynecol 2012;119:647–55.

18. Halford JC, Boyland EJ, Blundell JE, Kirkham TC, Harrold JA. Pharmacological management of appetite expression in obesity. Nat Rev Endocrinol 2010;6:255–69.

19. Eisenegger C, Naef M, Snozzi R, Heinrichs M, Fehr E. Prejudice and truth about the effect of testosterone on human bargaining behaviour. Nature 2010;463:356–9.

Chapter 6

Efficacy of testosterone combined with a PDE5 inhibitor and testosterone combined with a serotonin $_{1A}$ receptor agonist in women with SSRI-induced sexual dysfunction

A preliminary study

Kim van Rooij, Saskia Poels, Petra Worst, Jos Bloemers, Hans Koppeschaar, Andrew Goldstein, Berend Olivier and Adriaan Tuiten

European Journal Of Pharmacology 2014;
doi:10.1016/j.ejphar.2014.10.061

ABSTRACT

Selective serotonin reuptake inhibitors (SSRIs) are known to cause sexual dysfunction, such as decreased sexual motivation, desire, arousal, and orgasm difficulties. These SSRI-induced sexual complaints have a high prevalence rate, while there is no approved pharmacological treatment for SSRI-induced sexual dysfunction. It is hypothesized that a polymorphisms in the androgen receptor gene, encoded by the nucleotides cysteine, adenine, and guanine (CAG), influence the effect of testosterone on sexual functioning. In an explorative, randomized, double-blind, placebo-controlled, crossover study we investigated the possible effects of sublingual testosterone combined with a serotonin (5-HT)$_{1A}$ receptor agonist, and of sublingual testosterone combined with a phosphodiesterase type 5 inhibitor (PDE5-i) on sexual functioning in women with SSRI-induced sexual dysfunction. Furthermore, we did an exploratory analysis to assess if the CAG polymorphism influences this effect. 21 pre- and postmenopausal women with SSRI-induced sexual dysfunction participated and underwent the following interventions: a combination of testosterone (0.5 mg) sublingually and the PDE5-inhibitor sildenafil (50 mg) and a combination of testosterone (0.5 mg) sublingually and the 5-HT$_{1A}$ receptor agonist buspirone (10 mg). The results show that women who use a low dose of SSRI and have relatively long CAG repeats report a marked improvement in sexual function in response to both treatments compared to placebo.

This explorative study and preliminary results indicate that in women with SSRI-induced sexual dysfunction, a combination of testosterone sublingually and a PDE5-inhibitor or testosterone sublingually and a 5-HT$_{1A}$ receptor agonist might be promising treatments for certain subgroups of women with this condition.

INTRODUCTION

Antidepressant therapy has been frequently associated with negative sexual side effects, such as decreased sexual motivation, desire, arousal, and orgasm difficulties [1,2]. These negative sexual side effects can produce psychological distress and an impaired quality of life. Selective serotonin reuptake inhibitors (SSRIs) are the most notorious for causing sexual dysfunction (SD) with an estimated prevalence rate of 20-70% [3,4]. Although some beneficial effects have been reported with the use of buspirone [5,6], bupropion [7-9] or type 5 phosphopdiesterase (PDE5) inhibitors (e.g., sildenafil) [10-12], to date no FDA approved pharmacological treatment for SSRI-induced SD is available.

Of the different serotonin (5-HT) receptors, 5-HT_{1A} and 5-HT_2receptors are well accepted to be involved in sexual functioning [13,14]. The SSRI-induced elevated synaptic serotonin levels, and by inference the increased serotonergic neurotransmission, are thought to inhibit the sexual excitatory effects of dopamine and norepinephrine in the mesolimbic reward system [13,15,16] via a tonic increased serotonergic activity in the prefrontal cortex (PFC) [17,18]. 5-HT_{1A} [14] receptor agonists, e.g., buspirone, decrease serotonergic activity for a short time after a single dose administration via activation of the somatodendritic autoreceptor [19-21]. Accordingly, acute treatment with a 5-HT_{1A} receptor agonist might decrease, during a relatively short period of time, the serotonergic inhibitory control in the PFC, which might lead to an increased activity in the mesolimbic system involved in sexual motivation. Therefore the use of a 5-HT1A receptor agonist could be part of treatment in patients with SSRI-induced SD.

In sexual functioning it is well recognized that testosterone plays an important role [22,23]. Tuiten et al. reported that a single dose of 0.50 mg sublingual testosterone increased genital arousal and experiences of sexual lust and genital sensation in premenopausal sexually functional women 3-4 hours after the induced testosterone peak [24,25]. This delay in effect of sublingual testosterone has been frequently replicated in several studies regarding cognitive and affective functions, including sexual functioning and social behavior [26-38]. In addition, it has been demonstrated that the combined use of sublingual

testosterone with a PDE5-inhibitor (PDE5i), and of sublingual testosterone with a 5-HT_{1A} receptor agonist are two treatments which are effective in different subgroups of women with Hypoactive Sexual Desire Disorder (HSDD). The first combination increased sexual satisfaction in women who have a relatively insensitive brain system for sexual cues [39], while the second combination was most suitable for women with enhanced activity of sexual inhibitory mechanisms [40]. The sequence and timeframe of the delivery of the compounds in both combination-treatments were such that the pharmacological effects of the PDE5-inhibitor and 5-HT_{1A}ra coincide with the time-window of the testosterone-induced behavioral effects [39,40]. The behavioral effects of sublingual testosterone are mediated, at least in part, by binding to the androgen receptor (AR). It is our hypothesis that polymorphisms in the AR gene are of influence in this behavioral effect. The polymorphic polyglutamine stretch in the aminoterminal domain of the AR, encoded by the nucleotides cysteine, adenine, and guanine (CAG), is known to influence the function of the receptor as a transcription factor, so that relatively long CAG repeat lengths are associated with a low level of receptor function [41-43]. It can be hypothesized that women with relatively long CAG repeat lengths are less sensitive to sexual cues because their AR has a low level of receptor functioning. These women may need higher levels of circulating testosterone to activate intracellular processes after binding to the AR. Therefore, they could benefit more from sublingual testosterone in increasing their brain's sensitivity compared to women with relatively shorter CAG repeats.

In this explorative study we investigated the possible effects of sublingual testosterone combined with a 5-HT_{1A} receptor agonist, and of sublingual testosterone combined with a PDE5-inhibitor on sexual functioning in pre- and postmenopausal women with SSRI-induced SD.

METHODS

Study subjects

Twenty-one women, aged ≥ 21, pre- or postmenopausal with a diagnosis of SSRI-induced SD (comorbidity with other SDs was allowed) according to the Diagnostic and Statistical Manual of Mental Disorders-Fourth Edition (Text Revision) (DSM-IV-TR) criteria [44] were randomized (the diagnosis SSRI-induced SD falls under the diagnosis substance-induced SD). This diagnosis was made by a trained psychologist. In order to meet the criteria of this diagnosis it was evaluated to what extend depressive or anxious feeling contributed to the sexual complaints and it was assessed how these subjects experienced sex prior to the start of the SSRI. Participants used an SSRI for at least 3 months and were on a stable dose for a minimum of 6 weeks. Participants used the following SSRIs: citalopram (n=5), paroxetine (n=11), venlafaxine (n=3), fluvoxamine (n=1) and sertraline (n=1).

Exclusion criteria included a history of serotonin syndrome, endocrine disease, neurological problems, a current serious psychiatric disorder (e.g., schizophrenia, psychosis or treatment for obsessive compulsive disorder, anorexia nervosa, bulimia nervosa and/or social anxiety neurosis), cardiovascular condition, hypertension, and abnormal liver or renal function.

Blood samples for determination of baseline levels of total testosterone, Sex Hormone Binding Globulin (SHBG), albumin, Thyroid Stimulating Hormone (TSH), Follicle Stimulating Hormone (FSH), estrogen and CAG repeat length were collected at the screening visit. A urine pregnancy test was applied to all premenopausal women.

Twenty-one women participated after providing written informed consent. This study was approved by the local medical ethics committee and carried out in agreement with the International Conference on Harmonisation- Good Clinical Practice (ICH-GCP).

Study design

Study setting and design are the same as described in Poels et al. and van Rooij et al. [39,40]. In this manuscript only the results of the event questionnaire (see further on), which was filled out following each sexual event, will be reported.

In a randomized, double-blind, placebo-controlled, crossover design, twenty one women underwent three different medication regimes: (i) **placebo**: placebo for testosterone (cyclodextrin solution without testosterone) and placebo for the PDE5 inhibitor (PDE5i = sildenafil)/ 5-HT_{1A} receptor agonist (5-HT_{1A}ra= buspirone) (powder-filled gelatine capsule without sildenafil/buspirone); (ii) **T+PDE5i:** the combination of testosterone (0.5 mg) sublingually with cyclodextrin as carrier and sildenafil (50 mg, hidden in a powder-filled gelatine capsule); (iii) **T+5-HT_{1A}ra**; the combination of testosterone (0.5 mg) sublingually with cyclodextrin as carrier and buspirone (10 mg, hidden in a powder-filled gelatin capsule). Each medication regime lasted four weeks and the order of the three medication regimes was randomized [39,40].

Participants were instructed to take the cyclodextrin solution (testosterone or placebo) sublingually four hours prior to each measurement session or sexual event and rinse it under the tongue for one minute. They were instructed to ingest the capsule (sildenafil, buspirone or placebo) 2.5 hours later. When they had experienced a sexual event they were asked to fill out a secure web-based event questionnaire (EQ) within 24 hours following the sexual event. The EQ comprised of ten questions (one open-ended, four multiple-choice, five 5-point Likert scale items) concerning the type, duration, pleasantness and intensity of the sexual event.

In order to monitor changes in psychological wellbeing, the Beck Depression Inventory-II (BDI-II) and the State-Trait Anxiety Inventory - Disposition 2 (Trait) version Y (STAI-DY2) questionnaires were filled out at baseline and final follow up visit as well as after each treatment period.

Statistical analysis

Based on high Cronbach's alpha's of 5 items measuring desire and intensity of the sexual events (measured by the EQ) during the different drug conditions [Placebo: α=0.92; T + PDE5i: α=0.90; T+5-HT_{1A}ra: α=0.92] the mean of these items were calculated as a qualitative measure of "sexual satisfaction".

Two participants did not fill out their EQs in the placebo treatment period, however they did report sexual events in the T + PDE5i and T+5-HT_{1A}ra period. Because of the small study population their missing placebo data was imputed in the following manner: the correlation between placebo and the mean of both drug conditions (((T + PDE5i) + (T+5-HT_{1A}ra))/2) was high (r= .617, p = 0.005). Because all participants had (T + PDE5i) and (T+5-HT_{1A}ra) data, z-scores were made of the ((T + PDE5i) + (T+5-HT_{1A}ra))/2 variable. The two subjects with the missing placebo data comprised of one subject with a negative z-score and one subject with a positive z-score based on the ((T + PDE5i) + (T+5-HT_{1A}ra))/2 variable. The mean of sexual satisfaction during placebo of the subjects with a negative z-score in the ((T + PDE5i) + (T+5-HT_{1A}ra))/2 condition was imputed in the placebo condition for one subject and the mean of sexual satisfaction during placebo of the subjects with a positive z-score in the ((T + PDE5i) + (T+5-HT_{1A}ra))/2 condition was imputed in the placebo condition for the other subject.

Regarding the comparability of dosages of the SSRI use, the dosing classification of Gartlehner and colleagues [45] was used in this study. We divided the subjects into two subgroups on the basis of their SSRI dose: a low and medium-high dose group. SSRI dosages that were classified as low (n=16) were: citalopram <30 mg, fluvoxamine <75 mg, paroxetine <30 mg, sertraline <75 mg and venlafaxine <156.3 mg. Higher dosages of each SSRI (n=5) were classified as medium-high dosages.

For the CAG repeat length, we divided the subjects into two subgroups based on the mean of the CAG repeat length: subject with a relative short CAG repeat length (CAG ≤ 22 repeats) and subjects with relative long CAG repeat length (CAG length > 22 repeats). This cut-off point corresponds to other studies with CAG repeats in women (e.g., the comparison between women with breast

cancer and controls) [46,47] . Unfortunately, the CAG results of two subjects were missing due to an error in the laboratory analysis. In total, the mean of the CAGs of 19 subjects were used for the analysis: 8 subjects with relatively short CAG repeats and 11 subjects with relatively long CAG repeats.

Demographic data were analyzed to investigate possible group differences (low vs. medium-high SSRI dose and short vs. long CAG repeat length) with an independent t-test if the data were normally distributed. For non-normally distributed data, the Mann-Whitney test was used. Categorical data was compared between the groups with a Chi-square test. For each of the dependent variables, separate 2x2x2 repeated measures analyses of variance (repeated measures ANOVA) were carried out. The within-subject factor was drug response, and had two levels (T+PDE5i versus Placebo, and T+5-HT_{1A}ra response versus placebo). The between subject factor was SSRI dosage (low SSRI dose vs. medium-high SSRI dose) and CAG repeat length (short CAG repeat length vs. long CAG repeat length). To calculate the relative increase in sexual satisfaction, placebo was subtracted from the T+PDE5-inhibitor treatment and this was divided by placebo and multiplied by 100%. The same calculation was done for the relative increase in sexual satisfaction after T+5-HT_{1A}ra treatment. An alpha level of 0.05 was set for all analyses.

RESULTS

Twenty-one women completed the study. All baseline hormonal values were in the normal female reproductive and/or postmenopausal range. There were no clinical significant changes in the scores of the BDI-II and STAI-DY2 questionnaires during the study.

The baseline characteristics and hormone levels of the twenty-one women are outlined in Table 1.

Sexual satisfaction during sexual events

Overall, treatment with T+PDE5i or treatment with T+5-HT_{1A}ra produced no statistically significant increase in sexual satisfaction as compared with placebo. Subsequently, the participants were divided on the basis of their SSRI dose (in low SSRI dose (n=16) and medium-high SSRI dose (n=5)) and CAG repeat length (n=8 with short repeat length and n=11 with long repeat length), the results showed that there was a highly significant interaction effect between placebo versus T+PDE5i and these groups. The interaction between placebo and T+5-HT_{1A}ra and the two groups (SSRI dose and CAG repeat length) was also statistically significant. In the following sections each effect for the different drugs will be described separately.

Table 1. Demographics and baseline hormonal values of the participating women.

	Subjects	**Low dose SSRI**	**Medium-high dose SSRI**	***P***
N	*21*	*16*	*5*	
Age, years	38.81 ± 11.9	40.38 ± 12.5	33.80 ± 9.4	n.s.*
Body mass index, kg/m²	24.4 ± 3.3	23.91 ± 3.5	26.12 ± 2.9	n.s.*
Race, no (%)				
Caucasian	21 (100)	16	5	
SSRI-antidepressant, no (%)				n.s. †
Citalopram	5 (24)	4	1	
Paroxetine	11 (52)	8	3	
Venlafaxine	3 (14)	3	0	
Fluvoxamine	1 (5)	1	0	
Sertraline	1 (5)	0	1	
Menopausal status, no (%)				n.s. †
Premenopausal	16 (76)	11	5	
Postmenopausal	5 (24)	5	0	
Contraception, no (%)				n.s. †
Hormonal	12 (57)	9	3	
Non-hormonal	5 (24)	3	2	
None	4 (19)	4	0	
Total testosterone (ng/mL)	0.34 ± 0.5	0.31±0.5	0.45±0.6	n.s.^
SHBG (nmol/L)	98.7 ± 66.2	100.6±67.5	92.8±69.2	n.s.*
Mean CAG length	22.4 ± 2.3	22.1±2.4	23.2±2.4	n.s.*

Age and relationship duration are represented in means ± standard deviation. The body mass index (BMI) is the weight in kilograms divided by the square of the height in meters. Testosterone values <0.7 ng/mL are analysed as 0.01 ng/mL, the SHBG levels values > 200 nmol/L are analysed as 200 nmol/L.

*T-test

†Chi-square test

^ Mann–Whitney test

Placebo versus T+PDE5i

The interaction between drug (placebo versus T+PDE5i) and the two groups (SSRI dose and CAG repeat length) was statistically significant F(1,15) = 14.17, *P* = 0.002. In figure 1 it is shown in which group there is an increase or decrease in sexual satisfaction (relative increase in sexual satisfaction compared to placebo).

