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Development of a single-dose intranasal testosterone administration paradigm for use in men and women

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ABSTRACT

For over two decades, researchers in the field of human social neuroendocrinology have been using single-dose pharmacological challenge protocols to determine the causal effects of testosterone on psychological, behavioural, and neural processes. Most of these single-dose administration studies have so far used (1) single-sex samples and (2) varying modes of testosterone administration (intramuscular, transdermal, sublingual, and intranasal) that produced vastly different dose-response curves. Moreover, whereas studies with male participants increased men's testosterone concentrations within the high normal physiological range, studies with women typically increased testosterone concentrations to supraphysiological levels. The purpose of this study was to develop a single-dose administration protocol using intranasal testosterone that would produce a proportionally similar rise in testosterone for both sexes. We found that an 11 mg intranasal testosterone dose in men and a 0.3 mg dose in women raised testosterone concentrations to the high normal physiological range for each sex, producing similar dose-response dynamics in both sexes. This paradigm will allow researchers to design studies with mixed-sex samples that test physiologically plausible sex differences/similarities in the causal effects of testosterone. It will also provide a replicable protocol to examine the possible adaptive functions of acute increases in testosterone in both sexes.

1. Introduction

Testosterone is a key hormone in the human endocrine system, and it affects the physiology and behaviour of both women and men. Individual differences in baseline levels of testosterone are often only weak predictors of individual differences in social behaviours, such as dominance behaviours (see Archer et al., 2005 for a meta-analysis). However, acute increases in testosterone concentrations within the context of social threat and/or competition may be more strongly associated with social behaviours that are relevant to survival and reproduction (e.g., mate-seeking, intrasexual competition, aggression; for a review see Geniole and Carré, 2018). Indeed, theoretical models suggest that such rapid changes in testosterone might be adaptive as they appear to activate behaviours that allow individuals to be successful in specific competitive and/or mating-relevant social contexts, thus potentially increasing the individuals' reproductive fitness (Geniole and Carré, 2018; Zilioli and Bird, 2017). Experiments that manipulate testosterone concentrations are essential to assess testosterone's causal effects and its adaptive functions.

In human social neuroendocrinology, researchers have been using pharmacological challenge studies with a single-dose administration of testosterone to explore whether acute increases in testosterone have a causal effect on various psychological, behavioural, and neural processes (see Bos et al., 2012; Carré and Robinson, 2020 for reviews). These single-dose paradigms were initially developed and tested in healthy young women. Tuiten et al. (2000) were the first to assess the effects of a single, sublingual dose of testosterone (0.5 mg) on vaginal pulse amplitude in women. They reported that serum testosterone concentrations increased rapidly (within 15 min) and substantially (≈2300% above baseline) after drug application. In this work, testosterone administration increased vaginal pulse amplitude, but only 3.5–4 h after drug administration, and well after serum testosterone concentrations returned to baseline (Tuiten et al., 2000).

The relatively long delay with which testosterone modulated vaginal

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pulse amplitude suggests that testosterone's effects on this physiological response may have been modulated via a genomic mechanism of action (see Foradori et al., 2008 for a review of genomic vs. non-genomic mechanisms of steroid hormones). Specifically, the delayed effects on vaginal pulse may be mediated by testosterone binding to androgen receptors (or estrogen receptors after conversion by the enzyme aromatase), and ultimately affecting downstream molecular processes (e.g., gene transcription, translation, and protein formation). Dozens of studies with healthy young women have since used the same experimental paradigm (0.5 mg sublingual testosterone dose) and time lag between drug application and measures of various psychological, behavioural, and neural processes (see Bos et al., 2012 for review of sublingual studies).

More recently, researchers have developed single-dose testosterone administration paradigms for use in young men. In contrast to the almost exclusive use of the sublingual approach in women, single-dose studies with male participants have utilized different administration paradigms, including transdermal, intramuscular, and intranasal applications. Except for intramuscular application (see Dreher et al., 2016), single-dose transdermal and intranasal studies typically lead to a relatively rapid rise in serum testosterone concentrations (within 15–60 min) that remain elevated 3–4 h post-application (e.g., Geniole et al., 2019; Puiu et al., 2019). These studies indicated that a single transdermal (100–150 mg; Puiu et al., 2019) and/or intranasal (11 mg; Geniole et al., 2019) dose of testosterone gel acutely increases serum testosterone to the high-normal physiological range (reference range of testosterone in healthy young men 2.6–9.2 ng/ml; Travison et al., 2017).

