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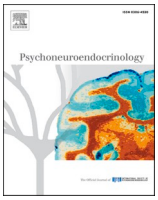

Plasma androgens and the presence and course of depression in a large cohort of men

Anouk E. de Wit [a,*], Erik J. Giltay [b], Marrit K. de Boer [a], Willem A. Nolen [a], Fokko J. Bosker [a], Brenda W.J.H. Penninx [c], Robert A. Schoevers [a]

[a] Department of Psychiatry, University of Groningen / University Medical Center Groningen, 9700 RB, PO Box 30.001 (CC 43), Groningen, The Netherlands
[b] Leiden University Medical Center, Department of Psychiatry, 2300 RC B1-P, Leiden, The Netherlands
[c] Department of Psychiatry, Amsterdam UMC, VU University, Amsterdam, The Netherlands

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ABSTRACT

Background: Hypoandrogenic men showed a higher prevalence of major depressive disorder (MDD), which could be ascribed to overlapping symptoms such as sexual dysfunction, or additionally to core emotional symptoms such as sadness and anhedonia. We examined whether androgen levels 1) differ between men with and without MDD cross-sectionally, 2) are associated with an elevated risk for onset of MDD prospectively, and 3) associate with all individual MDD symptoms, or only with hypogonadism overlapping symptoms.
Methods: In 823 men (mean age 43.5 years), baseline plasma levels of total testosterone, 5α-dihydrotestosterone (5α-DHT), and androstenedione were determined with liquid chromatography–tandem mass spectrometry, and dehydroepiandrosterone-sulphate (DHEAS) and sex hormone binding globulin with radioimmunoassay, whereas free testosterone was calculated. MDD status was assessed at baseline and after two years using structured interviews and individual MDD symptoms were self-rated at baseline, and after one and two years.
Results: None of the androgen levels were associated with current or onset (incidence or recurrence) of MDD. Free testosterone was only inversely associated with interest in sex. Also, androstenedione and DHEAS were positively associated with some individual MDD symptoms, and 5α-DHT levels showed non-linear associations (both with low and high levels) with MDD symptom severity and several individual MDD symptoms.
Conclusions: These results support the idea that circulating androgens synthesised by the testes are of limited clinical relevance to MDD in adult men, but levels of androstenedione, DHEAS and 5α-DHT may be associated with some individual MDD symptoms.

1. Introduction

The idea that adequate testosterone levels serve a protective function for depression in men, was originally rooted in the observation that hypogonadism is associated with more depressive symptoms (Hore, 1969). Such a relationship may be a consequence of sex steroid receptor bindings of testosterone that result in serotonin neurotransmitter function alterations or other genomic and non-genomic changes in the limbic system (Heberden, 2017; Höfer et al., 2013). However, such a relationship may also be the indirect result of shared symptoms of hypogonadism and major depressive disorder (MDD), such as sexual dysfunction, apathy and fatigue (Bhasin et al., 2018; Corona et al., 2020; Wu et al., 2010), that may additionally also have an effect on mood. To date, the association between testosterone and MDD remains disputed because studies examining the association between testosterone and MDD have yielded conflicting results.

The majority of cross-sectional studies that used structured diagnostic assessment procedures to identify MDD symptomatology and diagnoses reported no association between total or free testosterone and MDD (Markianos et al., 2007; Matsuzaka et al., 2013; Seidman et al., 2002) or severity of MDD symptoms (Barrett-Connor et al., 1999a; Monteagudo et al., 2016; T'Sjoen et al., 2005). These findings were confirmed by prospective studies showing that neither free nor total testosterone levels, nor a change in these levels, predicted the severity of MDD symptoms at two- or three-years follow-up (Giltay et al., 2017; T'Sjoen et al., 2005). However, some other studies have shown that men

* Corresponding author.
E-mail addresses: a.e.de.wit@umcg.nl (A.E. de Wit), e.j.giltay@lumc.nl (E.J. Giltay), m.k.de.boer@umcg.nl (M.K. de Boer), w.a.nolen@umcg.nl (W.A. Nolen), f.j.bosker@umcg.nl (F.J. Bosker), b.penninx@amsterdamumc.nl (B.W.J.H. Penninx), r.a.schoevers@umcg.nl (R.A. Schoevers).

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with low or lower levels of free or total testosterone are more often depressed (Giltay et al., 2017; McIntyre et al., 2006), have increased severity of MDD symptoms (Barrett-Connor et al., 1999a; Hintikka et al., 2009), or have an increased risk for first onset of MDD after two years (Shores et al., 2005, 2004).

