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Research paper

Associations of plasma androgens with suicidality among men and women: A 9-year longitudinal cohort study

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ABSTRACT

Background: Testosterone has been implicated in suicidality in cross-sectional studies. Stress that coincides with a suicide attempt may alter androgen levels, so prospective studies are needed to exclude reverse causation. We aimed to examine the associations of plasma androgens with concurrent and future suicidality, and if present, whether these associations were mediated by a behavioral trait like reactive aggression.

Methods: Baseline plasma levels of total testosterone, 5α − dihydrotestosterone, and androstenedione were determined with liquid chromatography–tandem mass spectrometry, and dehydroepiandrosterone-sulphate with a radioimmunoassay. Suicidality was assessed using the Suicidal Ideation Scale at baseline and after 2-, 4-, 6-, and 9-year follow-up. Men and women were analyzed separately, and potential confounders were considered.

Results: Participants (N = 2861; 66.3% women) had a mean age of 42.0 years (range 18–65) and almost half (46.9%) fulfilled criteria for a major depressive or anxiety disorder. At baseline 13.2% of men and 11.2% of women reported current suicidal ideation. In participants who were non-suicidal at baseline, slightly more men than women reported suicidal ideation during follow-up (14.7% vs. 12.5%), whereas the reverse pattern was observed for suicide attempts (3.6% vs. 4.2%). None of the associations between androgens and current and future suicidality were significant.

Limitations: Androgens were determined once, which may have been insufficient to predict suicidality over longer periods.

Discussion: The lack of associations between plasma levels of androgens determined by 'gold-standard' laboratory methods with suicidality do not support previous cross-sectional and smaller studies in adult men and women with values within the physiological range.

1. Introduction

Suicidal ideation and suicide attempts (together also known as suicidality), are a common mental health concern (Nock et al., 2008). They do not only cause a heavy emotional burden on individuals, but also on relatives as well as clinicians (Hendin et al., 2000; WHO, 2014). Approximately twice as many men as women commit suicide (WHO, 2014). Though reasons for this sex difference remain largely unknown, one could postulate that differences in the circulating levels of androgens between the sexes, are implicated in this phenomenon (Lenz et al., 2019). They are known to alter and to be altered by the hypothalamic-pituitary-adrenal-axis (HPA-axis), and serotonergic system, systems that are likely dysregulated in suicidality (Ludwig et al., 2017; Oyola and Handa, 2017; van Heeringen and Mann, 2014). Furthermore, these androgens can induce direct genomic and non-genomic effects by binding to sex steroid receptors in the brain (McHenry et al., 2014; Schmidt et al., 2000).

Support for a link between androgens and suicidality has come from cross-sectional studies, mostly conducted in men. One study revealed that plasma and cerebral spinal fluid (CSF) testosterone levels were higher in nine male suicide attempters measured two weeks after the attempt relative to the levels in 12 healthy controls (Stefansson et al., 2016). Opposite results were reported in two other studies showing lower plasma testosterone levels in 15 and 80 suicide attempters

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compared to an about equal number of controls (Markianos et al., 2009; Tripodianakis et al., 2007). However, three other studies with 31–112 suicide attempters found no association (Butterfield et al., 2005; Perez-Rodriguez et al., 2011; Zhang et al., 2015). Studies in women are more scarce, but results are similarly inconsistent. A study in 51 women with bipolar disorder reported that high plasma testosterone was weakly associated with more suicide attempts, but only in an adjusted analysis that also included 16 men (Sher et al., 2012). Other studies in 17 and 344 women that measured CSF and/or plasma testosterone found no significant associations with suicide attempts (Stefansson et al., 2016; Zhang et al., 2015). No study showed a statistically significant link between testosterone and suicidal ideation, neither in men nor in women (Butterfield et al., 2005; Sher et al., 2012, 2014; Zhang et al., 2015).

The above-described heterogeneity in findings may be related to the acute stress that accompanies an attempt. Due to the tight coupling between the hypothalamic-pituitary-gonadal (HPG-) and the HPA-axis, testosterone levels will be altered after such an event (Oyola and Handa, 2017). Hence, we cannot conclude from cross-sectional studies whether the association suggests a mechanism for suicidality or whether it is a result of experienced stress, thus reflecting reverse causation. Two studies acknowledged this, and examined whether testosterone levels predicted suicide attempts during a follow-up of 2.5 and 21 years (Sher et al., 2014; Stefansson et al., 2016). Higher baseline plasma testosterone levels predicted for suicide attempts in one, but not in the other study (Sher et al., 2014; Stefansson et al., 2016). However, the sample sizes were limited to 51 and 47 participants, and the analyses were not adjusted for previous attempts nor stratified for sex.