Recent work in our lab has revealed that a single dose of testosterone (one study using transdermal and the other intranasal testosterone administration) has a relatively weak positive effect on aggressive behaviour in men (Carré et al., 2017; Geniole et al., 2019). Both studies, however, found that testosterone's potentiation of aggression in men depends upon variability in personality traits. Specifically, testosterone increased aggression, but only in men scoring high in dominance, impulsivity, and/or self-construal (Carré et al., 2017; Geniole et al., 2019). For men scoring low on these traits, testosterone had no effect on aggressive behaviour. Research from other labs has also shown causal effects of a single dose of testosterone on other social behaviours in men (see Carré and Robinson, 2020 for a review).

An important feature of this single-dose testosterone manipulation used for men is that it produces a rise in testosterone that mimics natural changes in testosterone that occur in the context of competitive interactions and/or mating opportunities (see Geniole and Carré, 2018; Gleason et al., 2009). This enables researchers to study the potential functional role of acutely elevated testosterone concentrations. This design feature is quite different from the sublingual approach used primarily in women which produces supraphysiological changes in testosterone that would not be observed in nature.

Therefore, one major limitation in the field of human social neuroendocrinology is that standardized protocols that produce proportionally similar rises in testosterone in women and men have not been developed for mixed-sex samples. This limitation prevents researchers from effectively studying sex differences/similarities in the effects of testosterone on physiological, psychological, and behavioural processes. The studies that have experimentally manipulated testosterone in women have so far used a single dose of testosterone that increased serum testosterone concentrations in women to well above their normal physiological range (see discussion in Carré and Robinson, 2020). To our knowledge, only one study (van Wingen et al., 2009) used intranasal testosterone administration with female participants (0.9 mg dose); this intranasal dose increased women's serum testosterone again to supraphysiological levels (≈400% above baseline testosterone values; reference range for testosterone in women 0.2–0.9 ng/ml; Pesant et al., 2012), but it was a smaller increase compared to the sublingual administration developed by Tuiten et al. (2000).

Our primary aim for this experiment was to develop a pharmacological challenge paradigm that yields a proportionally similar rise in testosterone in men and women. Based on previous work (Geniole et al., 2019), we know that an 11 mg dose of intranasal testosterone gel reliably increases men's serum testosterone concentrations to the high-normal physiological range (men's normal physiological range = 2.6–9.2 ng/ml; Travison et al., 2017) and that this effect should be significant between 15 and 180 min after administration. Here, we develop a pharmacological challenge protocol for use in healthy young women, whereby we test a relatively low dose of intranasal testosterone (0.3 mg) with the goal of increasing women's serum testosterone within the high-normal physiological range. Results from testosterone administration studies in women so far are difficult to interpret due to the supraphysiological increase in testosterone caused by the doses they used. The results from these studies might thus not be relevant for understanding the extent to which physiologically plausible changes in testosterone map onto ecologically and evolutionarily relevant outcomes. The development of this standardized protocol that is biologically appropriate for both sexes will allow researchers to design mixed-sex studies and make possible meaningful comparisons between men and women when assessing testosterone's impact on psychological, behavioural, and neural processes.

1.1. Hypotheses

Based on the reviewed evidence, we proposed the following hypotheses on the effects of a single dose of intranasal testosterone in women (0.3 mg) and men (11 mg):

Hypothesis 1. Serum testosterone concentrations of male participants will be significantly higher after testosterone gel relative to placebo gel at 15, 30, 60, and 120 min after drug application. We expect no baseline differences in testosterone across drug conditions.

Hypothesis 2. Serum testosterone concentrations of female participants will be significantly higher after testosterone gel relative to placebo gel at 15, 30, 60, and 120 min after drug application. We expect no baseline differences in testosterone across drug conditions.

Hypothesis 3. The dose-response curve for intranasal testosterone in healthy young women will be proportionally similar to that in healthy young men (if the 0.3 mg dose increases women's testosterone within high-normal physiological levels, as we expect).