Besides testosterone, there are also other endogenous androgenic compounds circulating in the blood. An example includes 5α-dihydrotestosterone (5α-DHT) which is substantially more potent than testosterone as an agonist of the androgen receptor (Gao et al., 2005). However, studies examining 5α-DHT in relation to depression are limited (Barrett-Connor et al., 1999b), mainly because the previous gold-standard method for measuring androgens (radioimmunoassays) was less reliable for this compound (Wang et al., 2018). However, the currently widely available liquid chromatography–tandem mass spectrometry (LC-MS/MS) method is capable of reliable measuring 5α-DHT as well as other androgens, including the lower levels that are present in hypogonadal men (Kushnir et al., 2010).

To clarify the possible role of androgens in depression among men, future research should examine which specific symptoms drive the association between androgens and depression. Knowing if, and if so, which specific MDD symptoms are related to altered androgen levels in men, may provide insight into the pathophysiological mechanisms underlying this type of depression in men (Bhasin and Seidman, 2018). If anything, future research should also overcome previous (methodological) limitations of studies that hampered the ability to make definitive conclusions about the association of androgens and depression. These limitations include scarcity of results reported on the full range of testosterone levels as many examined hypo- versus eugonadal levels and the focus on free and total testosterone while many more androgenic compounds circulate in the blood. Additionally, limited number of studies have had prospective designs, precluding the assessment of temporal relationships. Finally, future research should also examine non-linear effects as both low and high levels of androgens, may be related to MDD symptoms (Booth et al., 1999; Wu et al., 2010).

In this study, we examined whether androgens levels 1) differ between men with and without MDD cross-sectionally; 2) are associated with an elevated risk for onset of MDD prospectively; and 3) are associated with all individual MDD symptoms or only with subset of symptoms. We used data from a large cohort of men from the Netherlands who were representative of men with depressive disorders in different health care and population settings and stages of the developmental history and in whom total testosterone, androstenedione, and 5α-DHT were determined with LC-MS/MS and DHEAS with radioimmunoassay. Given the predominantly negative findings in previous research, we a priori hypothesised that lower androgen levels are: 1) equally present in men with and without MDD; 2) not associated with an elevated risk for new onset of MDD; and 3) only associated with some overlapping symptoms of MDD and hypogonadism such as less sexual interest and more fatigue.

2. Material and methods

2.1. Study population

Netherlands Study on Depression and Anxiety (NESDA) is a large multicenter study designed to investigate the long-term course and consequences of depressive and anxiety disorders. Between 2004 and 2007, men and women (aged 18–65 years) were recruited from different health care settings (community, primary care and specialised mental health care) and in different stages of the developmental history of disorders (healthy controls, high familial risk, subthreshold disorders, first and recurrent episodes) in order to be representative of those with depressive and anxiety disorders in different health care settings and stages of the developmental history. Exclusion criteria included not speaking Dutch fluently, or being diagnosed with one of the following psychiatric diseases: psychotic, obsessive compulsive, bipolar, or substance use disorder (Penninx et al., 2008). For the baseline assessment, 26,393 men and women were contacted of whom 2981 (11.3%) participated. The ethical committees of the participating centres approved (VU Medical Centre, University Medical Centre Groningen, Leiden University Medical Centre) the study design and all participants gave verbal and written informed consent.

For this study, 1002 men were considered for inclusion. Exclusion criteria included men who had missing plasma androgen measurements (n = 24), extreme androgen values (>8 standard deviation [SD] below or above the mean, n = 8), who were transgender (n = 2), or in whom a measurement or sampling error was likely (n = 1). Men who used androgens World Health Organisation Anatomical Therapeutic Chemical (ATC) G03B and E, gonadotrophins (ATC G03G), antiandrogens (ATC G03H), gonadotrophin-releasing hormone analogues (ATC L02AE), or antiandrogens (ATC L02BB) at the time of the blood draw were also excluded (n = 4). Finally, men who were diagnosed (lifetime) with an anxiety disorder but not with MDD (n = 140) were excluded as well. Exclusion of these men following the above-mentioned criteria resulted in a baseline sample of 823 participants (82.1% of all men) to test the first hypothesis. This sample was categorised into three groups according to their psychopathology status; without lifetime MDD (neither currently nor in the past depressed, n = 246), remitted MDD (lifetime MDD, but currently not depressed, n = 318), and current MDD (currently depressed, n = 259). Of the sample of men with remitted MDD or without lifetime MDD (n = 564), 504 men (89.4% of the non-depressed baseline sample) did participate in the follow-up assessment after two years and were used to test the second hypothesis. Compared to men with follow-up, men without follow-up (n = 60, 10.6%) more often had lifetime MDD (76.7% vs. 54.0%) and low free testosterone levels (18.3% vs. 8.9%; Supplement 1). Finally, at baseline and after one and two years of follow-up, the severity of individual MDD symptoms was assessed. Out of the baseline sample, 751 men (91.3%) had responded to at least two out of three of the items during these assessments and were therefore included in the analyses to test the third hypothesis. Men without follow-up (n = 72, 8.7%) were comparable to men without follow-up regarding psychopathology, but smoked more often (62.5% vs. 39.5%; Supplement 1). A flow diagram of men included in the analyses to test each of the three hypotheses is shown in Supplement 2.