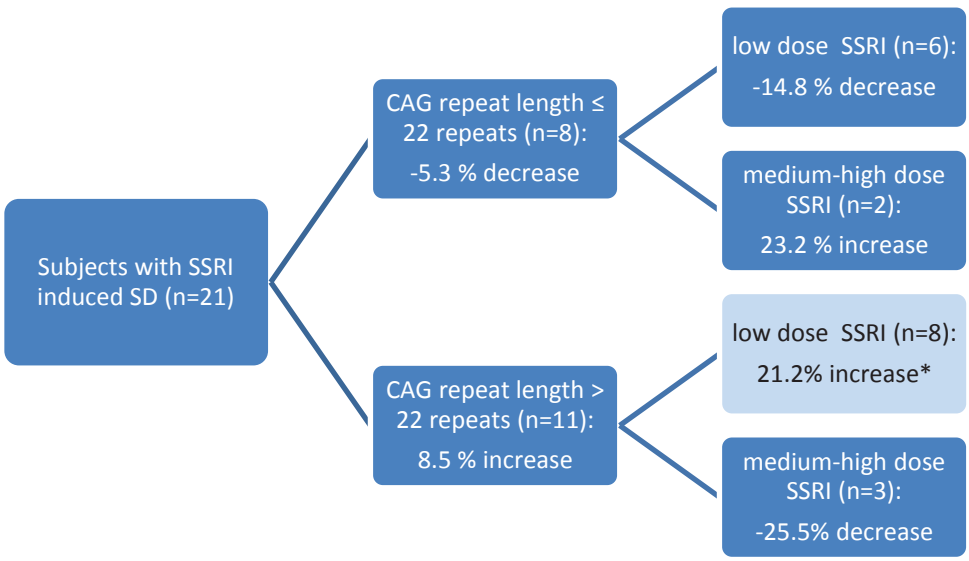

Figure 1. Overview of subjects in the different groups and the relative increase or decrease after T+PDE5i treatment compared to placebo. Relative increase is calculated as T+PDE5i-placebo/placebo*100%.
* p <0.05

As shown in figure 1, only women with relatively long CAG repeats and using relatively low SSRI doses (n=8) reported statistically significant more sexual satisfaction in the T+PDE5i (M = 3.53,SE = 0.26) condition compared to placebo (M = 2.92, SE = 0.21 [F(1,7) = -4.67, *P* = 0.002]).

Placebo versus T+5-HT$_{1A}$ra

The interaction between drug (placebo versus T+5-HT$_{1A}$ra) and the two groups (SSRI dose and CAG repeat length) was statistically significant F(1,15) = 13.37, *P* = 0.002. In figure 2 it is shown in which group there is an increase or decrease in sexual satisfaction (relative increase in sexual satisfaction compared to placebo).

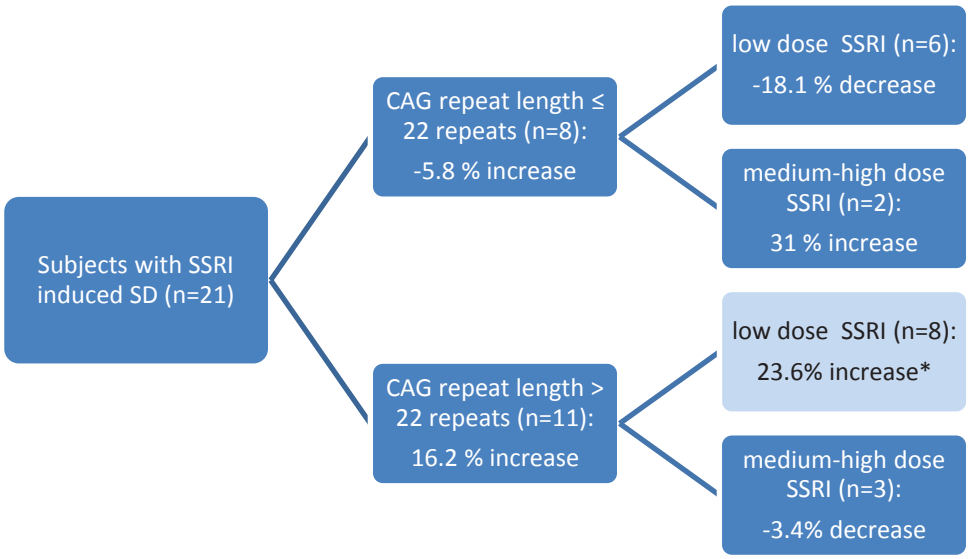

Figure 2. Overview of subjects in the different groups and the relative increase or decrease after T+5-HT$_{1A}$ra treatment compared to placebo. Relative increase is calculated as T+5-HT$_{1A}$ra-placebo/placebo*100%.
* p <0.05

As shown in figure 2 and comparable with the results after T+PDE5i administration, only women with relatively long CAG repeats and using relatively low SSRI doses (n=8) reported statistically significant more sexual satisfaction in the T+5-HT$_{1A}$ra (M = 3.58,SE = 0.27) condition compared to placebo (M = 2.92, SE = 0.21 [F(1,7) = -3.50, *P* = 0.010]).

Two women with short CAG repeat length and a medium-high SSRI dose seem to respond more to T+5-HT$_{1A}$ra (31% increase in sexual satisfaction), although not significant.

In figure 3 the relative increase of sexual satisfaction for both treatments compared to placebo is shown in the women with long CAG repeats and a relatively low dose of SSRI (n=8).

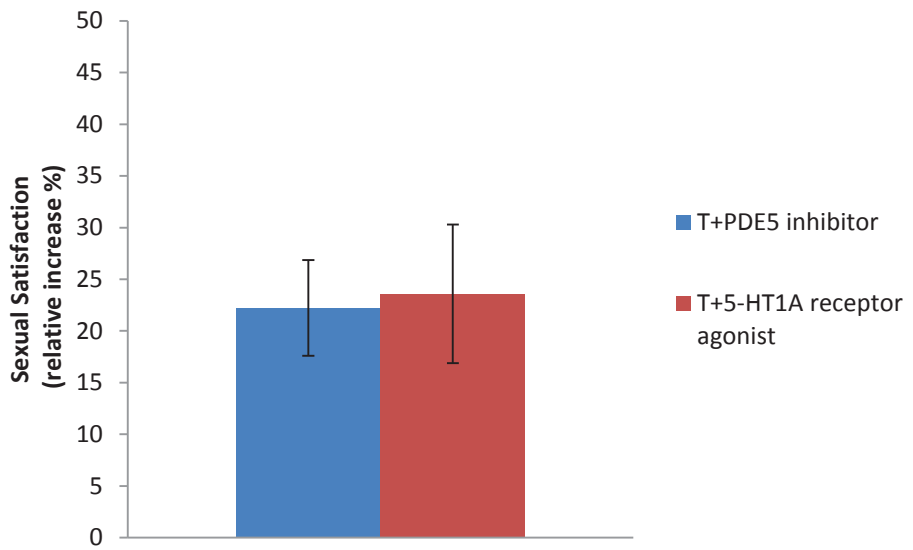

Figure 3. The increase in sexual satisfaction following T+PDE5i and T+5-HT$_{1A}$ra treatment in subjects with long CAG repeat length who use a low dose of SSRI (n=8). Treatment with T+PDE5-inhibitor increased sexual satisfaction with 22%, relative to placebo and sexual satisfaction was increased by 24% after T+5-HT1Ara administration. Relative increase is calculated as T+5-HT$_{1A}$ra-placebo/placebo*100% and T+PDE5-i-placebo/placebo*100%.

Treatment with T+PDE5i and T+5-HT$_{1A}$ra was well tolerated.

DISCUSSION

This is the first study in which a combination of testosterone with either a PDE5i (sildenafil) or a 5-HT_{1A}receptor agonist (buspirone) are used in women with SSRI-induced Sexual Dysfunction. Although both individual treatments showed no significant main effects on sexual functioning compared to placebo, our analyses revealed a significant interaction effect between SSRI dosage (low and medium-high SSRI dose, CAG repeat length (short and long length), and treatment response (placebo versus drug), on sexual satisfaction. Subsequent analyses showed that women with relatively long CAG repeat lengths and who use a low dose of SSRI, experienced an increase in sexual satisfaction after T+PDE5i administration compared to placebo (the relative increase in sexual satisfaction was approximately 22%). Moreover, for this subgroup of women our analyses revealed also an increase in sexual satisfaction after T+5-HT_{1A}ra administration compared to placebo (approximately 24%). In this study an increase in sexual satisfaction is found only in women with relatively long CAG repeat length and who use a low dose of SSRI. Here we will discuss possible hypotheses for the observed drug response patterns although they only can be considered as very preliminary.

It may be hypothesized that women with relatively long CAG repeat lengths are less sensitive to sexual cues because their AR has a low level of receptor functioning and would therefore benefit more from sublingual testosterone in increasing their brain's sensitivity compared to women with relatively shorter CAG repeats.

In this study statistically significant differences are only found in women with relatively long CAG repeat lengths using a low dose of SSRI. It can be hypothesized that women who use a low SSRI dose have a relatively modest elevation of synaptic serotonin levels and therefore treatment with sublingual testosterone is sufficient to increase the sensitivity of the brain, thereby increasing sexual motivation. Under this condition of increased sexual motivation, the PDE5i enhances physiological sexual responding. In case of the T+5-HT_{1A}ra treatment, the on-demand intake of the 5-HT_{1A}ra component lowers the serotonin firing activity for a relatively short period and thereby allows the

sublingual testosterone component to increase the sensitivity of the brain for sexual cues, thereby increasing sexual satisfaction.

It was expected that for women who use a medium-high SSRI dose, T+5-HT_{1A}ra administration would be beneficial in increasing sexual satisfaction since acute 5-HT_{1A}ra administration will lower tonic serotonin levels and thereby decreases the sexual complaints during its active behavioral window. However, there was no statistically significant increase in sexual satisfaction after T+PDE5i and T+5-HT_{1A}ra administration, irrespective of CAG repeat length in these women. One possibility is that this non-significant difference is due to the small numbers of subjects in this study. Only 5 women used a medium-high SSRI dose, of which two women with relatively long CAG repeats report an increase in sexual satisfaction of 31% although not significant. Another explanation is that in women who use a medium-high SSRI dose, synaptic serotonin levels and serotonergic neurotransmission are more profoundly increased resulting in higher tonic activity of serotonin, at which level the buspirone dosage studied might be too low.

In earlier studies we have shown that women with HSDD can be divided in two groups: women who have a relative insensitive brain system for sexual cues (who responded on T+PDE5i) and women with enhanced activity of sexual inhibitory mechanisms (who responded on T+5-HT_{1A}ra). In the present study population, women who use a low SSRI dose and have relatively long CAG repeats respond to T+PDE5i and T+5-HT_{1A}ra treatment, however it is not known which women can be subtyped into women who have a relative insensitive brain system for sexual cues or women with enhanced activity of sexual inhibitory mechanisms. There are many factors that could influence the brain's sensitivity in this population; the use and dosage of an SSRI, genetic factors such as serotonin receptor polymorphisms, and other polymorphisms. Furthermore, an individual's response to certain psychological factors (e.g., a negative sexual experience) is influenced by their brain's sensitivity and therefore these factors should also be taken into account. Also, it is not known if CAG repeat length itself influences the risk of developing SSRI induced female sexual dysfunction. To identify specific subgroups in this population that could benefit from treatment with T+PDE5i and T+5-HT_{1A}ra, future research will focus on a

personalized medicine approach through knowledge of more genetic polymorphisms, other biological markers, and other dosages of the compounds. In the present study a small number of women was included with a variety of SSRI molecules and dosages, therefore future studies on a larger scale are warranted.

Conclusively, this explorative study and preliminary results indicate that in women with SSRI-induced SD, T+PDE5i or T+5-HT_{1A} might be promising treatments for certain subgroups of women with this condition, depending on genetics, SSRI dose, and psychological factors.

REFERENCES

1. Rosen RC, Lane RM, Menza M. Effects of SSRIs on sexual function: a critical review. Journal of clinical psychopharmacology 1999 Feb;19(1):67-85.

2. Montgomery SA, Baldwin DS, Riley A. Antidepressant medications: a review of the evidence for drug-induced sexual dysfunction. Journal of affective disorders 2002 May;69(1-3):119-40.

3. Serretti A, Chiesa A. Treatment-emergent sexual dysfunction related to antidepressants: a meta-analysis. Journal of clinical psychopharmacology 2009 Jun;29(3):259-66.

4. Montejo AL, Llorca G, Izquierdo JA, Rico-Villademoros F. Incidence of sexual dysfunction associated with antidepressant agents: a prospective multicenter study of 1022 outpatients. Spanish Working Group for the Study of Psychotropic-Related Sexual Dysfunction. The Journal of clinical psychiatry 2001;62 Suppl 3:10-21.

5. Landen M, Eriksson E, Agren H, Fahlen T. Effect of buspirone on sexual dysfunction in depressed patients treated with selective serotonin reuptake inhibitors. Journal of clinical psychopharmacology 1999 Jun;19(3):268-71.

6. Norden MJ. Buspirone treatment of sexual dysfunction associated with selective serotonin re-uptake inhibitors. Depression 1994;2(2):109-112.

7. Labbate LA, Pollack MH. Treatment of fluoxetine-induced sexual dysfunction with bupropion: a case report. Ann Clin Psychiatry 1994 Mar;6(1):13-15.

8. Safarinejad MR. Reversal of SSRI-induced female sexual dysfunction by adjunctive bupropion in menstruating women: a double-blind, placebo-controlled and randomized study. J Psychopharmacol 2011 Mar;25(3):370-378.

9. Clayton AH, Warnock JK, Kornstein SG, Pinkerton R, Sheldon-Keller A, McGarvey EL. A placebo-controlled trial of bupropion SR as an antidote for selective serotonin reuptake inhibitor-induced sexual dysfunction. J Clin Psychiatry 2004 Jan;65(1):62-67.

10. Nurnberg HG, Hensley PL, Heiman JR, Croft HA, Debattista C, Paine S. Sildenafil treatment of women with antidepressant-associated sexual dysfunction: a randomized controlled trial. JAMA 2008 Jul 23;300(4):395-404.

11. Nurnberg HG, Hensley PL, Gelenberg AJ, Fava M, Lauriello J, Paine S. Treatment of antidepressant-associated sexual dysfunction with sildenafil: a randomized controlled trial. Jama 2003 Jan 1;289(1):56-64.

12. Fava M, Rankin MA, Alpert JE, Nierenberg AA, Worthington JJ. An open trial of oral sildenafilin antidepressant-induced sexual dysfunction. Psychother Psychosom 1998;67(6):328-331.

13. Clayton AH, Hamilton DV. Female sexual dysfunction. Obstetrics and gynecology clinics of North America 2009 Dec;36(4):861-76, xi.

14. Berger M, Gray JA, Roth BL. The expanded biology of serotonin. Annual review of medicine 2009;60:355-66.

15. Meltzer HY, Young M, Metz J, Fang VS, Schyve PM, Arora RC. Extrapyramidal side effects and increased serum prolactin following fluoxetine, a new antidepressant. J Neural Transm 1979;45(2):165-175.

16. Done CJ, Sharp T. Evidence that 5-HT2 receptor activation decreases noradrenaline release in rat hippocampus in vivo. Br J Pharmacol 1992 Sep;107(1):240-245.

17. Evers EA, Cools R, Clark L, van der Veen FM, Jolles J, Sahakian BJ, et al. Serotonergic modulation of prefrontal cortex during negative feedback in probabilistic reversal learning. Neuropsychopharmacology 2005 Jun;30(6):1138-1147.

18. Goldapple K, Segal Z, Garson C, Lau M, Bieling P, Kennedy S, et al. Modulation of cortical-limbic pathways in major depression: treatment-specific effects of cognitive behavior therapy. Arch Gen Psychiatry 2004 Jan;61(1):34-41.

19. Liu YP, Wilkinson LS, Robbins TW. Effects of acute and chronic buspirone on impulsive choice and efflux of 5-HT and dopamine in hippocampus, nucleus accumbens and prefrontal cortex. Psychopharmacology (Berl) 2004 Apr;173(1-2):175-185.

20. Sprouse JS, Aghajanian GK. Electrophysiological responses of serotoninergic dorsal raphe neurons to 5-HT1A and 5-HT1B agonists. Synapse 1987;1(1):3-9.