2. Method

2.1. Participants

The sample consisted of 20 healthy individuals (50% female) recruited from a database of research participants who had previously agreed to be contacted for research studies. Participants ranged in age from 20 to 39 years (M = 30.5, SD = 5.31). Their body mass index ranged from 18.1 to 47.0 (M = 26.9, SD = 8.09). All participants self-identified as White/Caucasian and were fluent in English. Participants were not currently receiving medications for medical conditions affecting hormone concentrations, did not have a diagnosed psychiatric disorder (e.g., anxiety, depression, schizophrenia, bipolar disorder), were not pregnant, did not have a drug and/or alcohol dependency, and were not members of teams/organizations (e.g., student-athletes) for whom testosterone is a banned substance. All participants provided informed consent to take part in the study before starting the experiment. They were paid at a rate of $25/h, for a total of $150 ($25 × 3 h × 2 sessions). See the pre-registration for more details about the enrollment processes and inclusion/exclusion criteria. This project received ethics approval from the Nipissing University Research Ethics Board.

2.2. Dosage

A previous study showed that serum testosterone concentration in women increased by more than 400% above baseline levels after a 0.9 mg dose of intranasal testosterone and rapidly modulated threat-related amygdala function (van Wingen et al., 2009). Because we wanted to increase testosterone levels, but within a normal physiological range, we opted for a much lower dose of intranasal testosterone for women. The 0.9 mg dose used by van Wingen et al. (2009) increased serum testosterone levels from approximately 0.18 ng/ml to 0.98 ng/ml (444% above baseline). We speculated that a dose half this size (i.e., 0.45 mg) would increase serum testosterone levels to approximately 0.49 ng/ml (with baseline levels of 0.18 ng/ml), yielding a 172% increase in testosterone concentrations above baseline ([0.49 ng/ml − 0.18 ng/ml] / 0.18 ng/ml * 100). This percentage increase in testosterone is still far above the increase in serum testosterone that we have observed in previous work involving healthy young men (≈60%). Therefore, we opted for a dosage of 0.3 mg, anticipating that this dosage would increase serum testosterone levels to 0.33 ng/ml (with baseline levels of 0.18 ng/ml), thus yielding an 82% increase in serum testosterone concentrations above baseline ([0.33 ng/ml − 0.18 ng/ml] / 0.18 ng/ml * 100). For men, we used an 11 mg dose of intranasal testosterone as this dosage was found to acutely increase testosterone concentrations to the high-normal range (see Geniole et al., 2019).

2.3. Study design

The procedures and experimental design were identical for female and male participants. Using a cross-over, within-subject design (randomized and counterbalanced), participants received intranasal testosterone gel (0.3 mg for women, 11 mg for men) or placebo. Each participant came in for two 3-hour sessions, at least 7 days apart from each other. Approximately half of the participants were tested between the hours of 9 am and 12 pm, and the other half between the hours of 1 pm and 4 pm. In one session a participant was administered testosterone and in the other placebo. The order in which testosterone and placebo were assigned to each participant was random and counterbalanced. Randomization was performed using random.org and ensured that half of the men tested received testosterone on day 1, and half of the women tested received testosterone on day 1. To test whether participants were subjectively aware of the treatment they received, on the second day of testing, participants were asked in which session they thought they received testosterone.

Female participants were asked to first take a urine HCG test to confirm they were not pregnant. Participants had an initial blood draw to assess their baseline serum testosterone levels. Heart rate and blood pressure were also obtained at baseline. Next, participants completed a demographics questionnaire, the Positive and Negative Affect Schedule, and a dot-probe attentional engagement task. The dot-probe task was used for exploratory purposes and hypothesis generation, and thus, results from this task are not reported in the current manuscript. Next, participants self-administered gel from two syringes containing either testosterone or placebo (each syringe contained one-half of the total dose; i.e., 5.5 mg per syringe for men and 0.15 mg per syringe for women). Under the supervision of a research assistant, participants were asked to apply the gel to the lateral sides of their left and right nostrils (using one syringe per nostril) and to then pinch the bridge of their nose to evenly distribute the gel around the nostril walls, where it remained for absorption.

After administration, blood samples, heart rate and blood pressure were collected at 15, 30, 60, and 120 min post-administration to track changes in testosterone concentrations and cardiovascular function. Participants repeated the mood questionnaire and dot-probe task for a second time after the 60-minute measurements, and finally for a third time after the 120-minute measurements. Participants returned to repeat the protocol one week after the first experimental session. Neither the participants nor the experimenters who ran the study were aware of the experimental treatment participants were randomly assigned to in each session (double-blind design). Data collection occurred between November and December 2020. All data were collected at a medical office by trained staff from a clinical trials research team in Northern Ontario. See Fig. 1 for a flowchart of the experimental design and timeline.