2.2. Assessment of androgens and SHBG

Blood plasma collection took place in the morning (mean time 08:46 h SD 32 min) after an overnight fast (success rate 94.4%). Total testosterone, 5α-dihydrotestosterone (5α-DHT), and androstenedione were determined by LC–MS/MS at the Clinical Chemistry department of the University Medical Centre Groningen. The lower limit of detection was 0.04 nmol/L for total testosterone and androstenedione, and 0.12 nmol/L for 5α-DHT. The inter-assay coefficients of variation for all androgens were low (total testosterone: 3.1%, 5α-DHT: 7.8%, androstenedione: 2.2%). Dehydroepiandrosterone-sulphate (DHEAS) and sex hormone binding globulin (SHBG) were determined using ARCHITECT (ABBOTT, Wiesbaden, Germany), a one and two-step radioimmuneassay with chemiflex assay protocols. The lower limit of detection of DHEAS was 0.3 μmol/L with a calibration range of 0.00–40.71 μmol/L, and 0.1 nmol/l for SHBG with a calibration range of 0.0–250 nmol/L. Free testosterone was calculated based on SHBG and total testosterone levels, and an assumed albumin level of 43 g/L with the formula of Vermeulen (Vermeulen et al., 1999).

2.3. Assessment of depressive disorders

The presence of MDD, currently or in the past, was ascertained using the lifetime version of the Composite International Diagnostic Interview (CIDI), version 2.1, a validated instrument with high interrater reliability (any depressive disorder kappa=0.95) and high validity for

depressive and anxiety disorders (APA, 2001; Wittchen, 1994). Current MDD was ascertained according to Diagnostic and Statistical Manual of Mental Disorders (DSM)-IV-based CIDI criteria within the past month, remitted MDD was determined when participants had MDD earlier in life, but not within in the last month. First or recurrent onset of MDD between baseline and 2 years follow-up, was ascertained when men without MDD at baseline did fulfil the criteria of MDD during follow-up. Finally, at baseline and after one and two years of follow-up, the severity of individual MDD symptoms was assessed with the self-report Inventory of Depressive Symptoms (Rush et al., 1996). This questionnaire consists of 30 items that assess the symptom severity over the last seven days. Besides the core MDD symptoms, this questionnaire also assesses the severity of symptoms that overlap with hypogonadism such as interest in sex (measured as no or little desire, derived pleasure or interest in sex) and fatigue. Each item is scored with a value between 0 and 4, with higher values indicating increased severity. This questionnaire had excellent internal consistency in previous research (Rush et al., 1996) and at our baseline assessment (Cronbach's alpha = 0.92).

2.4. Covariates

The following covariates measured at baseline were taken into account in the models described below the next header: age (continuously), waist circumference (cm), number of treated chronic somatic disorders (continuously), smoking status (dichotomously), alcohol use (dichotomously), physical activity (continuously), and the presence of a current anxiety disorder (dichotomously). Age was measured in years and waist circumference in cm.

Smoking status was dichotomised in smoker and no smoker, and alcohol use was dichotomised in $\leq$ or $>$ 7 units a week. Physical activity was measured as the total sum of MET-minutes for vigorous, moderate and walking activities as measured with the International Physical activity Questionnaire. Finally, presence of current anxiety disorders was, like for MDD, measured with the lifetime version of the CIDI, version 2.1. Missing values on alcohol use (n = 13; 2.9%), waist circumference (n = 2, 0.2%), and physical activity (n = 44, 5.3%) were imputed with the mean. Other covariates had no missing values.

2.5. Statistical analyses

At baseline, group characteristics of without lifetime MDD, current MDD, and remitted MDD were compared using one-way analyses of variance for independent samples or Kruskal Wallis in case of continuous variables with normal or non-normal distribution, respectively, and with $\chi 2$ tests in case of categorical variables.

To test our first hypothesis, we ran two-way analyses of covariance to test whether androgen levels varied by group status, and used two one-sided tests procedures to reject the presence of a smallest effect size of interest (Lakens, 2017). The latter was done since non-significant p-values obtained via classical testing would not declare the absence of meaningful effects. We considered 0.20 Cohen's d as the minimally clinically meaningful important difference (Cohen, 1977). To test our second hypothesis, we ran logistic regression analyses with baseline androgen levels as predictors and the presence or absence of first or recurrent onset of MDD between baseline and two years as the outcome measure in men without MDD at baseline. To test our third hypothesis, we ran 186 linear mixed models considering baseline androgen and SHBG levels as fixed factors, and total and individual MDD symptom severity scores as outcome measures. A random intercept and random slope were added to the model as they lowered the Akaike information criterion. A random intercept accounted for between-participant variability of severity of MDD symptoms and the random slope (time) allowed for individual slopes for MDD symptoms over time. An interaction between time and androgens was not added as it decreased the model fit. Mixed models with unstructured covariance structures were fitted using restricted likelihood estimation, but the final model was estimated with maximum likelihood estimation. All models for the testing hypotheses 1, 2, and 3, were adjusted for the above-described covariates. The models for examining hypotheses 2 were additionally adjusted for presence of remitted MDD.