21. Hamon M, Fattaccini CM, Adrien J, Gallissot MC, Martin P, Gozlan H. Alterations of central serotonin and dopamine turnover in rats treated with ipsapirone and other 5-hydroxytryptamine1A agonists with potential anxiolytic properties. J Pharmacol Exp Ther 1988 Aug;246(2):745-752.

22. Auger AP. Steroid receptor control of reproductive behavior. Hormones and behavior 2004 Mar;45(3):168-72.

23. Hull EM, Dominguez JM. Sexual behavior in male rodents. Hormones and behavior 2007 Jun;52(1):45-55.

24. Tuiten A, Van Honk J, Koppeschaar H, Bernaards C, Thijssen J, Verbaten R. Time course of effects of testosterone administration on sexual arousal in women. Arch Gen Psychiatry 2000 Feb;57(2):149-53; discussion 155-6.

25. Tuiten A, van Honk J, Verbaten R, Laan E, Everaerd W, Stam H. Can sublingual testosterone increase subjective and physiological measures of laboratory-induced sexual arousal? Archives of general psychiatry 2002 May;59(5):465-6.

26. Postma A, Meyer G, Tuiten A, van Honk J, Kessels RP, Thijssen J. Effects of testosterone administration on selective aspects of object-location memory in healthy young women. Psychoneuroendocrinology 2000 Aug;25(6):563-75.

27. Aleman A, Bronk E, Kessels RP, Koppeschaar HP, van Honk J. A single administration of testosterone improves visuospatial ability in young women. Psychoneuroendocrinology 2004 Jun;29(5):612-7.

28. Schutter DJ, van Honk J. Decoupling of midfrontal delta-beta oscillations after testosterone administration. Int J Psychophysiol 2004 Jun;53(1):71-3.

29. van Honk J, Schutter DJ, Hermans EJ, Putman P, Tuiten A, Koppeschaar H. Testosterone shifts the balance between sensitivity for punishment and reward in healthy young women. Psychoneuroendocrinology 2004 Aug;29(7):937-43.

30. van Honk J, Peper JS, Schutter DJ. Testosterone reduces unconscious fear but not consciously experienced anxiety: implications for the disorders of fear and anxiety. Biological psychiatry 2005 Aug 1;58(3):218-25.

31. van Honk J, Schutter DJ. Testosterone reduces conscious detection of signals serving social correction: implications for antisocial behavior. Psychol Sci 2007 Aug;18(8):663-7.

32. van Honk J, Tuiten A, Hermans E, Putman P, Koppeschaar H, Thijssen J, et al. A single administration of testosterone induces cardiac accelerative responses to angry faces in healthy young women. Behavioral neuroscience 2001 Feb;115(1):238-42.

33. Hermans EJ, Putman P, van Honk J. Testosterone administration reduces empathetic behavior: a facial mimicry study. Psychoneuroendocrinology 2006 Aug;31(7):859-66.

34. Hermans EJ, Putman P, Baas JM, Gecks NM, Kenemans JL, van Honk J. Exogenous testosterone attenuates the integrated central stress response in healthy young women. Psychoneuroendocrinology 2007 Sep-Nov;32(8-10):1052-61.

35. Hermans EJ, Ramsey NF, van Honk J. Exogenous testosterone enhances responsiveness to social threat in the neural circuitry of social aggression in humans. Biological psychiatry 2008 Feb 1;63(3):263-70.

36. Bos PA, Terburg D, van Honk J. Testosterone decreases trust in socially naive humans. Proceedings of the National Academy of Sciences of the United States of America 2010 Jun 1;107(22):9991-5.

37. Bos PA, van Honk J, Ramsey NF, Stein DJ, Hermans EJ. Testosterone administration in women increases amygdala responses to fearful and happy faces. Psychoneuroendocrinology 2013 Jun;38(6):808-817.

38. Eisenegger C, Naef M, Snozzi R, Heinrichs M, Fehr E. Prejudice and truth about the effect of testosterone on human bargaining behaviour. Nature 2010 Jan 21;463(7279):356-9.

39. Poels S, Bloemers J, van Rooij K, Goldstein I, Gerritsen J, van Ham D, et al. Toward personalized sexual medicine (part 2): testosterone combined with a PDE5 inhibitor increases sexual satisfaction in women with HSDD and FSAD, and a low sensitive system for sexual cues. J Sex Med 2013 Mar;10(3):810-823.

40. van Rooij K, Poels S, Bloemers J, Goldstein I, Gerritsen J, van Ham D, et al. Toward personalized sexual medicine (part 3): testosterone combined with a Serotonin1A receptor agonist increases sexual satisfaction in women with HSDD and FSAD, and

dysfunctional activation of sexual inhibitory mechanisms. J Sex Med 2013 Mar;10(3):824-837.

41. Chamberlain NL, Driver ED, Miesfeld RL. The length and location of CAG trinucleotide repeats in the androgen receptor N-terminal domain affect transactivation function. Nucleic Acids Res 1994 Aug 11;22(15):3181-3186.

42. Kazemi-Esfarjani P, Trifiro MA, Pinsky L. Evidence for a repressive function of the long polyglutamine tract in the human androgen receptor: possible pathogenetic relevance for the (CAG)n-expanded neuronopathies. Hum Mol Genet 1995 Apr;4(4):523-527.

43. Tut TG, Ghadessy FJ, Trifiro MA, Pinsky L, Yong EL. Long polyglutamine tracts in the androgen receptor are associated with reduced trans-activation, impaired sperm production, and male infertility. J Clin Endocrinol Metab 1997 Nov;82(11):3777-3782.

44. American Psychiatric Association. Diagnostic and statistical manual of mental disorders. 4th ed. Washington, DC; 2000.

45. Gartlehner G, Hansen R, Thieda P, DeVeaugh-Geiss A, Gaynes B, Krebs E, et al. Comparative effectiveness of second-generation antidepressants in the pharmacologic treatment of adult depression. Comparative effectiveness review, no 7. 2007; 7.

46.Spurdle AB, Dite GS, Chen X, Mayne CJ, Southey MC, Batten LE, et al. Androgen receptor exon 1 CAG repeat length and breast cancer in women before age forty years. J Natl Cancer Inst 1999 Jun 2;91(11):961-966.

47. Haiman CA, Brown M, Hankinson SE, Spiegelman D, Colditz GA, Willett WC, et al. The androgen receptor CAG repeat polymorphism and risk of breast cancer in the Nurses' Health Study. Cancer Res 2002 Feb 15;62(4):1045-1049.

Chapter 7

Pharmacokinetics of a prototype formulation of sublingual testosterone and a buspirone tablet, versus an advanced combination tablet of testosterone and buspirone in healthy premenopausal women

Kim van Rooij, Leo de Leede, Henderik W. Frijlink, Jos Bloemers, Saskia Poels, Hans Koppeschaar, Berend Olivier and Adriaan Tuiten

Drugs in R&D 2014; 14(2):125-132

ABSTRACT

The study aimed to compare the kinetics of two novel combination drug products for Female Sexual Interest/Arousal Disorder (FSIAD). Thirteen women received testosterone via the sublingual route followed 2.5 hours later by a buspirone tablet, versus a single combination tablet swallowed at once. The first clinical prototype consisted of a sublingual solution containing testosterone (0.5 mg) complexed with cyclodextrin and a tablet containing 10 mg buspirone, in a gelatin capsule to ensure blinding during the clinical studies. The innovative fixed-combination tablet consists of an inner-core component of 10 mg buspirone coated with a polymeric time-delay coating and an outer polymeric coating containing testosterone with hydroxypropyl-beta cyclodextrin. We observed an immediate testosterone pulse absorption from both formulations. We also demonstrated that there was adequate absorption of buspirone (>80 % relative to the conventional tablet) and a time delay in release of buspirone of 3.3 hours, close to the 3.0 hours of the reference formulation that showed clinical efficacy in early proof-of-principle studies. The newly developed combination tablet fulfils its design criteria and is a convenient tablet for further clinical studies in FSIAD.

INTRODUCTION

Human sexual behavior is extensively studied in biology, medicine and psychology, but so far there is limited success in the development of drugs for the treatment of sexual dysfunction in women. Low sexual desire, with or without sexual arousal problems, is the most common sex-related complaint reported by women [1–3]. As a result, many women suffer from sexual dissatisfaction, which often negatively interferes with psychological well-being [4]. This has been classified as a clinical condition, referred to as Hypoactive Sexual Desire Disorder (HSDD) [5] or, as recently renamed, Female Sexual Interest/Arousal Disorder (FSIAD) [6]. We have developed two new promising potential treatments for HSDD/FSIAD which are based on the premise that this disorder can have (at least) two different causes [7, 8].

For women who have a low sensitivity to sexual cues, Lybrido is indicated. Lybrido is the combination of sublingual testosterone and a phosphodiesterase type 5 (PDE-5) inhibitor, which is absorbed from the gastro-intestinal tract. Sublingual testosterone (0.5 mg) produces an increase in sexual motivation and desire in sexually functional women, about 4 hours after its peak plasma levels (time to maximum concentration [T_{max}] = 15 min) [9]. The testosterone and the PDE-5 inhibitor are released in such a timeframe that the peak plasma concentration of the PDE-5 inhibitor coincides with the 4-hour delay in behavioral effects of the testosterone. In women with low sensitivity to sexual cues, this combination showed superiority over placebo in increasing sexual satisfaction [7, 10]. For women who have a dysfunctional activation of sexual inhibitory mechanisms during sexual stimulation, Lybridos is developed. Lybridos is the combination of sublingual testosterone and a 5-HT_{1A} receptor agonist (buspirone), released in such a timeframe that the pharmacological effects of the 5-HT_{1A} receptor agonist coincide with the behavioral window induced by the testosterone administration [8]. This combination in women with dysfunctional activation of sexual inhibitory mechanisms increased sexual satisfaction compared with placebo [8]. In previous clinical trials, the two components (sublingual testosterone in combination with a PDE-5 inhibitor or 5-HT_{1A} receptor agonist) were administered separately; however, these components have been developed into one single combination tablet in recent phase IIb trials.

Both products are intended for use on a 'per need' (i.e., not continuous or chronic) basis before anticipated sexual activity. Studies performed by various researchers have clearly indicated a time lag of about 3–4 hours in the pharmacodynamics effect of sublingual testosterone on genital arousal in women and other cognitive and affective functions [9, 11–23]. Therefore, either the PDE5 inhibitor (Lybrido) or (5-HT1a) receptor agonist (Lybridos) component needs to be administered approximately 2–3 hours after administering the testosterone. In the above-mentioned clinical studies, this was obtained by administering the testosterone sublingually as a solution, followed 2.5 hours later by a PDE-5 inhibitor (sildenafil) or a 5-HT_{1A}receptor agonist (buspirone) as a tablet (to ensure blinding, the tablet was administered in a gelatin capsule), thus creating overlapping peaks in effect of testosterone and sildenafil or buspirone. Because this kind of administration is not suitable and rather cumbersome for daily use in practice, we developed a single oral combination tablet that will deliver testosterone sublingually and, approximately 2.5 hours later in the gastro-intestinal tract, the sildenafil or buspirone component, allowing women with FSIAD to take just one single tablet 3–6 hours before the anticipated sexual activity.

The objective of this study was to see if the pharmacokinetic profile of testosterone given sublingually followed 2.5 hours later by a tablet of buspirone (administered in a gelatin capsule) mimics the pharmacokinetic properties of the newly developed single combination tablet as described above. The results of the pharmacokinetic study of the combination of testosterone and sildenafil will be described separately.

At frequent time points, plasma samples were taken and the following pharmacokinetic parameters were determined: the time to maximum concentration (T_{max}), half-life ($T_{½}$), maximum concentration (C_{max}), and area under the curve (AUC) for total testosterone, free testosterone, buspirone, and buspirone's main metabolite (1-(2-pyrimidinyl)-piperazine) for each formulation.

METHODS

Study Subjects

Eligible women were aged between 18 and 35 years, premenopausal, and had a body mass index (BMI) between 18 and 30 kg/m^2. Exclusion criteria included an endocrine disease, neurological problems, a cardiovascular condition, hypertension, abnormal liver or renal function, and a history of a hormone-dependent malignancy. Women taking medications that interfere with the metabolism of sex steroids (e.g., oral contraceptives containing anti-androgens or (anti)androgenic progestogens), or who used serotonergic drugs or who had used testosterone therapy within 6 months before study entry were also excluded.

Women were recruited and enrolled from advertisements, and via a database of a contract research organization (QPS in the Netherlands). Recruitment started in June 2012 and the study was ended in November 2012. To determine eligibility, participants were screened approximately 4 weeks prior to study entry. In addition to an assessment of medical history, all subjects received a physical examination including a 12-lead electrocardiogram, standard biochemistry, serology, and hematological laboratory tests. Blood samples for determination of baseline levels of total testosterone, sex hormone-binding globulin (SHBG), albumin, thyroid-stimulating hormone (TSH), follicle-stimulating hormone (FSH) and estrogen were collected at the screening visit. A urine pregnancy test was applied to all women.

Thirteen healthy young women participated after providing written informed consent. This study was approved by the local medical ethics committee (Stichting BEBO, Assen, the Netherlands) and carried out in agreement with the International Conference on Harmonisation-Good Clinical Practice (ICH-GCP).

Study Design

This was a single-center, investigator-blind, randomized, cross-over controlled study investigating two different modes of administration of a combination of

testosterone and buspirone. The first mode (F1) consisted of the administration of a sublingual solution containing testosterone (0.5 mg) complexed with cyclodextrin, followed 2.5 hours later by an orally administered tablet containing 10 mg buspirone hydrochloride in a gelatin capsule. The second mode of administration (F2) consisted of an innovative fixed-combination tablet with an inner-core component of 10 mg buspirone hydrochloride coated with a polymeric ethylcellulose-based time-delay coating and an outer coating containing 0.5 mg testosterone complexed with hydroxypropyl-β cyclodextrin.

All 13 subjects received the investigational drug formulation in random order. Wash-out between treatments was at least 7 days. Subjects had serial blood samples drawn via an intravenous catheter. Pharmacokinetic parameters were monitored at baseline (−10 min) and (at 5, 10, 15, 20, 25, 30, 60, 90, 120, 135, 145, 165, 180, 195, 210, 225, 240, 270, 300, 330, 360, 390, 450, 570, 690, 810, 930, 1,590 min) after dosing.

Measurement of total testosterone, free testosterone, and dihydrotestosterone were performed at −10, 5, 10, 15, 20, 25, 30, 60, 90, 120, 145, 180, 240 and 1,590 minutes after dosing; buspirone and metabolite 1-(2-pyrimidinyl)-piperazine at −10, 10, 30, 60, 90, 120, 135, 145, 165, 180, 195, 210, 225, 240, 270, 300, 330, 360, 390, 450, 570, 690, 810, 930, 1,590 minutes after dosing.

For each admission period, subjects were instructed to come to the study site on the evening prior to dose administration where vital signs were checked (including ECG) and urine drug test, pregnancy test, and alcohol breath analysis were performed. During the admission period, the subjects received low calorie meals on site and decaffeinated coffee and tea to minimize the influence on pharmacokinetic parameters. Drug, alcohol, and pregnancy tests were performed prior to experimental sessions.

Medication and Dosing

The combination tablet is a menthol-flavored white tablet of 9 mm in diameter for sublingual administration followed by oral administration. The quickly dissolving outer coating, applied by film coating the tablet, delivers cyclodextrin-complexed testosterone (0.5 mg) sublingually, and the time-delayed-release

core delivers buspirone (10 mg) 2.5 hours later. The outer coating comprises testosterone, excipients, and a menthol flavor to guide the disappearance of the coating. The testosterone coating is designed to fully dissolve and to obtain a fast and complete absorption via the mucosal membranes under the tongue. The time-delayed-release core containing the buspirone has been designed on the basis of in-vitro release studies of US Pharmacopeia (USP) II and III, to release the buspirone in a pulsatile manner, approximately 2.5 hours after oral administration. This method of release is accomplished through the use of a polymer coating of ethylcellulose which allows for a slow permeation of water in a pH-independent manner. At the predetermined time, the polymer coating ruptures at the edge of the tablet. The complete disintegrated core of the inner tablet is released immediately, after which there is no delay for the dissolution of the buspirone in the surrounding fluid.

The two formulations were administered by a trained research associate and controlled by a second research associate.