Reactive aggression refers to aggressive behaviour evoked by a certain situation. It has been hypothesized that besides the suggested direct effect of testosterone on suicidality, reactive aggression mediates this association (Lenz et al., 2019). This follows from studies that showed increased rates of suicidality among those with aggressive traits (McCloskey and Ammerman, 2018; Turecki and Brent, 2016), and from studies that showed -though more consistently in other primates than humans- associations between androgen levels and aggression (Archer et al., 2004; Carré et al., 2011; Coccaro, 2017). The revised Leiden Index of Depression Sensitivity (LEIDS-R) measures cognitive changes that may occur during depressed mood (for the purpose of this paper, reactive aggression will refer to aggressive cognitive reactivity) (Solis et al., 2017). As suicidality is especially prevalent among depressed individuals (Bostwick and Pankratz, 2000), this questionnaire provides the unique opportunity to study whether a phenotype with more aggressive behavioral traits or cognitive styles mediates the association between androgens and suicidality.

The reported inconsistencies and the paucity of large and prospective studies prompted us to examine the role of androgens in suicidality. To accomplish this, androgens were determined by liquid chromatography–tandem mass spectrometry (XLC–MS/MS) in a large study (N = 2861) of men and women with depressive and anxiety disorders and healthy controls who were followed over nine years. Using this data, we sought to examine the association between androgen levels and a) concurrent suicidal ideation, and b) suicidal ideation and suicide attempts during nine years of follow-up, and c) whether these potential associations are mediated by reactive aggression.

2. Methods

2.1. Study sample

Data were derived from the Netherlands Study of Depression and Anxiety (NESDA), an ongoing longitudinal observational cohort study that started in 2004 (Penninx et al., 2008). Current and remitted depressed participants and healthy controls aged 18–65 years were recruited from the Dutch general population (19%), primary health care (54%) and specialized mental health care (27%). Ethics committees of participating universities approved the research design. After giving written informed consent, 2981 participants were included. Exclusion criteria were a poor comprehension of the Dutch language, and having a primary clinical diagnosis of psychotic disorder, obsessive–compulsive disorder, bipolar disorder or severe addiction disorder. Presence of mood disorders (within the past month) were ascertained using the Composite International Diagnostic Interview (CIDI version 2.1) administered by trained researcher assistants (Wittchen, 1994). Participants were reassessed after 2 (wave 3 [W3]), 4 (W4), 6 (W5), and 9 years (W6). All follow-up assessments were included in this study and had high retention rates (the proportion of the baseline sample participating) of 87.1% (W3), 80.6% (W4), 75.7% (W5), and 69.4% (W6).

The present study included participants of whom plasma androgens levels were available (N = 2917). Seventeen participants were excluded because they were using sex hormones (World Health Organization Anatomical Therapeutic Chemical [ATC code] G03) or hormone antagonists (ATC code L02) (World Health Organization Collaborarting Centre for Drug Statistic, 2007). Another 39 participants were excluded, as they were pregnant, transgender, had severely aberrant testosterone levels probably due to a measurement error, or had missing values on the outcome measure. Altogether, this resulted in a final sample of 2861 participants at baseline (96.0% of the total sample). Excluded participants (N = 120) drank less alcohol (percentage that drinks more than one unit a day 19.8% vs. 12.5%, p = 0.046), and had more often attempted suicide than included participants (18.8% vs. 15.8%, p = 0.04; see Supplementary Table 1).

2.2. Androgens

Blood was drawn in the morning (mean 08:48 AM ± 0:25 h) after an overnight fast (success rate 95.6%). Total testosterone, androstenedione, and 5α−dihydrotestosterone (5α-DHT) were determined with XLC–MS/MS at the Clinical Chemistry department of the University Medical Centre Groningen. In case insufficient biomaterial was available to obtain 200 uL (15%), samples were pooled. The lower limit of quantitation was 0.04 nmol/L for total testosterone and androstenedione, and 0.12 nmol/L for 5α-DHT. Participants with values below the detection limit (n = 1 [0.03%] for total testosterone and n = 178 [6.2%] for 5α-DHT) were given values of 0.01 below the lower limit of quantitation. Dehydroepiandrosterone-sulphate (DHEAS) was determined using ARCHITECT (ABBOTT, Wiesbaden, Germany) a radioimmunoassay with chemiflex assay protocol. The detection rate of DHEAS was ≤0.3 μmol/L with a calibration range of 0.00–40.7 μmol/L. The inter-assay coefficient of variation was estimated as ≤10%.