In sensitivity analyses, we examined whether the results of the first, second and third hypotheses testing changed when free and total testosterone levels were dichotomised into low and normal. In line with previous research, cut-off points of $<$ 11 nmol/L for total testosterone and $<$ 220 pmol/L for free testosterone were used (Wu et al., 2010). We used logistic regression analyses to test the first hypotheses with low free and total testosterone presence as the outcome measure, presence of current, remitted or without lifetime MDD (reference group) as predictor, and adjusted for the same covariates as mentioned above. The second and third hypotheses were tested in the same way as described above, except that continuous androgen levels were replaced with the presence of low free or total testosterone.

As a second sensitivity analyses, we examined whether the associations between androgens and individual MDD symptoms (hypothesis 3) were better described by a non-linear relationship by adding quadratic terms of androgen levels to the regression models.

Data were analysed using R (version 1.2.1335). In all models, the predictor and outcome (if not dichotomous) were standardised (into z-values) to ease comparability of effect sizes. In order to control for type-I-errors, we used the false discovery rate using the use the Benjamini-Hochberg procedure to adjust the p-values generated by the analyses examining hypotheses 1 and 2 (Benjamini and Yekutieli, 2001). For the analyses conducted for hypothesis 3, we considered a p-value of $<$ 0.05 being statistically significant given the more explorative character of this hypothesis.

3. Results

The characteristics of the 823 men are shown in Table 1. Men with current MDD presented with the highest mean waist circumference and

Table 1
Characteristics of 823 men at baseline.

	Without lifetime MDD n = 246	Remitted MDD n = 318	Current MDD n = 259	p[a]
Age, mean (SD)	42.3 (15.1)	44.4 (12.0)	43.7 (11.3)	.15
Education (years), mean (SD)	13.0 (3.2)	12.0 (3.3)	11.2 (3.0)	< 0.001
Waist circumference, mean (SD)	94.5 (12.6)	96.8 (12.7)	97.0 (13.3)	.04
Body Mass Index, mean (SD)	25.6 (4.3)	26.4 (4.6)	26.7 (4.7)	.02
Current smoking, no. (%)	78 (31.7)	143 (45.0)	121 (46.7)	< 0.001
> 1 alcohol unit/ day, no. (%)	81 (32.9)	91 (28.6)	75 (29.0)	.49
Physical activity, mean (SD)	3420 (1691–5062)	3052 (1386–5486)	2598 (1095–4418)	.02
≥ 1 treated chronic diseases, no. (%)	163 (33.7)	137 (43.1)	129 (48.8)	.08
Current antidepressant use, no. (%)	4 (1.6)	91 (28.6)	110 (42.5)	< 0.001
Current anxiety disorder, no. (%)	0 (0.0)	107 (33.6)	153 (59.1)	< 0.001
Severity of MDD symptoms, median (IQR)	5 (2–10)	19 (11–27)	36 (28–44)	< 0.001

Abbreviations: IQR, interquartile range; MDD, major depressive disorder; SD, standard deviation.

[a] Based on one-way analyses of variance for independent samples or $\chi 2$ tests for normally distributed variables and by Kruskal Wallis testing for non-normally distributed variables (severity of MDD symptoms).

the highest percentage of current smokers, followed successively by men with remitted MDD and without lifetime MDD.

3.1. Major depressive disorder

Mean total and free testosterone, SHBG, androstenedione, and DHEAS levels were not statistically different between men without lifetime, and men with remitted or current MDD (see Fig. 1), and equivalence testing showed that differences examined, were equal to zero (see Supplement 4). Only the results on 5α-DHT were inconclusive, as levels were not statistically different between the groups, but also not statistically equivalent to zero.

During two years of follow-up, 99 (19.6%) out of 504 men without MDD at baseline experienced a first or recurrent onset of MDD. However, none of the androgens statistically significantly predicted this event (see Table 2).

3.2. Individual major depressive disorder symptoms

None of the androgens examined was associated with the total MDD symptom severity score over two years. Lower free testosterone levels were associated with less interest in sex, but other individual MDD symptoms over two years were not associated with free or total testosterone, nor with SHBG or 5α-DHT levels (see Fig. 2). Higher androstenedione levels however, were associated with: pessimism, suicidality, anxiety or tension, and less increase in weight. Also, higher DHEAS levels were positively associated with lower self-esteem, suicidality, decrease in weight and pessimism.

Table 2
Odds ratio's for first or recurrent episode of MDD in 504 men according to plasma androgen and SHBG levels during up to two years of follow-up.