For the testosterone component of F1, a 1 mg/mL testosterone cyclodextrin complex solution was used; the solution was administered with a micropipette (e.g., Eppendorf) into the subject's mouth under the tongue, and the subjects were instructed to keep the solution sublingually for 60 seconds while moving the tongue slightly to optimize absorption. After 60 seconds the subject was instructed to swallow the solution. The buspirone component of F1 was administered orally, as an encapsulated tablet with a glass of water (approximately 200 mL) 150 minutes later.

For F2, the subject was instructed to keep the tablet in the mouth sublingually for 90 seconds, while moving the tongue slightly to optimize absorption. The amount of time that the tablet was in the mouth was timed so that the tablet was swallowed at exactly the right time. After 90 seconds, the subject was instructed to swallow the tablet as a whole, without chewing or otherwise disrupting the dosage form. If necessary, the subject could take a glass of water to enable swallowing.

Hormone Assays

The assay used for the determination of total testosterone and dihydrotestosterone was High Performance Liquid Chromatography with Mass Spectrometric detection (HPLC–MS/MS) (API 4000, Applied Biosystems, MDS SCIEX). Free testosterone was determined in plasma through ultra-filtration followed by HPLC–MS/MS. The method was validated with a lower limit of quantification (LLOQ) of 1.00 pg/mL for free testosterone with an intra-assay coefficient of variation (CV) of 5.2 % and an inter-assay CV of 12.6 %. The LLOQ for testosterone was 0.02 ng/mL with an intra-assay CV of 11.0 % and an inter-assay CV of 12.8 %. The LLOQ for dihydrotestosterone was 0.02 ng/mL with an intra-assay CV of 23.6 % and an inter-assay CV of 29.5 %. The HPLC–MS/MS assay is a reliable and sensitive method for the analysis of free testosterone and overcomes the known limitations of direct immunoassays in measurement of testosterone values in the lower range [24, 25].

Buspirone and 1-(2-Pyrimidinyl)-Piperazine Assay

The analytes buspirone and its major metabolite 1-(2-pyrimidinyl)-piperazine were determined in plasma by HPLC–MS/MS. The method was validated with a LLOQ of 0.01 ng/mL for buspirone with an intra-assay CV of 12.9 % and an inter-assay CV of 7.2 %. The LLOQ for 1-(2-pyrimidinyl)-piperazine was 0.20 ng/mL with an intra-assay CV of 9.4 % and an inter-assay CV of 4.7 %.

Statistical Analysis

The pharmacokinetic parameters were analyzed using the Watson 7.2 Bioanalytical LIMS software (Thermo Electron Corporation, Philadelphia, USA).

Pharmacokinetic parameters including AUC, C_{max}, T_{max} and $T_{½}$ were calculated based on actual and baseline corrected individual concentration–time curves. AUCs were estimated using the linear trapezoidal rule. C_{max} and T_{max} were taken from the measured values. $T_{½}$ was calculated from the unweighted linear regression of the log transformed data determined at the elimination phase of the pharmacokinetic profile of each subject.

The $AUC_{0-1,590}$ was determined as the area under the concentration versus time curve from the first time point to the last time point with measurable drug concentration with a linear/log-linear trapezoidal model. The $AUC_{0-\infty}$ was calculated from the $AUC_{0-1,590}$ by the addition of a constant (Cp/λz), where Cp is the last observed quantifiable concentration and λz is elimination rate constant. This was performed by dividing the Cp by λz determined using linear regression of Cp versus time data (standard extrapolation technique). The elimination rate constant and the corresponding elimination half-life was estimated by log-linear least squares regression of the terminal part of the plasma concentration versus time curve. Absorption lag time (Tlag) is determined as the first time point with a measurable concentration in plasma.

The demographic baseline levels of total and free testosterone, dihydrotestosterone, SHBG, and albumin were calculated by taking the mean of F1 and F2. For the baseline corrected pharmacokinetic parameters, the raw data of each subject was taken as baseline. Dependent on distribution of normality, paired-samples *t*-tests were used for the difference between the F1 and F2 pharmacokinetic parameters for the subjects of whom F1 and F2 data was obtained (n = 12). For all analyses a (two-sided) p value <0.05 was considered statistically significant. Statistical analyses were conducted with SPSS 19.0 (IBM SPSS Statistics for Windows, Version 19.0. Armonk, NY: IBM Corp).

RESULTS

The baseline characteristics and hormone levels of the 13 study participants are outlined in Table 1. Because one subject discontinued after F1 dose, an additional subject was included into the study in order to have F1 and F2 data from 12 subjects. Therefore, 13 subjects were included in F1 and 12 subjects were included in F2. Table 1 shows the baseline demographics of the 13 study participants, all subjects were Caucasian and the mean age was 25.8 years. Baseline levels (measured at screening) of testosterone, SHBG, and albumin were all in the normal female range.

Table 1. Baseline and clinical characteristics of the participants.

Characteristic	Value (*n* = 13)
Age (years)	25.8 ± 4.9
Race	
Caucasian	13
BMI (kg/m2)	22.9 ± 2.1
Contraceptive	
Hormonal	12
Combined oral contraceptive pill	8
IUD (levonorgestrel)	3
Vaginal ring (progestin and estrogen)	1
Non-hormonal	1
Total testosterone (ng/mL)	0.26 ± 0.1
SHBG (nmol/L)	92 ± 80
Albumin (g/L)	41.5 ± 2.8

Baseline levels of total testosterone, SHBG and albumin were measured at the screening visit

The values are mean ± SD. To convert total testosterone to nanomoles per liter, multiply by 3.467

BMI body mass index, IUD intrauterine device, SHBG sex hormone-binding globulin.

Pharmacokinetic Results

Testosterone, Free Testosterone and Dihydrotestosterone

Pharmacokinetic results of the two administrations show that from both products, testosterone was rapidly absorbed with a total testosterone T_{max} between 12 and 16 minutes (0.201–0.256 h) and a half-life between 36 and 44 minutes (0.598–0.726 h). Free testosterone reached the maximum concentration within 12–15 minutes (0.194–0.250 h) with a half-life between 40 and 48 min (0.674–0.798 h). C_{max} is significantly higher for total ($p = 0.003$) and free testosterone ($p = 0.003$) after F2 administration compared with F1 dosing. Furthermore, it is observed that the average AUC with F2 dosing is significantly higher for free testosterone ($p = 0.018$) and not statistically significant for total testosterone ($p = 0.078$) compared with the F1 dosing. The pharmacokinetic parameters of total and free testosterone and dihydrotestosterone after the different modes of administration are summarized in Table 2.

Table 2. Pharmacokinetic parameters for total testosterone, free testosterone, and dihydrotestosterone after F1 and F2 administration

Dosing	C_{max}(ng/mL)	T_{max} (h)	$AUC_{(0-1,590)}$(ng*h/mL)	$T_{½}$ (h)
F1 total testosterone (ng/mL)	5.65 ± 2.35	0.256 ± 0.063	6.41 ± 2.23	0.726 ± 0.165
F2 total testosterone (ng/mL)	7.84 ± 3.69*	0.201 ± 0.043	8.10 ± 2.49	0.598 ± 0.080
F1 free testosterone (pg/mL)	36.2 ± 14.9	0.250 ± 0.083	35.1 ± 18.8	0.674 ± 0.187
F2 free testosterone (pg/mL)	52.4 ± 20.8*	0.194 ± 0.054	55.5 ± 31.1*	0.798 ± 0.247
F1 dihydrotestosterone (ng/mL)	0.519 ± 0.222	0.410 ± 0.105	1.39 ± 0.87	1.14 ± 0.49
F2 dihydrotestosterone (ng/mL)	0.578 ± 0.245	0.451 ± 0.066	1.17 ± 0.47	0.850 ± 0.336

For all calculations, the predose concentration is subtracted from the determined concentration after dosing. The values are mean ± SD. The means of F1 are calculated with the data of 13 women and the means of F2 are based on the data of 12 women. To convert total testosterone to nanomoles per liter, multiply by 3.467.
* $p < 0.05$, value at F2 is significantly different from F1.

AUC area under the curve, C_{max} maximum concentration, T_{max} time to maximum concentration, $T_{½}$ half-life

The mean concentrations of testosterone, free testosterone, and dihydrotestosterone measured after sublingual administration of a single dose of testosterone (0.50 mg) after F1 and F2 administration are shown in the Figures 1, 2 and 3.

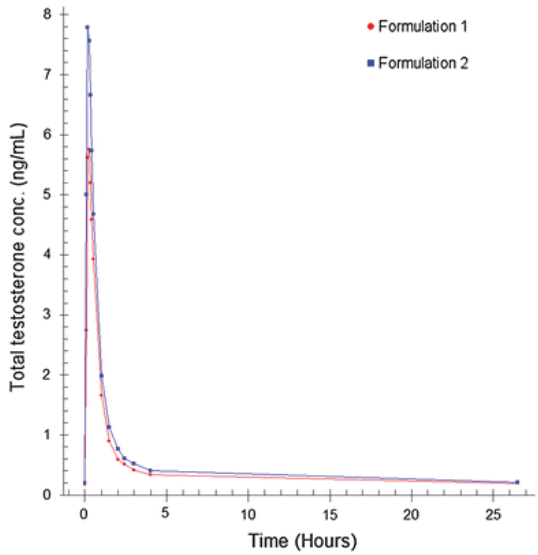

Figure 1. Mean total testosterone plasma concentration–time profile

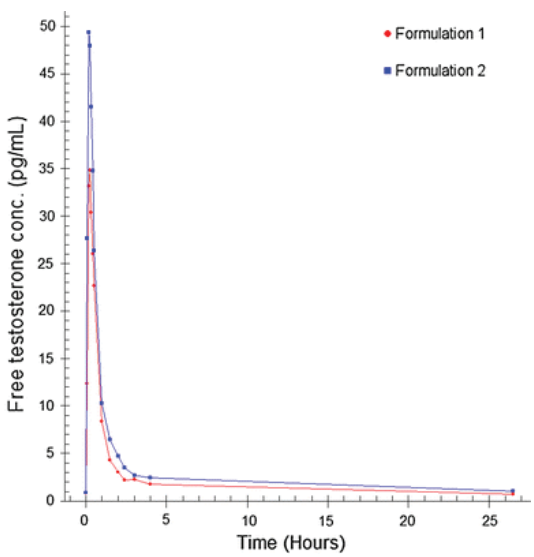

Figure 2. Mean free testosterone plasma concentration–time profile

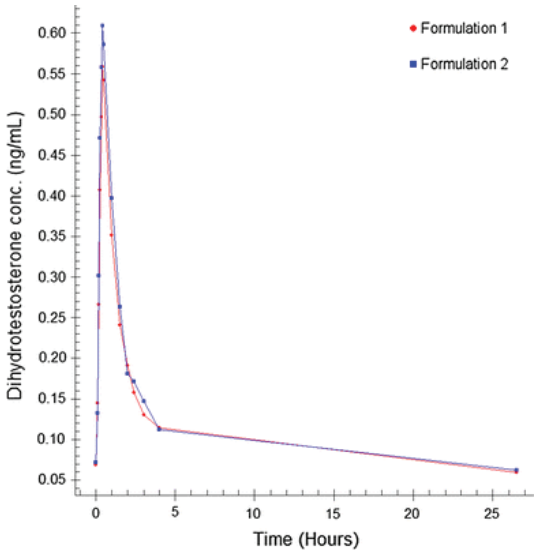

Figure 3. Mean dihydrotestosterone plasma concentration–time profiles

Buspirone and 1-(2-Pyrimidinyl)-Piperazine

Pharmacokinetic results of the two administrations show that from both products, buspirone was absorbed with a T_{max} between 3.69 and 3.95 hours and a half-life between 6.03 and 7.12 hours. Buspirone Tlag (median) was approximately 3 hours after F1 and approximately 3 hours and 20 minutes after F2 administration. Since for F1 the encapsulated tablet was taken after 150 minutes (2.5 h), the in vivo dissolution and absorption of buspirone took 30 minutes. The in vivo lag time for F2 was 200 – 30 = 170 minutes, which was well in line with in vitro observations of the tablet. 1-(2-pyrimidinyl)-piperazine reached the maximum concentration after approximately 4 hours (4.02–4.40 h) with a half-life between 4.84 and 4.86 hours. C_{max} and AUC were not significantly different between the F1 and F2 administration for buspirone as well as 1-(2-pyrimidinyl)-piperazine.

The pharmacokinetic parameters of buspirone and its primary metabolite 1-(2-pyrimidinyl)-piperazine after the F1 and F2 modes of administration are summarized in Table 3.

Table 3. Pharmacokinetic parameters for buspirone and 1-(2-pyrimidinyl)-piperazine after either F1 or F2 administration

Dosing	**C_{max} (ng/mL)**	**T_{max} (h)**	**$AUC_{(0-1,590)}$ (ng*h/mL)**	**AUC extrapolated $_{(0-\infty)}$ (ng*h/mL)**	**Tlag (h)**	**T ½ (h)**
F1 buspirone (ng/mL)	3.95 ± 4.38	3.69 ± 0.54	7.63 ± 8.07	8.02 ± 8.57	2.96 ± 0.14	6.03 ± 2.27
F2 buspirone (ng/mL)	2.16 ± 2.55	3.95 ± 1.82	5.14 ± 5.08	5.56 ± 5.24	3.33 ± 0.82	7.12 ± 2.33
F1 1-(2-pyrimidinyl)-piperazine (ng/mL)	4.35 ± 1.65	4.02 ± 0.68	25.4 ± 14.60	27.4 ± 17.8	3.27 ± 0.33	4.84 ± 2.11
F2 1-(2-pyrimidinyl)-piperazine (ng/mL)	3.99 ± 1.71	4.40 ± 2.27	21.6 ± 6.7	22.7 ± 7.4	3.58 ± 1.32	4.86 ± 1.66

The values are mean ± SD. The means of F1 are calculated with the data of 13 women and the means of F2 are based on the data of 12 women. AUC area under the curve, C_{max} maximum concentration, Tlag absorption lag time, T_{max} time to maximum concentration, T ½ half-life.

The mean concentration–time profiles of buspirone and 1-(2-pyrimidinyl)-piperazine measured after oral administration of a single dose of buspirone (10 mg) using the F1 and F2 modes of administration are shown in Figures 4 and 5.

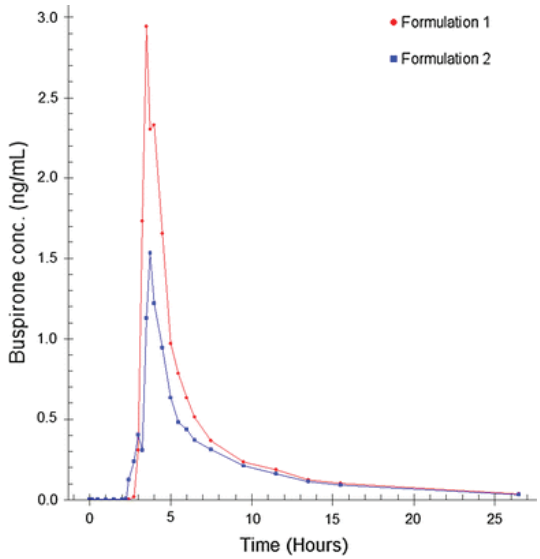

Figure 4. Mean buspirone plasma concentration–time profile

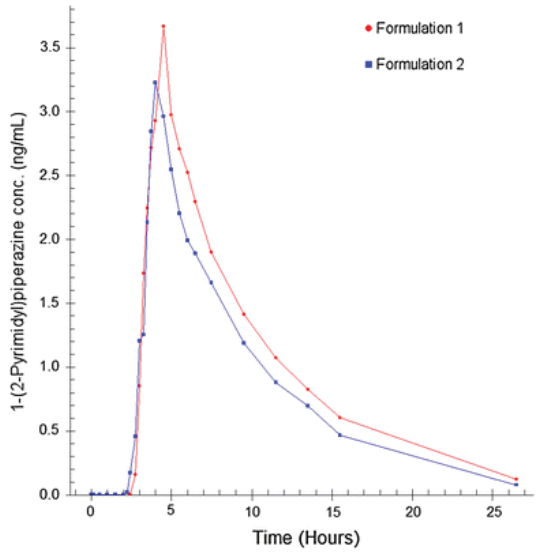

Figure 5. Mean 1-(2-pyrimidinyl)-piperazine plasma concentration–time profile

The two formulations were well tolerated.