2.3. Primary outcomes: suicidal ideation and attempts

Suicidal ideation and suicidal attempts at baseline and follow-up were assessed with the Suicidal Ideation Scale (SSI) (Beck, 1991; Beck et al., 1979). Suicidal ideation during the last week was determined with the first 5 items. With these items, participants' attitudes toward living and dying were examined with questions like 'Did you want to live and how strong was this wish?' and 'Did you feel the desire to harm or poison yourself?'. Suicidal ideation was considered present (i.e., a dichotomous outcome) when any form of ideation, ranging from death wish to suicide plans or threats, was expressed. Time to suicidal ideation was determined as the number of years until a participant reported suicidal ideation using the herein mentioned questionnaire. Suicidal attempts were assessed with item 6 'Have you ever seriously attempted to commit suicide?'. Time to suicidal attempt was ascertained as the number of years until a participant reported a suicide attempt during one of the follow-up assessments. When present, the date was specified and the number of years between baseline and the suicide attempt could be calculated.

2.4. Secondary outcomes: reactive aggression

Reactive aggression to sad mood was measured with the reactive aggression subscale of the revised Leiden Index of Depression Sensitivity (LEIDS-R)(Solis et al., 2017). This questionnaire intents to measure cognitive changes that may occur during depressed mood. Participants filling out the LEIDS-R were instructed to recall a situation when they felt sad and then rate how well they were able to imagine this situation (i.e. well, somewhat, or not at all). The reactive aggression subscale contains 6 items like "When I feel bad, I feel more like breaking things" and "When I feel down, I lose my temper more easily". Participants rated the extent to which each statement applied to them on a 5-point Likert scale (0, not at all; 1, a bit; 2, moderately; 3, strongly; 4, very strongly). The sum score of these items ranged from 0 to 24 with a higher score indicating stronger reactive aggression. The LEIDS-R reactive aggression subscale has good psychometric properties with a Cronbach α of 0.83(Solis et al., 2017).

2.5. Covariates

Age (in years), education (in years), married or with partner (yes/no), currently smoking (yes/no), alcohol use (≤ or > 7 units a week), measured Body Mass Index (BMI), and number of treated chronic somatic disorders were considered as covariates. In women, the models were also accounted for the use of oral contraceptives (yes/no).

2.6. Statistical analyses

All analyses were done in strata, separately for men and women. Baseline characteristics of participants were presented by means ± SD, geometric mean with 95% confidence interval, and frequencies with percentages, dependent on the type and distribution of the variable. Missing values on the covariate alcohol use (n = 39; 1.4%) and BMI (n = 2; 0.0007%) were imputed with the mean. Due to right skewedness, all androgen levels in women, and androstenedione and DHEAS levels in men, were log transformed. To ease comparability of effect sizes, levels of androgens were also standardized before the analyses (z-values).

In the primary analysis, we examined linear and non-linear associations of androgen levels with concurrent suicidal ideation using logistic regressions. Next, we analyzed linear and non-linear association between androgen levels and future suicidal ideation, and suicide attempts using Cox proportional hazard models. Participants with suicidal ideation (n = 340) or suicidal attempts (n = 328) at baseline were excluded. Participants who dropped out before the first follow-up assessment (n = 221) were also excluded for the analyses on suicidal ideation. Dropouts after 2 years of follow-up were censored. The Cox proportional hazards assumption was checked with log-minus-log (LML) curves.

In the secondary analysis, we examined the association between androgens and reactive aggression. Subsequently, we tested whether the significant associations in the primary analysis – if any – were mediated by reactive aggression, using the indirect method by Preacher and Hayes (2008). This method estimates the total, direct, and indirect effects of the independent variable on the dependent variable through the mediator variable.

All analyses were adjusted for the time-independent covariates as mentioned below the header 'covariates'. Data were analyzed using IBM SPSS Statistics (IBM Corp) version 25, using 2-sided tests. As multiple tests were performed, we calculated an adjusted False Discovery Rate [FDR] P cut-off value to avoid the inflation of false-positive findings. A P-value of <0.0005 was considered statistically significant.