	First or recurrent MDD			
	OR (95%CI)	p	OR (95%CI)[a]	p
Events	99 / 504 (19.6%)			
Total testosterone	1.03 (0.81–1.29)	.82	1.01 (0.78–1.30)	.93
SHBG	0.98 (0.77–1.23)	.88	0.98 (0.74–1.27)	.89
Free testosterone	1.01 (0.80–1.27)	.94	0.96 (0.72–1.28)	.80
5α-DHT	0.95 (0.76–1.19)	.68	0.94 (0.73–1.19)	.61
Androstenedione	1.03 (0.82–1.28)	.82	0.99 (0.77–1.27)	.92
DHEAS	1.00 (0.78–1.25)	.97	0.93 (0.69–1.24)	.65
Low total testosterone (< 11 nmol/L)	0.91 (0.45–1.76)	.80	0.94 (0.44–1.87)	.86
Low free testosterone (< 220 pmol/L)	0.59 (0.21–1.38)	.26	0.55 (0.19–1.37)	.23

Abbreviations: 5α-DHT, 5α-dihydrotestosterone; CI, confidence interval; DHEAS, dehydroepiandrosterone-sulphate; MDD, major depressive disorder; OR, odds ratio; SHBG, sex hormone binding globulin. Data are OR's for 1 standard deviation change in each biomarker based on logistic regression analyses. a Adjusted for age, waist circumference, number of treated chronic somatic disorders, smoking status, alcohol use, physical activity, the presence of a current anxiety disorder, and the presence of remitted MDD.

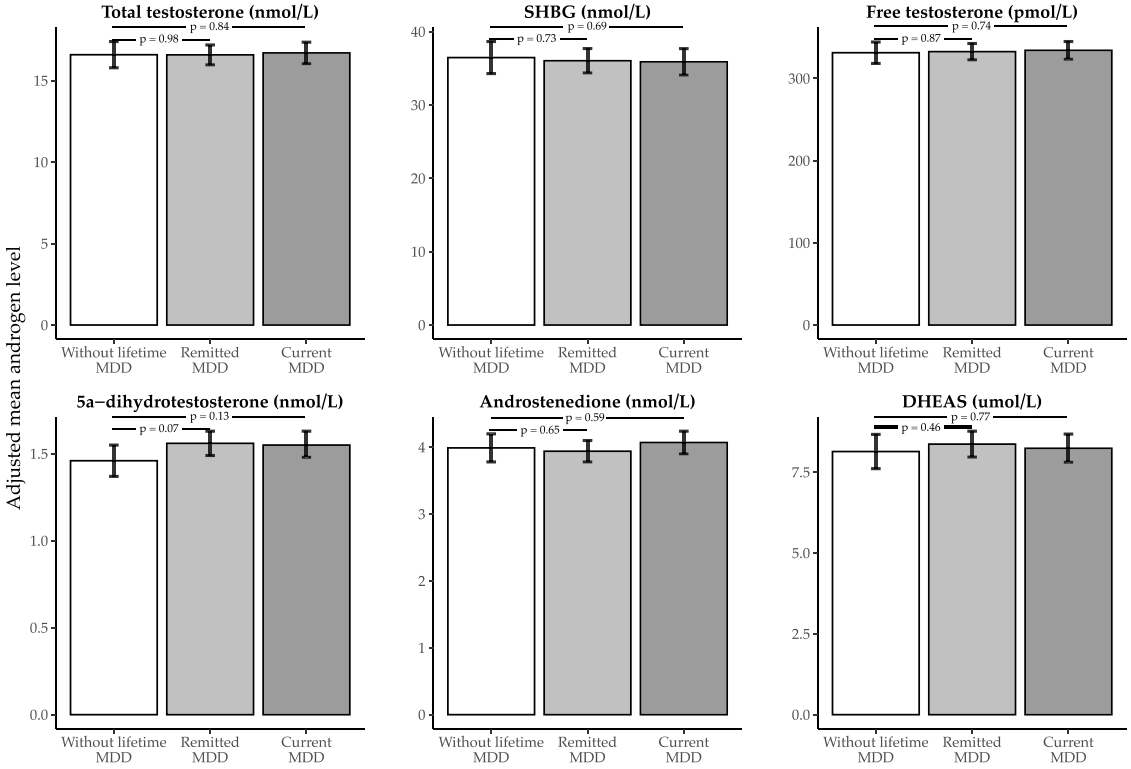

Fig. 1. Adjusted mean androgen and SHBG levels in 823 men according to their diagnosis at baseline. Abbreviations: 5a-DHT, 5α dihydrotestosterone; DHEAS, dehydroepiandrosterone-sulphate. Data are estimated means with 95% confidence intervals based two-way analyses of covariance models adjusted for age, waist circumference, number of treated chronic somatic disorders, smoking status, alcohol use, physical activity, the presence of a current anxiety disorder. To convert nmol/L to ng/dL for total testosterone, 5α-DHT, and androstenedione multiply by 28.84, 29.07, and 28.64, respectively. To convert pmol/L to pg/ml for free testosterone divide by 3.47. To convert μmol/L to μg/dl for DHEAS, multiply by 36.85. To convert to nmol/L to μg/ml for SHBG, multiply by 0.095. The data for this figure are given in Supplement 3.