DISCUSSION

Our results demonstrate that sublingual administration of testosterone in both formulations was followed by a very quick and steep increase of total and free testosterone levels; with peak levels reached between 10 and 20 minutes, which is in line with our previous studies [9, 26]. Serum levels of total and free testosterone rapidly declined to reach baseline levels by approximately 2.5 hours.

The total testosterone C_{max} following administration of 0.50 mg sublingual testosterone after the liquid dosing regimen showed consistency with the reported C_{max} of Tuiten et al. and van Rooij et al. [9, 26]; however, the C_{max} of total and free testosterone after administration of the tablet is higher. This is also reflected by the AUC for total and free testosterone after administration of the tablet compared with the liquid dosing, meaning very fast absorption from the solid polymeric matrix. Since there is no time delay or difference in absorption for the two formulations, the in vivo dissolution of testosterone from the tablet coating is not the rate-limiting step in the absorption process, which indicates that the driving force for dissolution in the saliva is high. It is surprising that the bioavailability of testosterone from the tablet is higher than from the liquid, since one would expect it to be the other way around, and certainly for an intrinsically insoluble compound such as testosterone. On the other hand, it should be taken into account that a small amount of the liquid testosterone (0.5 ml) may leak away to the esophagus and stomach which could explain the lower bioavailability of this dosage form compared with the combination tablet. In a previous study of van Rooij et al., three different doses of the liquid testosterone were investigated (0.25, 0.50, and 0.75 mg) and it was observed that the lowest testosterone dose (0.25 mg) had the highest bioavailability [26]. In that study, the 0.50 mg of sublingual testosterone solution had a relative availability to the lowest dose of 69 %. The AUC of the lowest dose was dose corrected equivalent to a 0.3 mg single pulmonal testosterone dose described by Davison and colleagues [27]. Due to the properties of testosterone, the low dose, and the large surface area of the lungs, it was anticipated that this was a near 100 % bioavailability, resulting in an approximate 70 % bioavailability for the 0.5 mg liquid sublingual dose. And since the new combination tablet with the

coating of testosterone has both a higher C_{max} and AUC, we assume that the absolute bioavailability of this tablet is above 70 and probably close to 80 %.

The metabolite dihydrotestosterone peak levels were reached within 30 minutes and levels returned to baseline levels within 4 hours, which is also consistent with our previous pharmacokinetic study [26].

Due to the high first-pass effect, the variability between the subjects for the buspirone levels was as expected very high. The Tlag time and the T_{max} for both buspirone and its metabolite 1-(2-pyrimidinyl)-piperazine were comparable for both formulations. This indicates that the in vivo rupture time of the tablet is within the set specification of 120–240 minutes (average 150 min).

Although the C_{max} for buspirone was not significantly different between the two formulations, the average C_{max} was somewhat lower for the combination tablet (F2) compared with the encapsulated tablet (F1) taken after 150 minutes. The encapsulated gelatin capsule of F1 is probably absorbed in the stomach, while the combination tablet is absorbed at a more distal location in the gastrointestinal tract (in the small intestines). Since the combination tablet will release its drug load after a 150-minute longer travel through the gastrointestinal tract, this could have influenced the C_{max} for buspirone. However, based on the AUC of the main first-pass metabolite of buspirone, there does not seem to be a significant incomplete absorption of the buspirone, but rather a more extensive first-pass effect with the tablet that resides longer and further in the gastrointestinal tract.

Conclusion

In conclusion, there was adequate absorption of both testosterone and buspirone and an adequate time delay for the release of buspirone after administration of the combination tablet, which is a well-tolerated, convenient and suitable formulation for Lybridos administration in a daily practical setting. All subsequent clinical studies, including dose-finding studies, will be done with this innovative tablet formulation.

REFERENCES

1. Laumann EO, Paik A, Rosen RC. Sexual dysfunction in the United States: prevalence and predictors. JAMA. 1999;281:537–44.

2. Fugl-Meyer KS. Sexual disabilities and sexual problems. In: Sex in Sweden. Stockholm: National Institute of Public Health; 2000. pp. 199–216.

3. Shifren JL, Monz BU, Russo PA, Segreti A, Johannes CB. Sexual problems and distress in United States women: prevalence and correlates. Obstet Gynecol. 2008;112:970–8.

4. Davison SL, Bell RJ, LaChina M, Holden SL, Davis SR. The relationship between self-reported sexual satisfaction and general well-being in women. J Sex Med. 2009;6:2690–7.

5.American Psychiatric Association. Diagnostic and statistical manual of mental disorders. 4th edn. Washington, DC; 2000.

6.American Psychiatric Association. Diagnostic and statistical manual of mental disorders. 5th edn. Arlington, VA: American Psychiatric Publishing; 2013.

7.Poels S, Bloemers J, van Rooij K, Goldstein I, Gerritsen J, van Ham D, van Mameren F, Chivers M, Everaerd W, Koppeschaar H, Olivier B, Tuiten A. Toward personalized sexual medicine (part 2): testosterone combined with a PDE5 inhibitor increases sexual satisfaction in women with HSDD and FSAD, and a low sensitive system for sexual cues. J Sex Med. 2013;10:810–23.

8.van Rooij K, Poels S, Bloemers J, Goldstein I, Gerritsen J, van Ham D, van Mameren F, Chivers M, Everaerd W, Koppeschaar H, Olivier B, Tuiten A. Toward personalized sexual medicine (part 3): testosterone combined with a Serotonin1A receptor agonist increases sexual satisfaction in women with HSDD and FSAD, and dysfunctional activation of sexual inhibitory mechanisms. J Sex Med. 2013;10:824–37.

9.Tuiten A, Van Honk J, Koppeschaar H, Bernaards C, Thijssen J, Verbaten R. Time course of effects of testosterone administration on sexual arousal in women. Arch Gen Psychiatry. 2000;57:149–53 discussion 155–156.

10.van der Made F, Bloemers J, Yassem WE, Kleiverda G, Everaerd W, van Ham D, et al. The influence of testosterone combined with a PDE5-inhibitor on cognitive, affective, and physiological sexual functioning in women suffering from sexual dysfunction. J Sex Med. 2009;6(3):777–90.

11.Postma A, Meyer G, Tuiten A, van Honk J, Kessels RP, Thijssen J. Effects of testosterone administration on selective aspects of object-location memory in healthy young women. Psychoneuroendocrinology. 2000;25:563–75.

12.Aleman A, Bronk E, Kessels RP, Koppeschaar HP, van Honk J. A single administration of testosterone improves visuospatial ability in young women. Psychoneuroendocrinology. 2004;29:612–7.

13.Schutter DJ, van Honk J. Decoupling of midfrontal delta-beta oscillations after testosterone administration. Int J Psychophysiol. 2004;53:71–3.

14.van Honk J, Schutter DJ, Hermans EJ, Putman P, Tuiten A, Koppeschaar H. Testosterone shifts the balance between sensitivity for punishment and reward in healthy young women. Psychoneuroendocrinology. 2004;29:937–43.

15.van Honk J, Peper JS, Schutter DJ. Testosterone reduces unconscious fear but not consciously experienced anxiety: implications for the disorders of fear and anxiety. Biol Psychiatry. 2005;58:218–25.

16.van Honk J, Schutter DJ. Testosterone reduces conscious detection of signals serving social correction: implications for antisocial behavior. Psychol Sci. 2007;18:663–7.

17.van Honk J, Tuiten A, Hermans E, Putman P, Koppeschaar H, Thijssen J, Verbaten R, van Doornen L. A single administration of testosterone induces cardiac accelerative responses to angry faces in healthy young women. Behav Neurosci. 2001;115:238–42.

18.Hermans EJ, Putman P, van Honk J. Testosterone administration reduces empathetic behavior: a facial mimicry study. Psychoneuroendocrinology. 2006;31:859–66.

19.Hermans EJ, Putman P, Baas JM, Gecks NM, Kenemans JL, van Honk J. Exogenous testosterone attenuates the integrated central stress response in healthy young women. Psychoneuroendocrinology. 2007;32:1052–61.

20.Hermans EJ, Ramsey NF, van Honk J. Exogenous testosterone enhances responsiveness to social threat in the neural circuitry of social aggression in humans. Biol Psychiatry. 2008;63:263–70.

21.Bos PA, Terburg D, van Honk J. Testosterone decreases trust in socially naive humans. Proc Natl Acad Sci USA. 2010;107:9991–5.

22.Eisenegger C, Naef M, Snozzi R, Heinrichs M, Fehr E. Prejudice and truth about the effect of testosterone on human bargaining behaviour. Nature. 2010;463:356–9.

23.Bos PA, van Honk J, Ramsey NF, Stein DJ, Hermans EJ. Testosterone administration in women increases amygdala responses to fearful and happy faces. Psychoneuroendocrinology. 2013;38:808–17.

24.Labrie F, Belanger A, Belanger P, Berube R, Martel C, Cusan L, Gomez J, Candas B, Castiel I, Chaussade V, Deloche C, Leclaire J. Androgen glucuronides, instead of

testosterone, as the new markers of androgenic activity in women. J Steroid Biochem Mol Biol. 2006;99:182–8.

25.Miller KK, Rosner W, Lee H, Hier J, Sesmilo G, Schoenfeld D, Neubauer G, Klibanski A. Measurement of free testosterone in normal women and women with androgen deficiency: comparison of methods. J Clin Endocrinol Metab. 2004;89:525–33.

26.van Rooij K, Bloemers J, de Leede L, Goldstein I, Lentjes E, Koppeschaar H, Olivier B, Tuiten A. Pharmacokinetics of three doses of sublingual testosterone in healthy premenopausal women. Psychoneuroendocrinology. 2012;37:773–81.

27.Davison S, Thipphawong J, Blanchard J, Liu K, Morishige R, Gonda I, Okikawa J, Adams J, Evans A, Otulana B, Davis S. Pharmacokinetics and acute safety of inhaled testosterone in postmenopausal women. J Clin Pharmacol. 2005;45:177–84.

Chapter 8

Discussion

DISCUSSION

In this thesis studies are presented which use a personalized medicine approach to investigate potential causal mechanisms, both biological and psychological, which might underlie sexual complaints such as low sexual desire, interest or arousal of women diagnosed with Female Sexual Interest/Arousal Disorder (FSIAD). We hypothesized that these causal mechanisms are partly the result of differences in sensitivity of the brain to sexual stimuli. The degree of this sensitivity for sexual cues or stimuli is influenced by biological factors, including hormonal levels and genetic factors. The results of the different studies described in this thesis suggest that there are at least two different subtypes among women who are diagnosed with HSDD/FSIAD; those who are low sensitive to sexual stimuli and those in which sexual stimuli elicit a dysfunctional activation of sexual inhibitory mechanisms.

We hypothesized that women who are classified as low sensitive would benefit from an on-demand treatment of sublingual testosterone (0.5 mg) in combination with a PDE5-inhibitor (e.g., sildenafil (50 mg)) and that women with dysfunctional activation of sexual inhibitory mechanisms would benefit from the combination of sublingual testosterone (0.5 mg) and the 5-HT_{1A} receptor agonist buspirone (10 mg). Besides the FSIAD population, these treatments could also be efficacious in another indication; women who developed sexual complaints as the result of the use of SSRI antidepressants. In these women in which synaptic serotonin levels are increased by the SSRIs, a 5-HT_{1A} receptor agonist such as buspirone, lowers serotonergic neuron firing activity for a short time after a single administration via activation of somatodendritic 5-HT_{1A} autoreceptors. Because of the pharmacodynamic properties of buspirone and other 5-HT_{1A} receptor agonists, we hypothesized that the combination of sublingual testosterone and buspirone could be beneficial in women diagnosed with SSRI-induced sexual dysfunction. However, the classification we used in the FSIAD population could also be of influence in women with SSRI-induced sexual dysfunction. The sensitivity of the brain to sexual stimuli before the start of SSRI therapy can influence a woman's response on our two treatments (sublingual

testosterone in combination with a PDE5 inhibitor and sublingual testosterone in combination with a 5-HT_{1A} receptor agonist).

Each treatment consists of a combination of two components, administered in such a time frame that during the behavioral window induced by sublingual testosterone (3-6 hours after the testosterone peak) the second component (PDE5-inhibitor or 5-HT_{1A} receptor agonist) becomes active. The increase in sensitivity to sexual stimuli in combination with the PDE5-inhibitor results in an increase in physiological sexual responding which might trigger a positive feedback loop: physical responses (such as genital arousal) are experienced more intensely and this experience might act as an enhancing (positive feedback) internal stimulus. Because the women are more aware of their physical responses, this might further increase sexual motivation and desire. The operating principle of the combination of sublingual testosterone and a 5-HT_{1A} receptor agonist is based on the following reasoning. In a subdivision of women with FSIAD an increase in sensitivity to sexual stimuli due to sublingual testosterone will elicit an exaggerated inhibitory sexual response. This inhibitory effect could have come about through a functional and a phasic increase of serotonergic activity in the dorsolateral PFC. Addition of the 5-HT_{1A} receptor agonist buspirone prevent this phasic increase of serotonergic neurotransmission which influences the inhibitory sexual response in the PFC. During exposure to sexual stimuli (internally or externally induced), women prone to sexual inhibition might then react to a combined treatment with testosterone and a 5-HT_{1A} receptor agonist with an increased physiological and subjective sexual response.

In this chapter, the main findings of the pharmacokinetic- and pharmacodynamic properties of these two treatments on sexual functioning in women with FSIAD and SSRI-induced sexual dysfunction, and future perspectives will be discussed.

Pharmacokinetic properties

On-demand sublingual testosterone administration forms the basis for the effect of our treatments for women diagnosed with FSIAD or SSRI-induced sexual dysfunction. After sublingual testosterone administration, plasma levels of total

and free testosterone rapidly increases with a maximum plasma concentration after 15 minutes. In order to obtain a peak in plasma level of free testosterone, which is the biological active substance, the dosage of sublingual testosterone should be high enough to surpass the Sex Hormone Binding Globulin (SHBG) threshold. By surpassing the SHBG threshold, unbound free testosterone increases, which has repeatedly shown to result in behavioral effects approximately 4 hours later. In our study described in chapter 2, we confirmed that the administration of 0.5 mg of sublingual testosterone is indeed sufficient to surpass this threshold.

In the majority of studies regarding the influence of testosterone on sexual behavior in women, chronic testosterone administration is utilized as the method of administration [1-3]. However, in contrast to on-demand sublingual testosterone administration, chronic testosterone will not lead to a steep and quick increase of free testosterone levels. During chronic treatment, the increase in the free levels of testosterone occurs slowly and much later in time, and therefore a behavioral (pharmacodynamics) effect on sexual functioning would follow later. Most importantly, chronic treatment with testosterone will result in a sustained increase of testosterone levels which subsequently might induce androgenic adverse events. After a single on-demand administration of 0.5 mg sublingual testosterone, plasma testosterone levels return to baseline within 3 hours. Thus, even if 0.5 mg sublingual testosterone is administered daily, this will not result in sustained supraphysiological testosterone levels. Long-term safety studies are needed, but it is expected that there will be no safety concerns with this dose and administration route.

In previous studies, the two components (testosterone and 5-HT_{1A} receptor agonist) were administered separately, which is not suitable for clinical practice. Chapter 7 describes the development of a new innovative combination tablet (a combination of 0.5 mg sublingual testosterone and 10 mg of the 5-HT_{1A} receptor agonist buspirone hydrochloride) which appears to have a comparable pharmacokinetic profile as the separate administration of 0.5 mg sublingual testosterone, followed 2.5 hours later by 10 mg of buspirone (the prototype). It is remarkable that the bioavailability of testosterone is even higher after administration of the combination tablet, compared to the sublingual

testosterone solution with cyclodextrin as a carrier that was used in the prototype. It is possible that a small amount of the sublingual testosterone solution with cyclodextrin as a carrier may leak away to the esophagus and the stomach, and results in a lower bioavailability of this administration method as compared to the combination tablet. The pharmacokinetics of buspirone for both administration methods was comparable. The combination tablet is a patient-friendly method of administering these treatments on-demand. For future studies, this on-demand combination tablet will be used.