3. Results

Data of 965 men and 1896 women with an age range from 18 to 65 years were included in the analyses. Both men and women were slightly overweight on average (mean BMI 26.2 ± 4.5 and 25.3 ± 5.2), and 41.8% and 37.0% were smokers, respectively. Almost half of the sample suffered from either a major depressive or an anxiety disorder in the preceding month (46.9%), and 99 men (10.3%) and 229 women (12.1%) had had a preceding suicide attempt. Men had almost 20 times higher total testosterone levels than women (geometric mean total testosterone 15.6; 95%CI, 14.6 to 16.6, vs., 0.79; 95%CI, 0.04 to 1.80; respectively). See also Table 1.

Table 1
Characteristics at baseline of the study samples of 2861 men and women

	Men n = 965	Women n = 1896
Age, mean (SD), y	43.6 ± 12.9	41.1 ± 13.1
Education, mean (SD), y	12.1 ± 3.3	12.2 ± 3.3
Married or with partner, no. (%)	675 (69.9)	1306 (68.9)
BMI, mean (SD)	26.2 ± 4.5	25.3 ± 5.2
No. smoking, no. (%)	403 (41.8)	702 (37.0)
No. alcohol intake >1 unit a day, no. (%)	307 (31.8)	262 (13.8)
Treated chronic diseases, geo. mean (95%CI)	0.5 (0.0–1.5)	0.4 (0.0–1.4)
No. oral contraceptive use, no. (%)	–	508 (26.8)
No. antidepressant use, no. (%)	238 (24.7)	498 (26.3)
No. MDD or anxiety disorder past month, no. (%)	453 (46.9)	889 (46.9)
No. suicide ideation in preceding week, no. (%)	127 (13.1)	213 (11.2)
No. suicide attempt in past, no. (%)	99 (10.3)	229 (12.1)
Reactive aggression, geo. mean (95%CI)	3.8 (2.8–4.8)	3.4 (2.4–4.4)
Predictors - Androgenic features		
Total testosterone (nmol/L), geo. mean (95%CI)	15.6 (14.6–16.6)	0.79 (0.04–1.80)
5α-DHT (nmol/L), geo. mean (95%CI)	1.5 (0.5–2.5)	0.36 (0.12–1.36)
Androstenedione (nmol/L), geo. mean (95%CI)	3.7 (2.7–4.7)	3.3 (2.3–4.3)
DHEAS (μmol/L), geo. mean (95%CI)	7.2 (6.2–8.2)	4.7 (3.7–5.7)

Abbreviations: 5α-DHT, 5α dihydrotestosterone; BMI, body mass index (calculated as weight in kilograms divided by height in meters squared); DHEAS, dehydroepiandrosterone-sulphate; MDD, Major Depressive 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.

3.1. Suicidal ideation and suicide attempts

As shown in Table 2, about similar rates of men and women experienced suicidal ideation (13.2% and 11.2%, respectively) at baseline. Higher levels of androstenedione were associated with increased odds of suicidal ideation in men (crude standardized odds ratio (OR), 1.42; 95%CI, 1.17–1.72; p = 0.0004), but not after consideration of covariates (adjusted standardized OR, 1.31; 95%CI, 1.06–1.62; p = 0.01). None of the other androgens was associated with suicidal ideation in men or women at baseline. See also Figure 1. Slightly more men than women reported suicidal ideation during follow-up (113 / 769 [14.7%] compared to 192 / 1531 [12.5%]), whereas the reverse was observed for suicidal attempts (29 / 866 [3.3%] compared to 64 / 1667 [3.8%]). As shown in Table 3, androgen levels were neither linear nor non-linearly associated with future suicidal ideation or suicide attempts in men nor in women.

3.2. Reactive aggression

We examined the association of androgens with reactive aggression. Only higher levels of androstenedione in women was associated more reactive aggression (crude standardized β, 0.362, 95%CI [0.166–0.559], p = 0.0003), but not after the adjustment for covariates (adjusted standardized β, 0.012, 95%CI [−0.219 to −0.243],

Table 2
Associations between baseline plasma androgen levels and concurrent suicidal ideation in men and women.