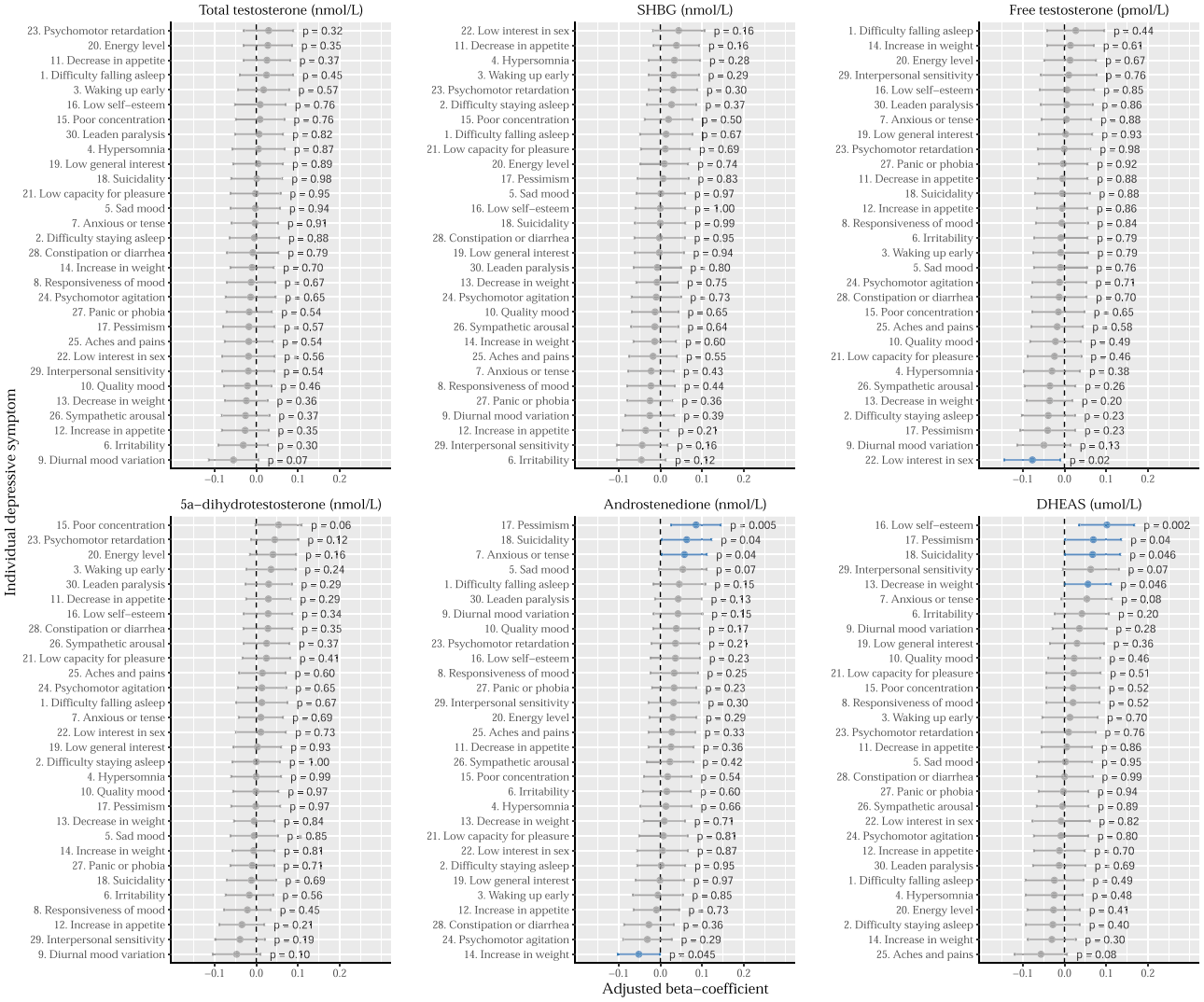

Fig. 2. Baseline plasma androgen and SHBG levels and their association with individual MDD symptoms over two years in 751 men. Abbreviations: 5α-DHT, 5α-dihydrotestosterone; DHEAS, dehydroepiandrosterone-sulphate; MDD, major depressive disorder. Figure shows adjusted standardised β's with 95% Confidence Interval (CI) for the association between androgen levels and the individual MDD symptom scores over 2 years examined with a linear mixed model. The blue dots and lines represent significant associations, whereas the non-significant associations are printed in grey. All models were adjusted for age, waist circumference, number of treated chronic somatic disorders, smoking status, alcohol use, physical activity, the presence of a current anxiety disorder. The data for these figures are given in Supplements 5 to 7.