2.4. Serum testosterone assay

Blood samples (10 ml per sample) were drawn by a licensed phlebotomist, allowed to clot, and then centrifuged at 3000 rpm to allow for the extraction of serum. The serum was then stored at −20 °C until assayed, in duplicate, using commercially available enzyme immunoassay kits from DRG International (EIA1559, Springfield Township, NJ). The intra- and inter-assays coefficients of variation were 5.41% and 10.14%, respectively.

2.5. Demographics questionnaire

Before drug administration, participants were asked to report their sex, age, weight (in pounds), height (in feet and inches), ethnic/cultural heritage, sexual orientation, highest level of education, whether they were taking any medications, whether they used any recreational drugs, whether they smoked, whether they were in a relationship and how long the relationship was (to see the list of demographics questions, see the pre-registration).

2.6. Positive and Negative Affect Schedule

Participants completed the 20-item PANAS questionnaire (Watson et al., 1988) before drug administration and 60 and 120 min after administration. The PANAS questionnaire consisted of 10 positively valenced items (e.g., excited, strong, enthusiastic) and 10 negatively valenced items (e.g., hostile, scared, irritable). Participants were asked to indicate the extent they felt each positive or negative emotion on a scale from 1 to 5 (1 – very slightly or not at all; 2 – a little; 3 – moderately; 4 – quite a bit; 5 – extremely). We then summed the scores on the positively valenced items and divided the sum by 10 to create a composite average Positive Affect score for each participant, and we did the same with the negatively valenced items to create a composite average Negative Affect score. Cronbach's alphas were 0.95 and 0.83 for Positive and Negative Affect, respectively.

2.7. Cardiovascular function

Heart rate and blood pressure were measured prior to each blood draw using standardized, automated blood pressure monitors that record systole and diastole (mmHg) and heart rate (beats/min).

2.8. Data analysis method

The data analysis plan for this project was pre-registered before we analyzed the data (see pre-registration; https://osf.io/r7eth/?view_only=d567c0f2fdfe4d11812afd8fdd25f2a6). A sample size of 10 participants for each sex was sufficient to detect the hypothesized relationships with ~87% power (see pre-registration). We planned to collect 100 serum testosterone measurements from male participants (10 men × 2 sessions × 5 measurements) and 100 measurements from female participants (10 women × 2 sessions × 5 measurements). However, during data collection, one female participant did not come back for the second session, leaving 95 measurements for women. There were missing measurements for two of the male participants, due to difficulty during phlebotomy, leaving 96 measurements for men. In contrast to our pre-registration, we did not exclude these participants from the data analysis, because linear mixed models are robust in handling missing