	Current suicidal ideation			
	Crude OR (95% CI)	P[b]	Adjusted[a] OR (95% CI)	P[b]
Men				
No. events (%)		127 / 965 (13.2)		
Total testosterone	1.01 (0.84–1.22)	.88	0.95 (0.76–1.17)	.60
5α-DHT	0.97 (0.81–1.18)	.78	0.92 (0.75–1.13)	.42
Androstenedione	1.42 (1.17–1.72)	<.0004	1.31 (1.06–1.62)	.01
DHEAS	1.07 (0.88–1.29)	.50	1.11 (0.87–1.41)	.39
Total testosterone	0.91 (0.74–1.11)	.33	0.81 (0.64–1.01)	.06
Total testosterone[2,c]	1.21 (1.02–1.44)	.03	1.29 (1.08–1.54)	.01
5α-DHT	093 (0.75–1.15)	.49	0.86 (0.68–1.08)	.20
5α-DHT[2,c]	1.04 (0.95–1.14)	.35	1.06 (0.96–1.16)	.23
Androstenedione	1.44 (1.17–1.78)	.001	1.34 (1.07–1.69)	.01
Androstenedione[2,c]	0.97 (0.85–1.11)	.66	0.95 (0.83–1.09)	.45
DHEAS	1.01 (0.81–1.26)	.92	1.01 (0.81–1.26)	.92
DHEAS[2,c]	0.88 (0.75–1.03)	.10	0.88 (0.75–1.03)	.10
Women				
No. events (%)	213 / 1896 (11.2)			
Total testosterone	1.15 (0.99–1.33)	.07	1.07 (0.92–1.25)	.40
5α-DHT	1.13 (0.98–1.30)	.11	1.05 (0.89–1.24)	.54
Androstenedione	1.22 (1.06–1.42)	.01	1.14 (0.95–1.36)	.16
DHEAS	1.08 (0.93–1.25)	.33	0.96 (0.81–1.15)	.67
Total testosterone	1.18 (1.02–1.35)	.02	1.10 (0.94–1.28)	.23
Total testosterone[2,c]	1.13 (1.00–1.28)	.05	1.11 (0.98–1.26)	.10
5α-DHT	1.12 (0.98–1.28)	.09	1.04 (0.89–1.22)	.59
5α-DHT[2,c]	1.19 (1.07–1.34)	.002	1.19 (1.06–1.34)	.003
Androstenedione	1.25 (1.08–1.44)	.002	1.17 (0.98–1.39)	.09
Androstenedione[2,c]	1.08 (1.00–1.16)	.06	1.06 (0.97–1.14)	.20
DHEAS	1.13 (0.97–1.31)	.13	0.99 (0.82–1.20)	.93
DHEAS[2,c]	1.06 (0.98–1.15)	.14	1.03 (0.95–1.12)	.45

Abbreviations: 5α-DHT, 5α dihydrotestosterone; DHEAS, dehydroepiandrosterone-sulphate; MDD, Major Depressive Disorder; OR, odds ratio.
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.

[a] Adjusted for age, education, married or with partner, BMI, smoking, alcohol use, and number of treated chronic diseases, (and in women also for oral contraceptive use).

[b] Based on logistic regression analysis.

[c] Androgen level is squared to examine non-linear relationship with current suicidal ideation.

p = 0.92). See Supplementary Table 2. As none of the associations in the primary analyses was significant, no mediation analyses with reactive aggression was performed.

4. Discussion

In this large study among 965 men and 1896 women whose plasma androgen levels were measured with the best available laboratory methods, we could not demonstrate significant associations between plasma levels of androgens with current as well as suicidal ideation or suicide attempts during follow-up. Therefore, our results challenge findings from previous studies that plasma androgen levels might be a viable biomarker of suicidality.

Our analyses focused on associations of plasma androgen levels at baseline with future suicidal ideation and suicide attempts during follow-up, as our study was founded on the notion that acute stress that is experienced by the suicide attempter may increase androgen levels whereas chronic stress may decrease androgen levels. Hence, this might explain why we cannot confirm the either positive or negative associations between androgens and suicidality that were demonstrated in previous smaller studies that examined androgen levels directly after a suicide attempt (Butterfield et al., 2005; Markianos et al., 2009; Perez-Rodriguez et al., 2011; Roland et al., 1986; Sher et al., 2012; Stefansson et al., 2016; Tripodianakis et al., 2007; Zhang et al., 2015). These studies also rarely considered confounders in their analyses and used less reliable methods than XLC–MS/MS to determine androgen levels. As a consequence, this might have led to overestimation of true effect sizes. However, an alternative explanation for the discrepancies between our results and that of others might be lying in differences in study population regarding psychiatric diagnoses. We examined participants with depression and anxiety, and healthy controls, while previous significant positive associations were derived from patients with schizophrenia (Markianos et al., 2009; Tripodianakis et al., 2007), and significant negative associations from patients with bipolar disorder (Sher et al., 2012, 2014). Nevertheless, since this study included the largest sample of men and women so far, the absence of significant associations suggests that these are –if present at all– of weak strength and likely not clinically meaningful in a population of depressed and anxious patients. Finally, the positive findings in smaller previous studies may be false positive findings or the effect of publication bias. Studying testosterone during windows of development in which it may also cause permanent effects on cerebral functioning like antenatal or during puberty, may be an intriguing alternative direction (Lenz et al.,