3.3. Sensitivity analyses: low (hypogonadal) free and total testosterone

The sensitivity analyses confirmed the robustness of the results described above. Compared to men without lifetime MDD, men with current or remitted MDD were not more likely to have low free (adjusted odds ratio [OR] with 95%CI current MDD 1.27 [0.57; 2.47], p = 0.64, and remitted MDD 1.06 [0.55; 2.05], p = 0.87) or total testosterone levels (current MDD 1.41 [0.81; 2.50], p = 0.11, and remitted MDD 1.67 [0.90; 3.12], p = 0.23). Moreover, baseline presence of low free or total testosterone did not predict the onset of MDD during two years of follow-up (see Table 2). Furthermore, low free or total testosterone levels were not associated with increased total MDD symptom severity over two years in the adjusted analyses (see Supplement 8). Only low free testosterone levels were associated with hypersomnia, and low total testosterone levels were associated with difficulty staying asleep and diurnal mood variation (*see* Fig. 3). Although low free testosterone levels were not significantly associated with less interest in sex, the β was in the same direction and of similar strength as the association of lower free testosterone levels with less interest in sex.

3.4. Sensitivity analyses: non-linear analyses individual major depressive disorder symptoms

Non-linear associations between androgens and individual MDD symptom severity scores over two years were also mostly non-significant, except for the associations with 5α-DHT (see Supplement 9). In that specific case, the quadratic term was statistically significant with mainly positive effect estimates, indicating that both lower and higher 5α-DHT levels tended to be associated with increased total MDD symptom severity score, and with difficulty staying asleep, waking up early, low responsiveness of mood, pessimism, low energy level, low capacity for pleasure, psychomotor retardation, sympathetic arousal, and leaden paralysis.

4. Discussion

In this study on the relationship between androgens and depression in men, we showed that none of the levels of different androgens, including those of free testosterone, were associated with current or new onset MDD, which was in line with our first and second hypothesis. Also,

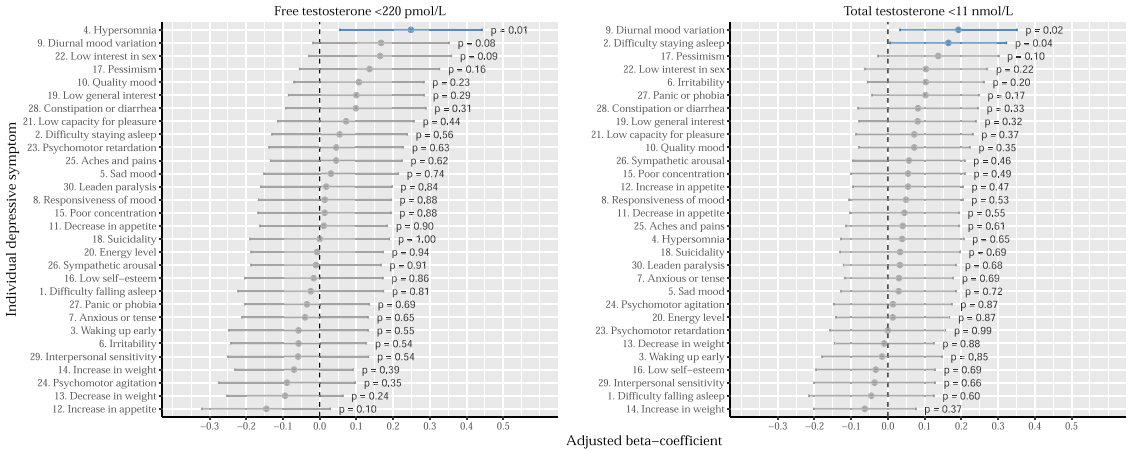

Fig. 3. Presence of low (hypogonadal) free and total testosterone levels at baseline and their association with individual MDD symptoms over two years in 751 men. Abbreviation: MDD, major depressive disorder. Figure shows adjusted standardised beta coefficients with 95% confidence interval for the association between androgen levels and the individual MDD symptom scores over two years examined with a linear mixed model. The blue dots and lines represent significant associations, whereas the non-significant associations are printed in grey. The analyses were adjusted for age, waist circumference, number of treated chronic somatic disorders, smoking status, alcohol use, physical activity, the presence of a current anxiety disorder. The data for these figures are given in Supplement 8.

in line with our third hypothesis, lower free testosterone levels were only associated with less interest in sex. However, higher levels of androstenedione and DHEAS, and both lower and higher levels of 5α-DHT were associated with several individual MDD symptoms. These results may suggest that, in contrast to what is often thought, testosterone is not of clinical relevance for MDD in men.