Effect on sexual functioning in women with HSDD/FSIAD

In chapter 3, the basis for different causal mechanisms in the etiology of FSIAD and the role of androgens and serotonin in sexual functioning is discussed. A rationale is given for the use of on-demand combined administration of sublingual testosterone and a PDE5 inhibitor for women with low sensitivity for sexual cues, and for the use of on-demand combined administration of sublingual testosterone and a 5-HT_{1A} agonist in women with a dysfunctional activity of sexual inhibitory mechanisms. These women suffer from low sexual desire and are therefore not motivated to initiate sexual activities. It seems counterintuitive that these women would take an on-demand drug; however, because they are distressed by their low desire, they are motivated to change and improve their sexual functioning and seek help.

In chapter 4 and chapter 5, the results of a randomized, double-blind, placebo-controlled cross-over study are presented in which 56 women with FSIAD took both on-demand treatments (testosterone 0.5 mg + sildenafil 50 mg, and testosterone 0.5 mg + buspirone 10 mg, respectively) and placebo in an at home setting. In the first week of each treatment, the women were instructed to take 3 administrations of study drug, and their subjective and physiological responses during exposure to erotic film clips were measured with the help of an ambulatory lab. In the following 3 weeks, the women could take the study drug on-demand (4 hours before anticipated sexual activity) and were instructed to fill out a sexual event diary after each sexual activity. The combination of sublingual testosterone and sildenafil, and the combination of sublingual testosterone and buspirone increased sexual functioning in the ambulatory

psychophysiological part as well as during the sexual activity at home. As hypothesized, the combination of testosterone and sildenafil significantly improved sexual functioning in the low sensitive women, while the combination of testosterone and buspirone improved sexual functioning in women with high inhibition.

The results did not show any interaction with menopausal status or oral contraceptive use. Apparently, the increase in plasma testosterone levels after the sublingual testosterone administration proved to be enough to saturate the high SHBG levels, observed in women who took combined oral contraceptives, and to increase free testosterone levels, thereby inducing behavioral effects 3-6 hours later. This has to be confirmed in larger trials.

As stated in the beginning of this chapter, the sensitivity of the brain for sexual stimuli is based, inter alia, on biological mechanisms. The chance of developing a sexual dysfunction in women with a low sensitive or normal/high sensitive brain system for sexual stimuli depends on all kinds of individual characteristics in the context of external stimuli. Women high sensitive for sexual stimuli, might be more sensitive for positive sexual experiences. The other side of this high sensitivity coin is that they also might be more sensitive for negative experiences (as is described in the introduction and in chapter 3). In the light of individual differences, women prone to inhibition might have an altered serotonergic neurotransmission making them more sensitive to negative sexual experiences which could lead to a dysfunctional activity of inhibitory mechanisms. In the study described in chapters 4 and 5, we did not observe a difference in the number of negative experiences between women who are low sensitive and women who are high sensitive based on the emotional Stroop task (described in chapter 4). However, we did observe that women who were high inhibitory reported significantly more negative sexual experiences compared to the low inhibitory group (described in chapter 5). One can imagine that high sensitive women, as compared to low sensitive women, might experience to a greater degree positive or negative sexual experiences, respectively. As a result, it could be expected that the frequency of negative sexual experiences would also be higher in the high sensitive group compared to the low sensitive women. However, because a subdivision of women responds to both treatments or does

not respond to any of the treatments, there is only in a part overlap between high sensitive and high inhibitory women. In this study, the women who reported a decrease in sexual functioning under the condition of sublingual testosterone combined with sildenafil were classified as high inhibitors. Based on this analyses, we made the subdivision between women who were low or high sexual inhibitors. It must be noted that negative sexual experiences per se do not have to cause learning of sexually inhibitory mechanisms, or FSIAD.

Women who are low sensitive and suffer from FSIAD lack an adequate activation of excitatory mechanisms, which could be partly the result of an inadequate activation of the androgen receptor and/or different activity of testosterone metabolism. These underlying mechanisms must be interpreted irrespective of absolute testosterone levels, which is currently under debate by various researchers [4,5]. These women, although biologically equipped with a low sensitive system, do not necessarily develop sexual complaints. We believe however, that these women are not experiencing the same amount of sexual motivation, desire, and maybe sexual pleasure as compared to high sensitive women. This does not have to create a problem, and therefore a low sensitive woman can be sexually functional although this is also dependent on her partner's sexual needs. If a low sensitive woman meets a high sensitive partner, it is possible that after a certain period of time the low sensitive woman experiences distress because of the interpersonal difference in sexual motivation and thereby is prone to develop a sexual dysfunction.

Effect on sexual functioning in women with SSRI-induced sexual dysfunction

Women who develop sexual complaints after the start of an SSRI do not fall under the diagnosis of FSIAD because their sexual complaints are caused by a pharmacological substance. However, these women meet the criteria of the DSM-5 diagnosis: substance (in this case an SSRI)-induced sexual dysfunction. We have conceptualized FSIAD as the result of interacting biological and psychological mechanisms; however, in women with SSRI-induced sexual dysfunction the effects of serotonin reuptake inhibition on serotonergic neurotransmission is likely to play an important role. SSRIs not only influence the serotonergic neurotransmission, but also other neurotransmitter systems (e.g.,

dopamine, noradrenalin). It is known that the prevalence of sexual complaints is very high in women taking SSRIs. In our studies we assume that testosterone administration could be an effective therapy for the sexual complaints of these women. In a preliminary, placebo-controlled, randomized, cross-over, exploratory study (described in chapter 6), 21 women with SSRI-induced sexual dysfunction received the combination of sublingual testosterone 0.5 mg and sildenafil 50 mg, and the combination of sublingual testosterone 0.5 mg and buspirone 10 mg. In addition to measures of sexual functioning, the androgen receptor CAG repeat length was assessed. It was hypothesized that women with relatively long CAG repeats, which implies a lower receptor function, are less sensitive to sexual cues. These women might need higher levels of circulating testosterone compared to women with relatively shorter CAG repeats to activate intracellular processes after binding to the androgen receptor, hereby increasing their sensitivity to sexual stimuli.

In this study, overall no main effect of either treatment on sexual satisfaction was found. However, a significant interaction effect between the SSRI dosage (low and medium-high dose), CAG repeat length (short and long length) and treatment response on sexual satisfaction was found. Women with a relatively long CAG repeat length who use a low dose of a SSRI reported an increase in sexual satisfaction with both drug combinations. Thus, only significant differences in sexual satisfaction were found in women with relatively long CAG repeats which confirmed our hypothesis partially. However, this difference was only observed in women who use a low dose of SSRI. It can be hypothesized that women who use a low dose of SSRI have relatively modest elevations of synaptic serotonin levels and therefore 0.5 mg sublingual testosterone would be sufficient to increase the sensitivity of the brain, thereby increasing sexual motivation, which enhances the physiological responsiveness due to sildenafil. These women also responded to sublingual testosterone in combination with buspirone, in which case buspirone seemed to lower serotonergic firing activity for a short period of time, thereby allowing testosterone to increase the sensitivity of the brain for sexual stimuli.

In women with a medium-high SSRI dose there were no differences in sexual satisfaction after treatment with either drug combinations compared to placebo.

This is probably due to the small sample size (only 5 women used a medium-high dose). Another explanation may be that the buspirone dose is relatively too low in case a medium-high SSRI dose is given to effectively decrease the synaptic serotonin levels. Another point of discussion is that we do not know in this population which women can be subtyped into low sensitive or prone to sexual inhibition because we do not have data regarding baseline sexual functioning prior to SSRI administration.

This preliminary study suggests that our treatments may be promising in certain subgroups with this indication, depending on genetics, SSRI dose, and psychological factors. More research in a larger study population is needed and more polymorphisms should be investigated that could have an influence on sexual functioning.

Future perspectives

In the studies described in chapters 3, 4 and 5, the ratio between low sensitivity and high sensitivity was determined by a masked version of the emotional Stroop task in which a woman's reaction time to erotic and neutral words are measured. Based on this task, women who are low sensitive are identified as having a longer reaction time to neutral words compared to erotic words (they are low sensitive for erotic stimuli (in this case erotic words), and therefore have less attention to these words). Based on this method, the ratio between the two subtypes is approximately 50/50. Although we believe that this ratio reflects two subdivisions of the FSIAD population, the Stroop task, however, is not a practical tool in a clinical setting. As a first step towards a more practical tool, we developed a demarcation formula which includes biological variables, genetic polymorphisms e.g., CAG repeats, other relevant neurotransmitter and hormonal systems and subjective variables such as questionnaires regarding sexual functioning. With the use of this demarcation formula, we performed two phase 2b trials in which 210 women with FSIAD participated in each trial. If a woman was low sensitive according to the demarcation formula, she could participate in the testosterone and sildenafil trial; high sensitive women (who are prone to sexual inhibition) could participate in the testosterone and buspirone trial. The women who were low sensitive were randomly assigned to 1

of the following treatment groups: placebo; sublingual testosterone 0.5 mg; sildenafil 50 mg; 0.25 mg testosterone+25 mg sildenafil; 0.25 mg of testosterone+50 mg sildenafil; 0.50 mg testosterone+25 mg sildenafil; 0.50 mg testosterone+50 mg sildenafil. Women who were high sensitive participated in another trial with also 7 treatment arms (placebo; testosterone 0.5 mg; buspirone 10mg; 0.25 mg testosterone+5 mg buspirone; 0.25 mg of testosterone+10 mg buspirone; 0.50 mg testosterone+5 mg buspirone; 0.50 mg testosterone+10 mg buspirone). The results of the study in low sensitive women confirmed our hypothesis that the dosage of 0.5 mg testosterone in combination with 50 mg sildenafil is the most efficacious. Analysis of the results of the high sensitive women is currently ongoing.

The demarcation formula based on the parameters we used in the phase 2b trials could be optimized and made more suitable for clinical practice if it could be based on only genetic polymorphisms. In this way, a practical kit (based on, for example, saliva of a finger prick) may be developed to predict almost immediately whether a women is low- or high sensitive.

It is very likely that there might be different levels of sensitivity for sexual stimuli (e.g., low sensitivity; medium-high sensitivity; high sensitivity), which are influenced by the difference in the sensitivity of the androgen system (e.g., CAG repeat length, testosterone metabolism), the serotonin system (5-HT receptor polymorphism) as well as other neurobiological systems (for example dopamine, prolactin, and norepinephrine systems). In recent years, genetic polymorphisms are more and more studied and new polymorphisms are identified in a broad area of medicine. In a personalized medicine point of view, analysis of relevant polymorphisms might be an excellent method to identify individual differences in sensitivity for sexual stimuli and response of our pharmacological treatments.

REFERENCES

1. Shifren JL, Braunstein GD, Simon JA, Casson PR, Buster JE, Redmond GP, et al. Transdermal testosterone treatment in women with impaired sexual function after oophorectomy. N Engl J Med 2000 Sep;343(10):682-688.

2. Sherwin BB. Randomized clinical trials of combined estrogen-androgen preparations: effects on sexual functioning. Fertil Steril 2002 Apr;77(4):S49-S54.

3. Simon J, Braunstein G, Nachtigall L, Utian W, Katz M, Miller S, et al. Testosterone patch increases sexual activity and desire in surgically menopausal women with hypoactive sexual desire disorder. J Clin Endocrinol Metab 2005 Sep;90(9):5226-5233.

4. Leusink P, Boeke AJ, Laan E. Toward Personalized Sexual Medicine: Where is the Evidence? J Sex Med 2014 Sep;11(9):2357-2358.

5. Tuiten A, Bloemers J, van Rooij K, Poels S, Gerritsen J, van Ham D, et al. Response to "Toward Personalized Sexual Medicine: Where is the Evidence?". J Sex Med 2014 Sep;11(9):2359-2363.

Chapter 9

Nederlandse samenvatting (Summary in Dutch)

Curriculum Vitae

List of publications

Dankwoord (Acknowledgements)

SAMENVATTING/SUMMARY IN DUTCH

Seksuele problemen bij vrouwen kunnen een negatieve invloed hebben op het psychisch welbevinden. De meest gerapporteerde seksuele klacht bij vrouwen is een verminderd seksueel verlangen, welke tot voorheen werd geclassificeerd als de diagnose Hypoactive Sexual Desire Disorder (HSDD) in het diagnostisch handboek voor statistische en psychische stoornissen, de Diagnostic and Statistical Manual for Mental Disorders (DSM, versie IV-tekst revisie) [1]. Vrouwen die problemen hebben met seksuele opwinding worden in deze manual geclassificeerd als Female Sexual Arousal Disorder (FSAD). Omdat verlangen en opwinding niet los van elkaar gezien kunnen worden, zijn deze twee diagnosen in de meest recente editie van dit handboek (DSM-5 uit 2013) samengevoegd onder één diagnose: Female Sexual Interest/Arousal Disorder (FSIAD) [2]. De belangrijkste criteria voor deze diagnose zijn dat vrouwen met FSIAD een afwezig of verminderde seksuele interesse/verminderde seksuele opwinding hebben gedurende minimaal 6 maanden en dat zij gebukt gaan onder deze klachten. Vrouwen voldoen niet aan deze diagnose wanneer de klachten veroorzaakt worden door een andere seksuele stoornis, psychische diagnose (zoals depressie of angst) of een medische conditie.

De prevalentie van seksuele klachten bij vrouwen is niet eenduidig en de percentages lopen uiteen van 10% tot 43%. Het exacte percentage vrouwen met HSDD/FSIAD[1] is onduidelijk omdat dit afhangt van de populaties die onderzocht zijn (bijvoorbeeld verschillende leeftijden, of de klachten gepaard gaan met psychisch lijden, duur van de klachten). Tot op heden is er voor vrouwen met FSIAD geen farmacotherapeutische behandeling geregistreerd door de regulatoire instanties (de Food and Drug Administration (FDA) in de Verenigde Staten en de European Medicines Agency (EMA) in Europa).

[1] In onze huidige en toekomstige studies zullen de vrouwen die gediagnosticeerd worden met FSIAD ook aan de volgende criteria moeten voldoen: “afwezig of verminderde interesse in seksuele activiteiten” en “afwezige/verminderde erotische gedachten of fantasieën”. Deze criteria vallen onder criterium A in de DSM-5 en komen overeen met de indicatoren zoals die beschreven zijn in de diagnose HSDD in de DSM-IV-TR. Dus alle vrouwen die aan criterium A van de diagnose FSIAD voldoen worden automatisch gediagnosticeerd als HSDD (maar niet vice versa). Om onduidelijkheden te voorkomen zal de HSDD populatie zoals we die in eerdere studies hebben beschreven aangepast worden als FSIAD in dit proefschrift.

Dit proefschrift beschrijft de ontwikkeling van twee verschillende 'on-demand' farmacologische behandelingen (dus deze medicijnen worden alleen ingenomen wanneer een vrouw en haar partner anticiperen op een seksuele activiteit) voor vrouwen met FSIAD. De basis voor dit ontwikkelingsprogramma is een gepersonaliseerde aanpak, oftewel een *personalized medicine approach*; verschillende individuele behandelmogelijkheden gebaseerd op verschillende onderliggende mechanismen. Deze onderliggende mechanismen zijn deels gebaseerd op het dual control model van Bancroft en Janssen (2007). In dit model beargumenteren Bancroft en Janssen dat verschillen in de seksuele respons beïnvloed worden door activatie van excitatoire en inhibitoire mechanismen en processen [3,4].

Naar onze mening, zijn deze excitatoire en inhibitoire mechanismen afhankelijk van de gevoeligheid van het brein voor seksuele stimuli. Vrouwen die een relatief ongevoelig brein hebben voor seksuele stimuli hebben onvoldoende activatie van excitatoire mechanismen die betrokken zijn bij seksuele motivatie en seksueel verlangen. Aan de andere kant, hebben vrouwen met een normaal of verhoogd gevoelig breinsysteem voor seksuele stimuli, een adequate excitatoire respons maar kunnen ook een verhoogde gevoeligheid hebben voor het ontwikkelen van een negatieve associatie ten aanzien van deze seksuele prikkels, waardoor een versterkte inhibitoire respons kan optreden. Met betrekking tot deze vrouwen poneren wij de hypothese dat de seksuele klachten beïnvloed worden door activiteit van de prefrontale cortex (PFC) welke betrokken is bij inhibitoire processen die een algemene rol spelen in gedrag, inclusief seksueel gedrag. We hebben de hypothese dat er in deze vrouwen een fasische toename van serotonerge activiteit in de PFC plaatsvindt in respons op de negatieve associaties die deze vrouwen hebben bij bepaalde seksuele prikkels. Deze fasische serotonerge toename in de PFC zorgt op zijn beurt dat er minder activiteit optreedt in de limbische gebieden die betrokken zijn bij seksuele motivatie, waardoor verminderd seksueel verlangen en opwinding kan ontstaan. Gebaseerd op deze onderliggende mechanismen hebben wij twee subtypes binnen de FSIAD populatie geclassificeerd: vrouwen met een relatief ongevoelig breinsysteem voor seksuele stimuli en vrouwen met een versterkte inhibitoire respons (of een overactief remsysteem) als reactie op seksuele stimuli.