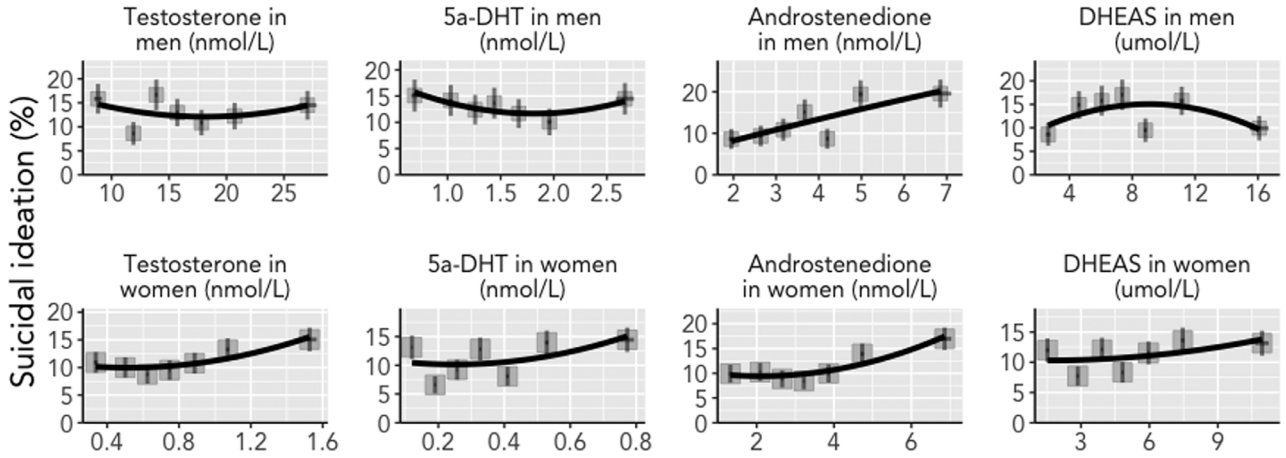

Fig. 1. Percentage of participants with suicidal ideation in the last week according to 7 septiles (i.e., quantiles that divided an ordered sample population into seven equally numerous subsets) with their mean levels of plasma androgens in 965 men and 1896 women. Abbreviations: DHT, 5α dihydrotestosterone; DHEAS, dehydroepiandrosterone-sulphate. 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. Vertical error bars represent standard errors of the mean (SE), and horizontal error bars represent 95% CI of the mean of each septile. A second order regression line was fitted.

Table 3
Associations between baseline plasma androgen levels and future suicidal ideation and suicide attempts over 9 years in men and women.