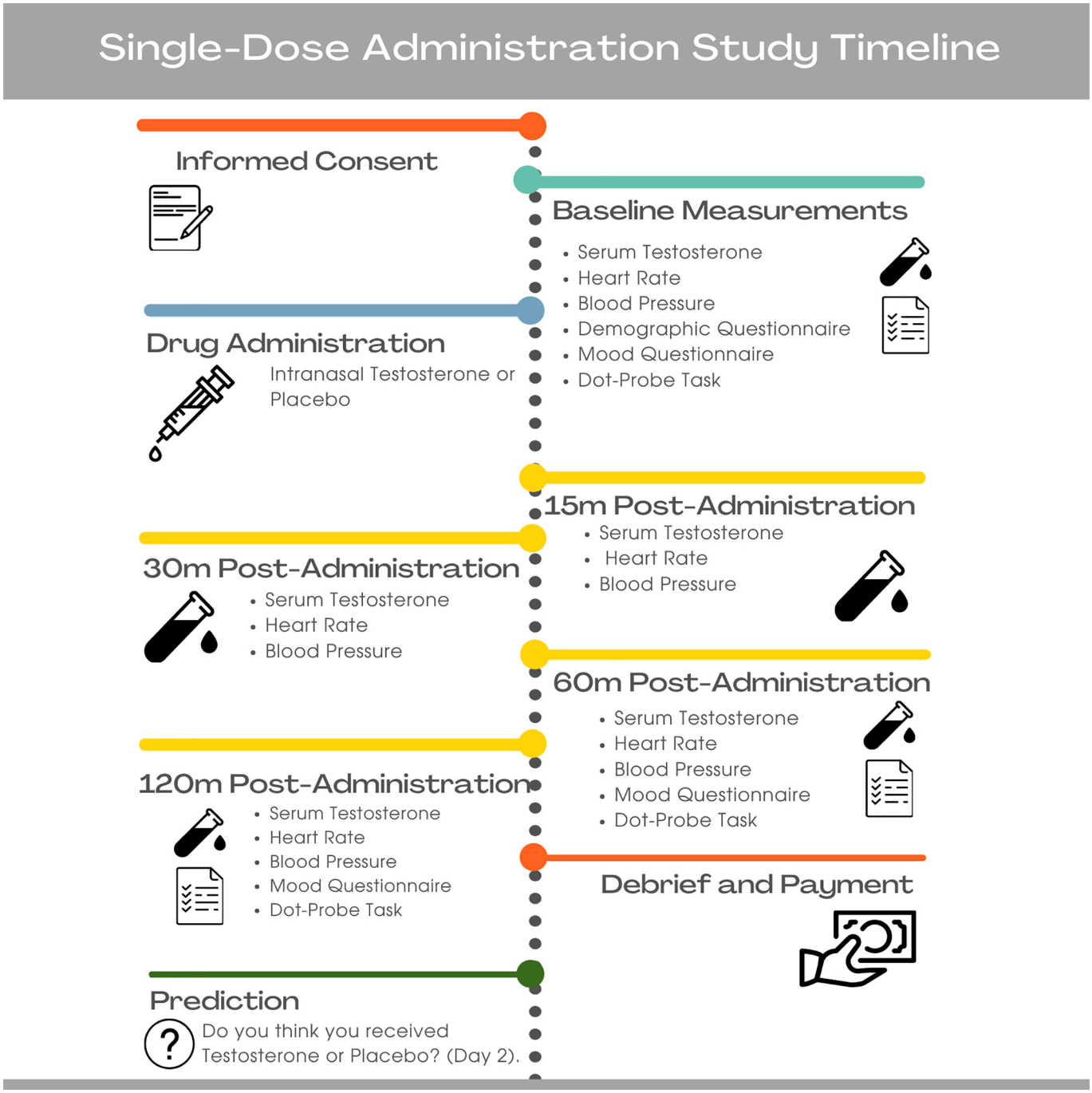

Fig. 1. The flowchart of the experimental design and study timeline.

data and we did not want to decrease the sample size by eliminating these three subjects.

Here, we repeat the description of the data analysis plan outlined in the pre-registration and indicate if the analysis steps deviated from the pre-registration. The data analysis was conducted separately on data from female and male participants, but the analysis steps for each sex were identical. The Drug Type independent variable was dummy coded as a categorical factor with "placebo" used as the control group/reference category (i.e., Placebo was coded as 0 and Testosterone as 1). The Time of Measurement independent variable was also dummy coded as a categorical factor with five levels and "Baseline" was used as the reference category (Baseline was coded as 0, 15 min as 1, 30 min as 2, 60 min as 3, and 120 min as 4). Participant ID was coded as a factor with 10 levels per sex, and unlike what we said in the pre-registration, here IDs 1–10 corresponded to Female Participant 1–Female Participant 10, whereas IDs 20–26 and 28–30 corresponded to Male Participants 1–7, and Male Participants 8–10, respectively (Participant 27 dropped out of the study after the first blood draw because of a vasovagal reaction; we did not keep any of their data). The serum testosterone concentration was the outcome variable for these analyses, and it was a continuous variable. We did not transform it, as the linear mixed models we ran satisfied linear mixed model assumptions (see Results).

We performed all analyses on R version 3.6.3. All figures were made using the ggplot2 (Wickham, 2019) package, version 3.3.0. We used the lme4 (version 1.1-26; Bates et al., 2015) and lmerTest (version 3.1-3; Kuznetsova et al., 2019) packages to run the linear mixed models that tested our hypotheses. First, we compared models with different random effects to find the best fit model to analyze these data. We used the

Akaike Information Criterion (AIC) to select the best fit model (Akaike, 1973). The model with the lowest AIC value was selected as the best fit model and used for further analyses. All the models specified Drug Type, Time of Measurement, and their interaction (Drug Type × Time of Measurement) as fixed effects. Participant ID was included in the model as a random factor. We compared models that differed in the random effects: (1) Model 1 accounted for random intercepts for each participant (i.e., each Participant ID); (2) Model 2 accounted for random intercepts for each participant and random slopes for the Drug Type predictor for each participant; (3) Model 3 accounted for random intercepts for each participant and random slopes for the Time of Measurement predictor for each participant; and (4) Model 4 accounted for random intercepts for each participant, and random slopes for both the Drug Type and Time of Measurement predictors for each participant.