Voor deze twee subtypes binnen de FSIAD populatie hebben wij twee verschillende farmacologische behandelingen ontwikkeld die on-demand ingenomen kunnen worden: een combinatie van sublinguaal testosteron (0.5 mg) met een fosfodiësterase-type 5 (PDE5)-remmer (sildenafil 50 mg) voor vrouwen met een relatief ongevoelig systeem voor seksuele prikkels en een combinatie van sublinguaal testosteron (0.5 mg) met een serotonine $(5\text{-HT})_{1A}$ receptor agonist (buspiron 10 mg) voor vrouwen met een overactief remsysteem.

De basis voor beide farmacologische behandelingen is sublinguaal testosteron. Testosteron is zowel betrokken bij sociaal gedrag als seksueel gedrag. Wanneer testosteron in het bloed komt bindt het aan seks hormoon bindend globuline (SHBG), het deel wat ongebonden blijft (het vrije testosteron) is het biologische actieve deel. Het nadeel van chronische testosteron toediening, welke in de meeste studies met vrouwen is onderzocht, zijn de androgene effecten (zoals hirsutisme en acne). Bovendien treden de effecten van chronische testosteron pas laat in de behandeling op omdat er voldoende toename moet zijn van het vrije testosteron wat vaak pas na dagen of weken optreedt bij chronische toediening. Bij sublinguaal testosteron, in de dosering die wij gebruiken (0.5 mg), is er een snelle piek van het vrije testosteron (na 15 min) en zijn de uitgangswaarden weer bereikt binnen 3 uur. Omdat er na on-demand sublinguaal testosteron toediening geen blijvende toename van testosteron ontstaat, is de kans op androgene bijwerkingen zeer gering. Het effect op gedrag treedt ongeveer 4 uur na inname van sublinguaal testosteron op, dus 4 uur na de piek van het vrije testosteron. Deze vertraging van het testosteron effect is voor het eerst ontdekt door Tuiten et al. (2000), en zeer vaak gerepliceerd in studies naar sociaal gedrag en cognitieve functies [5].

In een eerdere studie van onze onderzoeksgroep is onderzocht of alleen de combinatie van testosteron en een PDE5-remmer (vardenafil) effect had op de fysiologische respons (de vaginale puls amplitude) in vrouwen met FSIAD. Testosteron of vardenafil alleen had geen significant effect op deze fysiologische respons. De hypothese dat sublinguaal testosteron de seksuele motivatie zou kunnen verhogen wat een noodzakelijke voorwaarde is voor het effect van een PDE5- remmer werd bevestigd in deze studie [6]. Om een effectieve combinatie

van sublinguaal testosteron en de PDE5-remmer te krijgen moet het farmacodynamische effect van de PDE 5-remmer optreden tijdens het gedragseffect van sublinguaal testosteron (ongeveer 4 uur na inname). In een vervolg studie werd gevonden dat vrouwen die een relatief lage gevoeligheid hebben voor seksuele prikkels, een toename in het seksueel functioneren rapporteerde na de combinatie van sublinguaal testosteron en vardenafil. Bij vrouwen die hoog gevoelig waren voor seksuele prikkels (gebaseerd op een neuropsychologische taak) werd deze toename niet gerapporteerd [7]. Deze studie vormde de basis voor onze classificatie in vrouwen met FSIAD: vrouwen met een laag gevoelig brein-systeem voor seksuele prikkels en vrouwen met een overactief remsysteem.

De vrouwen met een hoog gevoelig brein-systeem reageerden niet op de combinatie van sublinguaal testosteron en vardenafil; de hypothese ontstond toen dat deze vrouwen baat zouden kunnen hebben bij een combinatie van sublinguaal testosteron en een 5-HT_{1A} receptor agonist. Zoals hierboven beschreven hebben wij de hypothese dat er in deze vrouwen een fasische toename van serotonerge activiteit in de PFC plaatsvindt, in respons op de negatieve associaties die deze vrouwen hebben bij bepaalde seksuele prikkels. Een 5-HT_{1A} receptor agonist zoals buspiron, zorgt na acute administratie voor een verlaging van de serotonine transmissie in de PFC wat bij deze vrouwen de remming van de PFC op de limbische gebieden zou remmen wat een positieve invloed zou kunnen hebben op het seksueel verlangen en de opwinding.

Hoofdstuk 2 beschrijft een gerandomiseerd, onderzoeker-blinde, crossover-studie waarin 16 premenopauzale vrouwen zonder seksuele klachten drie verschillende doseringen (0.25 mg, 0.50 mg en 0.75 mg) sublinguaal testosteron toegediend kregen. De farmacokinetische profielen van deze doseringen werden onderzocht, alsmede het effect van deze doseringen op de vrije fractie van testosteron (het gedeelte wat niet gebonden is aan SHBG). Het farmacokinetische profiel van de 0.50 mg dosering sublinguaal testosteron werd vergeleken met de studie van Tuiten et al. (2000) waarin de farmacokinetische eigenschappen van deze dosering al eerder werden onderzocht [5]. De resultaten lieten zien dat testosteron in alle drie doseringen zeer snel werd opgenomen in het bloed (maximale concentraties werden na 15 minuten

gemeten) en de totale en vrije testosteron niveaus waren binnen 3 uur weer op de beginwaarden. Deze resultaten komen overeen zoals deze eerder door Tuiten et al.(2000) zijn beschreven. De oppervlakte onder de curves (AUCs) en maximale concentraties (Cmax) verschilden significant tussen de doseringen en namen dosis-afhankelijk toe. Bovendien suggereren de resultaten dat er een SHBG drempel bestaat die de vrije testosteron fractie beïnvloedt.

In hoofdstuk 3 wordt de theoretische achtergrond van onze hypothesen beschreven en uitgelegd dat verschillende onderliggende mechanismen ten grondslag kunnen liggen aan de seksuele klachten bij vrouwen die gediagnosticeerd zijn met FSIAD. Een verminderd seksueel verlangen kan veroorzaakt worden door een relatief ongevoelig breinsysteem voor seksuele prikkels of ontstaan door een overactief remsysteem in respons op seksuele prikkels. In dit hoofdstuk wordt o.a. de invloed van androgenen en serotonine op seksueel gedrag beschreven. Verder wordt er een rationale gegeven voor onze verschillende farmacotherapeutische on-demand behandelingen en de rol van androgenen en serotonine hierin. Een combinatie van sublinguaal testosteron en een PDE5 remmer (sildenafil) voor vrouwen met een relatief ongevoelig brein voor seksuele prikkels en een combinatie van sublinguaal testosteron en een 5-HT_{1A} receptor agonist (buspiron) voor vrouwen met een overactief remsysteem in respons op seksuele prikkels.

In een placebo-gecontroleerde, dubbelblinde, cross-over studie, beschreven in hoofdstuk 4 en hoofdstuk 5, laten we zien dat de combinatie van sublinguaal testosteron (0.50 mg) in combinatie met sildenafil (50 mg) werkt in vrouwen met een laag gevoelig brein-systeem voor seksuele prikkels (*N* =29) en dat de combinatie van sublinguaal testosteron (0.50 mg) met buspiron (10 mg) effect heeft in vrouwen met een overactief remsysteem (*N* = 28). Het design van de studie was dat vrouwen een maand placebo, een maand een combinatie van sublinguaal testosteron met sildenafil en een maand sublinguaal testosteron met buspiron mee naar huis kregen (gerandomiseerd) waar ze deze medicijnen on-demand konden innemen (dus wanneer ze dat zelf wilden). In de eerste week van de maand waarin ze de specifieke medicatie mee naar huis hadden, werden ze geïnstrueerd om driemaal hun eigen fysiologische respons te meten middels een ambulant laboratorium. Met dit laboratorium konden de vrouwen zelf hun

vaginale puls amplitude meten gedurende het kijken naar erotisch stimulus materiaal. Voorts moesten ze tijdens het kijken ook een aantal vragen in de computer beantwoorden die aangaven in hoeverre de vrouw opgewonden was op dat moment. De overige drie weken konden de vrouwen on-demand de medicatie innemen en werd hen gevraagd om na een seksuele activiteit een dagboekje in te vullen waarin ze konden aangeven in hoeverre ze seksueel verlangen, opwinding en andere seksuele gevoelens ervoeren na inname van de medicatie.

De studie beschreven in hoofdstuk 4, beschrijft de resultaten van 56 vrouwen met FSIAD waarin aangetoond wordt dat wanneer vrouwen ingedeeld worden door middel van een neuropsychologische taak (de Stroop taak), de vrouwen met een laag gevoelig-brein systeem voor seksuele prikkels (in dit geval minder gevoelig zijn voor erotische woorden die gemaskeerd gepresenteerd worden in de Stroop taak) een verbetering in hun seksueel functioneren rapporteren na inname van sublinguaal testosteron in combinatie met sildenafil. Deze vrouwen rapporteerden een toename in de seksuele tevredenheid van 22% in vergelijking tot placebo. De vrouwen die na het innemen van sublinguaal testosteron in combinatie met sildenafil geen verbetering rapporteerden konden ingedeeld worden in vrouwen met lage inhibitie (lage remming, *N* = 26) en vrouwen met hoge inhibitie (*N* = 28). Op basis van deze indeling worden de uitkomsten in hoofdstuk 5 beschreven. De resultaten laten zien dat vrouwen met hoge inhibitie een significante verbetering in hun seksueel functioneren ervaren (o.a. een toename van 25% in de seksuele tevredenheid ten opzichte van placebo) na inname van de combinatie sublinguaal testosteron met buspiron, terwijl dit effect niet werd gevonden in vrouwen in de lage inhibitie groep.

Naast de vrouwen die seksuele klachten ervaren die onder de diagnose FSIAD vallen, zijn er ook vrouwen die seksuele problemen ervaren door het gebruik van selectieve serotonine-heropnameremmers (SSRI) antidepressiva. Van alle antidepressiva zijn de SSRIs het meest beducht in het ontwikkelen van seksuele klachten met een prevalentie van 20-70% in mannen en vrouwen. Deze negatieve seksuele bijwerkingen kunnen de kwaliteit van leven beïnvloeden alsmede de therapietrouw.

SSRIs zorgen er in de hersenen voor dat de synaptische serotonine concentratie omhoog gaat, welke de seksuele excitatie, gemedieerd door dopamine en noradrenaline, remt in het mesolimbische systeem via een tonische serotonerge toename in de prefrontale cortex. Een hogere serotonine concentratie werkt als een filter voor de verwerking van negatieve en positieve stimuli. Buspiron, een 5-HT_{1A} receptor agonist, verlaagt de serotonerge neuronale activiteit voor een korte periode na eenmalige toediening via activatie van somatodendritische 5-HT_{1A} autoreceptoren. Hierdoor gaat de serotonine concentratie in de PFC naar beneden en dit kan leiden tot een tijdelijke afname van de remmende werking van serotonine op het mesolimbische systeem dat betrokken is bij seksuele motivatie. Gebaseerd op de farmacokinetische eigenschappen van on-demand toegediend buspiron, hebben wij de hypothese geponeerd dat de combinatie van sublinguaal testosteron en buspiron effectief zou kunnen zijn in vrouwen met SSRI-geïnduceerde seksuele klachten. De classificatie die wij hebben gehanteerd in de FSIAD populatie zou ook van toepassing kunnen zijn in vrouwen die SSRIs gebruiken. Vrouwen kunnen een laag gevoelig brein-systeem hebben of een overactief remsysteem voorafgaand aan de start van de SSRI. Om de sensitiviteit voor testosteron te onderzoeken in deze populatie werd tevens bij iedere vrouw het polymorfisme van de androgeen receptor bepaald, gecodeerd door de nucleotiden cysteine, adenine en guanine (CAG) herhalings (repeat) lengte. Deze CAG herhalings lengte is geassocieerd met de functie van de androgeen receptor: een relatieve lange CAG herhaling is geassocieerd met een verminderde functie van de androgeen receptor. Onze hypothese is dat vrouwen met een relatieve lange CAG herhaling minder gevoelig zijn voor seksuele prikkels dan vrouwen met een relatief korte herhaling. Dus vrouwen met een relatief lange CAG herhalings lengte zouden meer baat kunnen hebben bij sublinguaal testosteron om hun sensitiviteit voor seksuele prikkels te verhogen in vergelijking met vrouwen met een relatieve korte CAG herhalings lengte.

Hoofdstuk 6 beschrijft een preliminaire studie waarin 21 vrouwen met SSRI-geïnduceerde seksuele klachten participeerden. Het design van de studie was een placebo-gecontroleerde, dubbelblinde, cross-over studie waarin gerandomiseerd iedere vrouw een maand placebo, een maand een combinatie van sublinguaal testosteron (0.50 mg) met sildenafil (50 mg) en een maand

sublinguaal testosteron (0.50 mg) met buspiron (10 mg) mee naar huis kregen waar ze deze medicijnen on-demand konden innemen (dus wanneer ze dat zelf wilden). Na een seksuele activiteit vulden de vrouwen een dagboekje in waarin ze de mate van seksueel verlangen, opwinding en andere seksuele gevoelens konden rapporteren. Ook de CAG herhalings lengte werd bepaald. De resultaten toonden een significant interactie effect tussen SSRI dosering, CAG herhalings lengte en het effect van de medicatie op het seksueel functioneren. Vrouwen met een relatief lange CAG herhalings lengte en een lage dosering SSRI (*N* = 8), rapporteerden een toename in het seksueel functioneren na 0.50 mg sublinguaal testosteron in combinatie met 50 mg sildenafil en ook na inname van 0.50 mg sublinguaal testosteron in combinatie met 10 mg buspiron. Gezien de kleine populatie in deze studie is meer toekomstig onderzoek nodig.

Bovenstaande onderzoeken werden allemaal verricht met een oplossing van 0.50 mg testosteron (in een cyclodextrine complex) die sublinguaal werd toegediend, gevolgd door een sildenafil of buspiron capsule 2.5 uur later zodat het effect van de sildenafil en buspiron maximaal is tijdens het farmacodynamische effect van testosteron (3-6 uur na inname). Omdat deze manier van toediening niet praktisch is voor de klinische praktijk werd er een combinatie tablet ontwikkeld. Deze combinatie tablet heeft een coating van testosteron (met hydroxypropyl-beta cyclodextrin), die na 1 minuut onder de tong opgelost is waarna de tablet doorgeslikt kan worden. Na 2.5 uur klapt de tablet open en komt de sildenafil of buspiron vrij in het maag-darm kanaal.
In hoofdstuk 7 worden de resultaten beschreven van een farmacokinetische studie waarin bij 13 vrouwen de farmacokinetiek werd vergeleken tussen de ‘oude’ manier van toediening (testosteron oplossing met apart een capsule van 10 mg buspiron) en de nieuwe combinatie tablet. Na beide formuleringen werd testosteron snel opgenomen. De resultaten laten ook zien dat buspiron adequaat wordt opgenomen en na ongeveer 3 uur na inname van de combinatie tablet vrij komt in het bloed wat vergelijkbaar is met de ‘oude’ formulering waarin sublinguaal testosteron en buspiron apart werden toegediend. De nieuwe combinatie tablet is goed vergelijkbaar met de ‘oude’ formulering en zal gebruikt worden in toekomstige onderzoeken bij vrouwen met FSIAD.