	Future suicidal ideation				Future suicide attempt			
	Crude HR (95% CI)	P[b]	Adjusted[a] HR (95% CI)	P[b]	Crude HR (95% CI)	P[b]	Adjusted[a] HR (95% CI)	P[b]
Men								
No. events (%)		113 / 769 (14.7)			29 / 866 (3.3)			
Total testosterone	0.87 (0.71–1.06)	.17	0.85 (0.68–1.06)	.15	1.01 (0.69–1.46)	.97	1.02 (0.67–1.55)	.94
5α-DHT	0.87 (0.71–1.06)	.16	0.86 (0.69–1.06)	.16	0.88 (0.60–1.30)	.53	0.85 (0.55–1.30)	.45
Androstenedione	1.04 (0.87–1.26)	.65	1.07 (0.89–1.30)	.47	1.13 (0.79–1.62)	.52	1.20 (0.82–1.75)	.36
DHEAS	0.98 (0.82–1.17)	.79	1.13 (0.90–1.41)	.28	0.91 (0.64–1.29)	.60	1.08 (0.70–1.66)	.73
Total testosterone	0.85 (0.69–1.05)	.13	0.82 (0.66–1.03)	.09	1.02 (0.67–1.57)	.91	1.02 (0.64–1.62)	.94
Total testosterone[2,c]	1.08 (0.83–1.40)	.58	1.12 (0.785–1.47)	.43	0.96 (0.55–1.66)	.87	1.00 (0.57–1.76)	1.00
5α-DHT	0.82 (0.66–1.00)	.05	0.80 (0.64–0.99)	.04	0.96 (0.59–1.57)	.87	0.92 (0.55–1.55)	.76
5α-DHT[2,c]	1.07 (0.99–1.16)	.11	1.08 (0.99–1.17)	.08	0.78 (0.50–1.21)	.27	0.78 (0.50–1.22)	.27
Androstenedione	1.06 (0.89–1.26)	.55	1.08 (0.89–1.31)	.45	1.12 (0.79–1.59)	.52	1.20 (0.82–1.75)	.36
Androstenedione[2,c]	1.05 (0.95–1.15)	.38	1.01 (0.92–1.12)	.81	1.03 (0.84–1.26)	.77	1.00 (0.82–1.22)	.98
DHEAS	1.03 (0.86–1.25)	.75	1.23 (0.97–1.55)	.09	0.85 (0.56–1.31)	.46	0.99 (0.58–1.66)	.95
DHEAS[2,c]	1.07 (0.97–1.18)	.18	1.08 (0.98–1.19)	.12	0.92 (0.70–1.20)	.52	0.91 (0.70–1.19)	.50
Women								
No. events (%)	192 / 1531 (12.5)				64 / 1667 (3.8)			
Total testosterone	0.92 (0.80–1.06)	.27	0.96 (0.83–1.11)	.57	1.09 (0.84–1.40)	.52	0.99 (0.75–1.29)	.92
5α-DHT	0.93 (0.80–1.07)	.30	0.99 (0.84–1.17)	.94	1.29 (1.00–1.66)	.05	1.24 (0.93–1.64)	.14
Androstenedione	0.97 (0.84–1.12)	.67	1.02 (0.87–1.20)	.82	1.14 (0.88–1.47)	.33	1.00 (0.74–1.35)	.99
DHEAS	0.83 (0.73–0.95)	.005	0.87 (0.75–1.02)	.09	0.99 (0.77–1.27)	.92	0.89 (0.66–1.20)	.43
Total testosterone	0.94 (0.81–1.09)	.42	0.96 (0.82–1.12)	.61	1.08 (0.84–1.41)	.55	0.98 (0.74–1.30)	.88
Total testosterone[2,c]	1.04 (0.93–1.17)	.51	1.00 (0.89–1.12)	1.00	0.96 (0.69–1.34)	.82	0.94 (0.67–1.32)	.74
5α-DHT	0.93 (0.80–1.07)	.31	0.99 (0.84–1.17)	.89	1.27 (0.99–1.63)	.06	1.22 (0.92–1.62)	.16
5α-DHT[2,c]	1.00 (0.88–1.14)	1.00	0.97 (0.85–1.11)	.68	1.05 (0.86–1.30)	.62	1.04 (0.85–1.27)	.72
Androstenedione	1.03 (0.89–1.19)	.69	1.07 (0.90–1.28)	.45	1.17 (0.93–1.49)	.18	1.04 (0.77–1.39)	.81
Androstenedione[2,c]	1.07 (1.01–1.14)	.02	1.04 (0.98–1.11)	.21	1.10 (0.98–1.23)	.11	1.08 (0.95–1.22)	.27
DHEAS	0.88 (0.75–1.03)	.10	0.89 (0.74–1.08)	.25	1.04 (0.80–1.35)	.76	0.94 (0.68–1.28)	.68
DHEAS[2,c]	1.04 (0.97–1.12)	.28	1.01 (0.94–1.09)	.72	1.07 (0.93–1.22)	.35	1.06 (0.92–1.22)	.43

Abbreviations: 5α-DHT, 5α dihydrotestosterone; DHEAS, dehydroepiandrosterone-sulphate; HR, hazard ratio; MDD, Major Depressive 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.

[a] Adjusted for age, education, married or with partner, BMI, smoking, alcohol use, and number of treated chronic diseases, (and in women also for oral contraceptive use).

[b] Based on cox regression analysis.