The finding that lower testosterone levels associate with some overlapping symptoms between MDD and hypogonadism but do not beget a higher risk of MDD, is in line with studies reporting that total and free testosterone levels did not differ between men with and without MDD (Matsuzaka et al., 2013) and had no predictive value for the development of MDD symptoms (Giltay et al., 2017; T'Sjoen et al., 2005). As similar results were found testing low (hypogonadal) levels of testosterone, our results add to the evidence of studies showing that sexual complaints, such as decreased libido, decreased morning erections or erectile dysfunction, are the most prominent symptoms in middle-aged and elderly men with low total or free testosterone levels (Rastrelli et al., 2016; Wu et al., 2010).

We observed for the first time a non-linear association between 5α-DHT level and (several individual) MDD symptoms. This suggests that both lower and higher levels rather than solely lower levels, tended to be associated. Importantly, 5α-DHT is the most potent androgen as an agonist of the androgen receptor, so also more potent than testosterone (Gao et al., 2005). These results therefore highlight the importance of examining non-linear relationships when studying the association between androgens and depression, and the importance of measuring 5α-DHT in future studies.

In contrast to the testosterone findings, androstenedione and DHEAS levels were associated with some individual MDD symptoms. The fact that both androstenedione and DHEAS are precursors of total testosterone and have little androgenic activity itself, may suggest that these levels were elevated as a consequence of (processes related to) MDD, rather than that they directly caused the symptoms. For example, we propose that an altered hypothalamic-pituitary-adrenal-axis (HPA-axis) with a chronic stress response could underlie this phenomenon. The HPA signalling pathways may be altered such that hyperactivity of the HPA-axis and impaired negative feedback responses have been described in those patients with current and remitted MDD (Anacker et al., 2011; Vreeburg et al., 2009). Aside from activating cortisol release, these pathways also have a stimulatory effect on the zona fasciculata and reticularis of the adrenal cortex to produce adrenal androgens, like DHEAS, and other androgens such as androstenedione which are not solely produced by the adrenals but still for a reasonable amount (Schweikert, 2019). This is apparent as those with central adrenal insufficiency who lack adrenocorticotropic hormone production, have a deficiency in cortisol and dehydro-epiandrosterone. Therefore, in a chronic stress-response model of MDD, it would seem that an enhanced HPA-axis activation of the adrenal cortex not only stimulates release of cortisol, but of adrenally produced androgens as well. Nevertheless, this and other mechanisms remain to be further explored.

Strengths of this study include the examination of a well-phenotyped sample where psychiatric diagnoses were based on structured diagnostic interviews and androgens determinations with LC-MS/MS, which is imperative given the detection of lower androgens levels in hypogonadal men (Kushnir et al., 2010). Furthermore, we were able to differentiate associations with individual MDD symptoms, which enabled us to determine which symptoms could drive an association between androgens and MDD. Our study has also some limitations. First, because we used observational data, we cannot conclude whether elevated androstenedione levels are a cause or an effect of MDD. Second, the number of men without MDD at baseline was small, which limited the power to detect an association between androgens and onset of MDD. Moreover, the majority of these men had a history of MDD. Hence, we studied both new onset and recurrence of MDD in relation to androgen levels. Third, although androgens can pass the blood-brain-barrier, binding proteins limit the extent which may consequently limit the sensitivity of peripherally measured androgens to reflect androgen levels in the brain. Fourth, a repeated measurement of androgen levels after a short interval would have increased the precision of the androgen levels measured. Fifth, the single measurement of androgens might have lacked the sensitivity to predict depression over a two-year course. Future studies should measure androgens at least twice as change levels provide more insights in the within-person variation. Alternatively, measurement of androgens in hair using LC-MS/MS would offer the unique chance to estimate androgen production over longer time periods (Gao et al., 2016; Noppe et al., 2015). Sixth, multiple testing may have induced type-I errors. Finally, free testosterone levels were calculated but not measured.

4.1. Conclusions

In conclusion, in our study we did not find differences in testosterone levels between adult men with and without MDD, nor an association of testosterone levels with individual MDD symptoms. Only lower free testosterone levels were associated with less interest in sex. Therefore, this study confirms the idea that single measured circulating androgens

synthesised by the testes have a limited clinical relevance to MDD in adult men, although they may affect some individual symptoms that overlap with hypogonadism.

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CRediT authorship contribution statement

Ms de Wit and Dr Giltay had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis; **de Wit, Giltay, de Boer, Schoevers**: Concept and design; **All authors**: Acquisition, analysis, or interpretation of data, Critical revision of the manuscript for important intellectual content; **de Wit**: Drafting of the manuscript; **de Wit, Giltay**: Statistical analysis; **Penninx, Giltay, Schoevers**: Obtained funding; **de Wit, Schoevers**: Supervision; **Giltay, de Boer, Schoevers**: Administrative, technical, or material support.

Declaration of Competing Interest

BWJH Penninx has received research grants from Jansen Research and Boehringer Ingelheim. The other authors: nonehip with any organisation that might have an interest in the submitted study in the last 3 years.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.psyneuen.2021.105278.

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