Tenslotte worden in hoofdstuk 8 de belangrijkste bevindingen van dit proefschrift bediscussieerd. Binnen de FSIAD populatie hebben wij 2 subtypes kunnen classificeren: vrouwen met een relatief ongevoelig breinsysteem voor seksuele stimuli en vrouwen met een versterkte inhibitoire respons (of een overactief remsysteem) als reactie op seksuele stimuli. Het zou natuurlijk heel goed kunnen dat er meerdere subtypes zijn te vormen gebaseerd op deze gevoeligheid, bijvoorbeeld lage, laag-normale gevoeligheid, normaal-hoge en hoge gevoeligheid. Hetzelfde zou kunnen gelden voor vrouwen die lijden aan SSRI-geïnduceerde seksuele klachten. De hypothese is dat de gevoeligheid van het brein voor seksuele prikkels in belangrijke mate wordt beïnvloed door het androgene systeem (zoals de CAG herhalings (repeat) lengte en het metabolisme van testosteron), het serotonine systeem (5-HT receptor polymorfismen) en andere neurobiologische systemen (zoals dopamine, prolactine). De laatste jaren wordt er steeds meer onderzoek gedaan naar genetische polymorfismen. In het licht van een gepersonaliseerde aanpak, zou analyse van relevante polymorfismen een zeer goede methode zijn om te kunnen differentiëren in de verschillende mate van gevoeligheid voor seksuele prikkels alsmede de respons op onze farmacologische behandelingen.

REFERENTIES

1. American Psychiatric Association. Diagnostic and statistical manual of mental disorders. 4th ed. Washington, DC; 2000.

2. American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders. 5th ed. Arlington, VA: American Psychiatric Publishing; 2013.

3. Janssen E, Bancroft J. The dual control model: the role of sexual inhibition and excitation in sexual arousal and behaviour. In: Janssen E, editor. The psychophysiology of sex Bloomington: Indiana University Press; 2007. p. 197.

4. Bancroft J, Graham CA, Janssen E, Sanders SA. The dual control model: current status and future directions. J Sex Res 2009 Mar-Jun;46(2-3):121-142.

5. Tuiten A, Van Honk J, Koppeschaar H, Bernaards C, Thijssen J, Verbaten R. Time course of effects of testosterone administration on sexual arousal in women. Arch Gen Psychiatry 2000 Feb;57(2):149-53; discussion 155-6.

6. Van der Made F, Bloemers J, Van Ham D, El Yassem W, Kleiverda G, Everaerd W, et al. Childhood sexual abuse, selective attention for sexual cues and the effects of testosterone with or without vardenafil on physiological sexual arousal in women with sexual dysfunction: A pilot study. J Sex Med 2009;6:429-439; 429.

7. van der Made F, Bloemers J, Yassem WE, Kleiverda G, Everaerd W, van Ham D, et al. The influence of testosterone combined with a PDE5-inhibitor on cognitive, affective, and physiological sexual functioning in women suffering from sexual dysfunction. The journal of sexual medicine 2009 Mar;6(3):777-90.

CURRICULUM VITAE

Kim van Rooij was born on the 25th of January in 1980 in Amsterdam, The Netherlands. In 1998, she graduated from secondary school (VWO) at the Montessori Lyceum in Amsterdam. She obtained her Bachelor degree in Biomedical Sciences at the Vrije Universiteit of Amsterdam in 2002. In 2002 she started studying Medicine at the Vrije Universiteit of Amsterdam. During this study she did a scientific internship in Zambia, to evaluate the local growth monitoring program for malnourished children. She received her medical degree in 2008.

In 2008, she started working at Emotional Brain as a research physician. Later that year, Kim commenced her PhD project at Emotional Brain under supervision of dr. A Tuiten, dr. H. Koppeschaar and prof. Dr. B. Olivier of which the results are described in this thesis. She concurrently worked as a research physician and medical coordinator at Emotional Brain and was heavenly involved in the design of several clinical studies, and was responsible for the medical content and safety monitoring of these studies. In 2013, she became the Director of Medical Affairs and co-authored regulatory submissions and presented for the U.S Food and Drug Administration and the European Medicines Agency. Besides her work as a Director Medical Affairs she started in 2014 as a general practitioner in training at the AMC in Amsterdam.

LIST OF PUBLICATIONS

Kim van Rooij, Jos Bloemers, Leo de Leede, Irwin Goldstein, Eef Lentjes, Hans Koppeschaar, Berend Olivier and Adriaan Tuiten. Pharmacokinetics of three doses of sublingual testosterone in healthy premenopausal women. *Psychoneuroendocrinology* 2012;37:773-781.

Jos Bloemers, **Kim van Rooij**, Saskia Poels, Irwin Goldstein, Walter Everaerd, Hans Koppeschaar, Meredith Chivers, Jeroen Gerritsen, Diana van Ham, Berend Olivier and Adriaan Tuiten. Toward Personalized Sexual Medicine (Part 1): Integrating the "Dual Control Model" into Differential Drug Treatments for Hypoactive Sexual Desire Disorder and Female Sexual Arousal Disorder. *Journal of Sexual Medicine* 2013;10:791-809.

Saskia Poels, Jos Bloemers, **Kim van Rooij**, Irwin Goldstein, Jeroen Gerritsen, Diana van Ham, Frederiek van Mameren, Meredith Chivers, Walter Everaerd, Hans Koppeschaar, Berend Olivier and Adriaan Tuiten. Toward Personalized Sexual Medicine (Part 2): Testosterone combined with a PDE5 inhibitor increases sexual satisfaction in women with HSDD and FSAD, and a low sensitive system for sexual cues. *Journal of Sexual Medicine* 2013;10:810-823.

Kim van Rooij, Saskia Poels, Jos Bloemers, Irwin Goldstein, Jeroen Gerritsen, Diana van Ham, Frederiek van Mameren, Meredith Chivers, Walter Everaerd, Hans Koppeschaar, Berend Olivier and Adriaan Tuiten. Toward Personalized Sexual Medicine (Part 3): Testosterone combined with a serotonin1A receptor agonist increases sexual satisfaction in women with HSDD and FSAD, and dysfunctional activation of sexual inhibitory mechanisms. *Journal of Sexual Medicine* 2013;10:824-837.

Kim van Rooij, Saskia Poels, Petra Worst, Jos Bloemers, Hans Koppeschaar, Andrew Goldstein, Berend Olivier and Adriaan Tuiten. Efficacy of testosterone combined with a PDE5 inhibitor and testosterone combined with a serotonin$_{1A}$ receptor agonist in women with SSRI-induced sexual dysfunction. A preliminary study. *European Journal Of Pharmacology* 2014, doi:10.1016/j.ejphar.2014.10.061.

Kim van Rooij, Leo de Leede, Henderik W. Frijlink, Jos Bloemers, Saskia Poels, Hans Koppeschaar, Berend Olivier and Adriaan Tuiten. Pharmacokinetics of a prototype formulation of sublingual testosterone and a buspirone tablet, versus an advanced combination tablet of testosterone and buspirone in healthy premenopausal women. *Drugs in R&D* 2014; 14(2):125-132.

Jos Bloemers, Steven Scholte, **Kim van Rooij**, Irwin Goldstein, Jeroen Gerritsen, Berend Olivier and Adriaan Tuiten. Reduced Gray Matter Volume and Increased White Matter Fractional Anisotropy in Women with Hypoactive Sexual Desire Disorder. *Journal of Sexual Medicine* 2014;11:753–767.

Saskia Poels, Jos Bloemers, **Kim van Rooij**, Hans Koppeschaar, Berend Olivier and Adriaan Tuiten. Een vernieuwende farmacotherapeutische behandeling voor vrouwen met Hypoactive Sexual Desire Disorder [A new pharmacotherapeutic treatment for women with Hypoactive Sexual Desire Disorder]. *Neuropraxis* 2014;18:32-45.

Saskia Poels, Jos Bloemers, **Kim van Rooij**, Hans Koppeschaar, Berend Olivier and Adriaan Tuiten. Two novel combined drug treatments for women with Hypoactive Sexual Desire Disorder. *Pharmacology, Biochemistry and Behavior* 2014;121:71-79.

Adriaan Tuiten, Jos Bloemers, **Kim van Rooij**, Saskia Poels, Jeroen Gerritsen, Diana van Ham, Leo de Leede and Walter Everaerd. Response to “Toward Personalized Sexual Medicine: Where is the Evidence?”. *Journal of Sexual Medicine* 2014;11:2357-2363.

DANKWOORD/ACKNOWLEDGEMENTS

Toen ik in 2008 net na mijn artsenbul bij Emotional Brain (EB) kwam werken werd ik al snel gegrepen door de bevlogenheid van Adriaan Tuiten en zijn team. Er was altijd veel ruimte om mee te denken over de inhoud van de klinische studies en om verantwoordelijkheid te nemen waardoor ik het werk als zeer uitdagend en leerzaam heb ervaren. Al snel kon ik een promotietraject ingaan bij EB in samenwerking met prof. Berend Olivier van de Universiteit Utrecht. In de afgelopen jaren heeft EB veel mijlpalen behaald en veel meegemaakt. Ik heb de afgelopen jaren dan ook als zeer fijn en leerzaam ervaren en hoop dat ik nog vele jaren verbonden kan blijven aan EB.

Ik wil graag iedereen in mijn omgeving bedanken en hoop dat ik niemand vergeet.

Beste Adriaan, we kennen elkaar nu bijna 7 jaar en hebben in deze jaren veel meegemaakt. Jij hebt mij laten zien hoe bevlogen onderzoek kan zijn, leren schrijven, hoofd en bijzaken leren onderscheiden en vooral laten zien hoe hard je soms moet zoeken en moet doorzetten om je doel te bereiken. Ik heb bewondering voor je zoals jij altijd creatieve oplossingen kan bedenken en hoe hard je vecht voor EB. We hebben veel spannende situaties meegemaakt en elkaar goed leren kennen in de afgelopen jaren. Jij bent mijn mentor maar ook een ware vriend. Ik hoop dat we nog veel mooi onderzoek en avonturen gaan beleven samen.

Beste Berend, ik wil je bedanken voor al je hulp en het feit dat je altijd laagdrempelig bereikbaar was voor alle vragen die ik over het proefschrift had en de regeldingen daaromheen. Wanneer ik jou stukken uit het proefschrift ter review stuurde was je altijd de eerste met commentaar. Ik hoop dat we nog lang contact houden en veel nieuwe artikelen gaan publiceren.

Beste Hans, ik heb heel wat uren bij jou op de kamer gezeten om samen je aanpassingen door te nemen voor de stukken uit mijn proefschrift en jij hebt mij geleerd om oog voor detail te hebben. Ook heb ik veel geleerd van jouw

expertise over de endocrinologie en ik hoop dat we nog veel stukken samen gaan schrijven.

Geachte leden van de leescommissie: prof. dr. Jack van Honk, prof. dr. Toine Egberts, prof. Dr. Daan Touw, prof. dr. Madeleine Drent en prof. dr. Willem Noolen ik wil jullie bedanken dat jullie de tijd hebben genomen om dit proefschrift kritisch te lezen.

Beste Leo, dank voor alle hulp bij de farmacokinetische artikelen en de gezelligheid bij alle buitenland bezoeken (sjoelen). Jij bent echt onderdeel van het EB team en het is heel fijn dat je er elke woensdag bent. Ik hoop dat we dit jaar mooie dingen gaan beleven.

Dear Members of the Advisory Board, and especially Marcel Waldinger, Len Derogatis and Andrew Goldstein, thank you for all the interesting discussions and input during our Advisory Board meetings. I've learned a lot from you expertise. Thank you Andrew, for your input in our publication regarding SSRI-induced sexual dysfunction.

Dear Don Rubin, thank you for your hospitality when we visited Cambridge, your input during the preparations for the FDA meetings and your enthusiasm. It's an honour that you will be present during my dissertation and I hope we will see each other often this year.

Dear Jim Pfaus, we know each other from the Advisory Board meetings but got to know each other a bit better when we visited your university in Montreal and had great talks and laughter in the coffee café nearby. It's great that you will be present during my dissertation and I look forward to our trip to ISSWSH.

Alle mensen van EB, ATIA en Flevoresearch wil ik graag bedanken voor de fijne werkomgeving en de samenwerking. Zeker het laatste jaar hebben we veel samengewerkt met de analyse en uitvoer van de studies. Lief EB team: Jeroen, Cliviante, Monique, Diana, Petra, Martine, Jos, Jasmijn, Yvonne, Rob (en ook de mensen die weg zijn: Saskia, Marijke, Crista, Eva Marijn, Frederiek), ik ben trots op ons over wat we allemaal voor elkaar hebben gekregen in de afgelopen jaren. Wanneer er binnen een (zeer) korte tijd een studie opgezet moest

worden draaide we daar onze handen niet voor om. Ik ben er helaas wat minder dan vroeger maar EB voelt als thuis en dat is door jullie, de gezellige lunches en het feit dat er altijd een luisterend oor was als er problemen waren. Ik hoop dat we nog veel samen gaan doen! Jos, dank voor de motiverende woensdagavonden en alle last-minute vragen die ik aan je kon stellen over het promoveren.

Er zijn een heleboel mensen met wie we samen gewerkt hebben de afgelopen jaren en die ik ook graag wil bedanken: Hansje Bokma van PSR Group (dank voor de samenwerking en alles wat je mij hebt bijgebracht over ICH-GCP, de gezellige lunches en het meedenken over de praktische uitvoer van de studies), Diny Laan van PSR Group (voor alle hulp met de database), Amy Criswell (thanks for all your help and we are gonna miss you, good luck with the new job!), Henrik Rasmussen (thanks for all your input during the clinical trials and the regulatory issues), Richard Hooijmaijers (voor alle statistische ondersteuning van de fase 2 onderzoeken).

Zonder de aandeelhouders van EB en de RvC waren we nooit zover gekomen en ik wil jullie bedanken voor jullie steun en geduld!

Graag wil ik de proefpersonen bedanken die aan onze studies hebben meegewerkt, zonder jullie interesse en motivatie waren we nooit zo ver gekomen!

Beste Felici Jeuken en John Mens, sinds een paar maanden werk ik met veel plezier bij jullie in het gezondheidscentrum Parkwijk als huisarts in opleiding en ik wil jullie bedanken voor jullie begrip en de ruimte die jullie hebben geboden zodat ik mijn proefschrift kon afronden in de afgelopen maanden.

Lieve vrienden, dank voor jullie steun en soms felle discussies die mij alleen maar meer gemotiveerd hebben om door te gaan en ervoor gezorgd hebben dat dit proefschrift klaar is. Een paar mensen wil ik graag even persoonlijk noemen: Lieve Moniek dank voor je steun en hulp wanneer alles soms even tegenzat, lieve Sarah Mei ik ben heel blij dat je weer terug bent uit China en er bij kan zijn, lieve Ireen jij weet mij altijd weer te motiveren en heb zin om weer eens samen naar Barcelona te gaan, lieve Just jij bent een onvoorwaardelijke steun en ik ben

trots op je, lieve Sarah zo jammer dat je er niet bij kan zijn nog eventjes en dan ga jij ook promoveren.

En dan tot slot mijn familie en paranimfen: Lieve Ruud dank voor alle ruimte die je mij hebt gegeven de afgelopen jaren om te kunnen promoveren en je goede zorgen voor onze Olivia. Lieve mamma en Carlijne, mijn paranimfen, ik vind het heel fijn dat jullie naast mij kunnen staan straks tijdens de verdediging en ik hou heel veel van jullie. Lieve mamma, ik wil je bedanken voor je steun en de opvoeding die je ons gegeven hebt, door jou hebben Carlijne, Sebastiaan en ik altijd genoeg zelfvertrouwen gekregen om ons hart te volgen en de dingen in het leven te doen die we graag willen ook al is het soms moeilijk. Lieve Carlijne, je bent mijn zusje maar ook mijn beste vriendin en de tweede moeder van Olivia. Lieve Sebastiaan, ik ben trots op je wat je al allemaal bereikt hebt in het leven en dank voor alle dagen en avonden dat je op Olivia paste, je bent de liefste oom die ze zich maar kan wensen. Lieve pappa dank voor de onbezorgde opvoeding die ik heb gehad en de humor die wij zo delen, jouw invloed heeft mij gevormd zoals ik ben. Lieve Marja en pappa, dank voor jullie goede zorgen voor Olivia en het wekelijkse diner waar ze zo van geniet.

Tot slot, draag ik dit boekje op aan mijn mooie prinses Olivia. Lieve Olivia, ik ben zo blij met je en geniet van elk moment dat we samen zijn. Ik hoop dat ik de komende tijd meer tijd heb om samen leuke dingen te doen en ik beloof je dat we samen naar Amerika gaan als je groot bent.