[c] Androgen level is squared to examine non-linear relationship with current suicidal ideation.

2019). Indirect measures of intra-uterine androgen exposure like second-to-fourth finger length (2D:4D), birth weight and maternal androgen levels, and thus masculinization of the brain, have been associated with increased risk of suicide completion during adulthood (Lenz et al., 2019). Future studies could also look into androgen receptor CAG trinucleotide repeats rather than plasma levels, as those better reflect androgen sensitivity (Mobasseri et al., 2018).

We also observed no association between levels of androgens and reactive aggression after the consideration of important covariates. Higher levels of aggressiveness have mostly been reported as a side effect of anabolic androgenic steroid abuse in men and women (Gruber and Pope, 2000; Onakomaiya and Henderson, 2016; Strauss et al., 1985), but those concerned supraphysiological 5α-DHT levels and might therefore not be comparable to the physiological levels in our cohort of men and natural cycling women. Otherwise, a recent randomized controlled trial revealed that testosterone could rapidly potentiate aggressive behaviour, but only among men with dominant or impulsive personality styles (Carre et al., 2017). Also, men who received testosterone were more likely to pay money to punish proposers who made unfair offers as well as to reward proposers who made fair offers in the ultimatum game (Dreher et al., 2016). Apparently, testosterone may evoke both prosocial and antisocial (e.g. aggressive) aspects depending on the context. Yet, these time- and situation depending differences were not considered in this study which might explain the lack of associations.

Strengths of this study include the examination of the largest sample of men and women up to date who were followed over prolonged periods of time with rigorously assessment of their phenotype with well-validated questionnaires (Beck et al., 1979; Van der Does, 2002). Previous studies were smaller, and included up to 490 participants in cross-sectional analyses (Zhang et al., 2015) and 51 participants in prospective analyses (Sher et al., 2014). Also, we present the first study that reliably measured testosterone in women as androgens were determined by the 'golden-standard' XLC–MS/MS method. Immunoassays were less reliable when measuring the low testosterone (< 5.0 nmol/L) levels that are typically present in women due to cross reactivity with other hormones (Kushnir et al., 2010). Finally, we were able to adjust for a wide variety of confounders that are important in the potential association between testosterone and suicidality. Nevertheless, this study has some limitations. Suicidality was assessed with a 5 item scale and may wax and wane over time. Therefore, it may have been undetected due to the relatively large time intervals between follow-up assessments in our study. However, if strong associations would exist, those would resist the long timespan that were present in the longitudinal analyses. Also, single measurements may have been insufficient to predict suicidality over longer periods of time. Yet, levels of testosterone, 5α-DHT and androstenedione tend to only slightly decrease over time in women, and be even stable after menopause (Burger et al., 2000; Davison et al., 2005; Elmlinger et al., 2005; Rothman et al., 2011). This decrease is most pronounced in women in their third decade (Davison et al., 2005), but this age category was only presented by 23% of our cohort. In middle aged men, a slow aging-related decline in total testosterone levels of 0.3–1.8% annually, is seen (Kaufman et al., 2019). Moreover, 5α-DHT levels tend to be stable in men until the age of 65, like the age range that was included this study (Handelsman et al., 2015). Likewise, reported intraclass correlation

coefficients for testosterone, 5α-DHT and DHEAS are good, ranging from 0.74 to 0.97, depending on the time between measurements (4 weeks to 5 years) and method used (radio immune assay or LC/MS–MS) (Cauley et al., 1991; Hsing et al., 2007; Jones et al., 2014). Altogether, this suggests that a single measure may be reliable for ranking men and women according to their androgen level for epidemiologic research. Furthermore, we were not able to account for recent perceived stress levels, which may be interesting since the HPA-axis and HPG-axis are known to be coupled (Oyola and Handa, 2017). Finally, we were not able to study completed attempts. Hence, our results are limited to the notion of the role of androgens in suicidal ideation and non-lethal attempts.

In conclusion, this study, that followed a large sample of participants over nine years, showed the absence of significant associations between plasma androgens and suicidality. Thus, our results do not support findings from previous cross-sectional and smaller studies on the role of plasma androgens in suicidality in adult men and women with values within the physiological range.

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Data for reference

Data of the NESDA study, on which the manuscript was based, can be requested through the website of the study: www.nesda.nlhttp://nesdo.amstad.nl.

Declaration of Competing Interest

None of the authors has conflict of interest to declare.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jad.2020.03.032.

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