Once we found the best fit model, we used Satterthwaite's method through the lmerTest package to test the statistical significance of each fixed effect estimate. We then calculated the estimated marginal means (EMMs)—and their standard errors (SEs) and 95% confidence intervals—for serum testosterone concentrations for each Drug and each Time of Measurement predicted by the best fit model. We also calculated pairwise contrasts for each Time of Measurement, separately for either Drug (Placebo or Testosterone). This analysis tested whether serum testosterone concentrations were significantly higher than baseline levels at 15, 30, 60, and 120 min after testosterone, but not placebo, administration (and whether there were significant differences in serum testosterone concentrations between any other two Time of Measurement levels for either Drug).

We then calculated contrasts for Drug Type, separately for each Time of Measurement. This analysis tested whether serum testosterone concentrations were significantly higher for testosterone gel than placebo gel at 15, 30, 60, and 120 min after drug administration, but not at Baseline (the key contrasts that tested Hypotheses 1 and 2). The emmeans function from the emmeans package (version 1.5.2-1; Lenth et al., 2018), which we used to calculate the EMMs and the contrasts, uses the Tukey method to adjust *p*-values for multiple comparisons. Finally, we also used the emmeans package to calculate polynomial contrasts to establish whether linear, quadratic, cubic, or quartic contrasts significantly predicted the serum testosterone dose-response curve. We used the standard $p < 0.05$ cut-off value to determine statistical significance for each test. For the main linear mixed models, we did not adjust for multiple comparisons, but we reported all tests conducted to ensure transparency.

As a robustness check, we repeated all described analysis steps including "Time of Day" as a random factor in the linear mixed models. Testosterone levels are normally higher in the morning vs. the afternoon (Dabbs, 1990), so data from participants tested in the morning and data from participants tested in the afternoon might be more like each other than data from participants across the two "Time of Day" groups. In the pre-registration, we said that the Time of Day variable would be dummy coded as 0 for the AM group and 1 for the PM group. However, during the data collection, Participant 9's measurements were taken in the afternoon for their first session and in the morning for their second session. Thus, we dummy coded the variable as 1 for participants who were tested in the morning for both sessions, 2 for participants who were tested in the afternoon for both sessions, and 3 for Participant 9. We compared 16 models that differed in their random effects to find the best fit linear mixed model for our data (see pre-registration for a complete list of the 16 models). Once we found the best fit model, analysis steps were identical as those previously described for the analysis without the Time of Day random factor.

In addition to these confirmatory analyses, we also compared men's and women's dose-response curves (see the Exploratory analyses section of the pre-registration). We suggested that the dose-response curve of women would not be significantly different from that of men in terms of proportional change in serum testosterone concentrations compared to baseline levels after testosterone administration. Because in raw serum testosterone units, both baseline values and number of units of change were larger for men than for women, we standardized the data within sex to compare the testosterone response in women to that in men, as described in our pre-registration. Our first step was to calculate, for each sex, the mean and standard deviation for baseline serum testosterone measurements on the day testosterone gel was administered. Then, each individual serum testosterone measurement from each participant at each time of measurement was standardized using the following formulas:

$$zscore_{iF} = \frac{x_{iF} - M_F}{SD_F}$$

$$zscore_{iM} = \frac{x_{iM} - M_M}{SD_M}$$

M_F (M_M) and SD_F (SD_M) were the mean and SD for the baseline serum testosterone measurements (10, one per participant) on the day testosterone gel was administered for women (men), x_{iF} (x_{iM}) the raw serum testosterone measurements for each woman (man) at each time point (10 participants × 5 time points, for a total of 50 measurements), and $zscore_{iF}$ ($zscore_{iM}$) the corresponding standardized measurements for women (men). We note that this standardization method pins the mean for the baseline values in the testosterone treatment at 0 for both women and men. After standardization, we ran a linear mixed model to examine whether the standardized means of the serum testosterone measurements at each time point after testosterone gel administration were significantly different for men and women.

The linear mixed model for this analysis step specified Participant Sex, Time of Measurement, and their interaction (Sex × Time of Measurement) as fixed effects, and Participant ID as the random effect (i.e., the model accounted for random intercepts for each participant; we also ran a model that, in addition to random intercepts for each participant, accounted for random slopes for the Time of Measurement predictor for each participant. However, the model did not compute, suggesting that the model that only accounted for random intercepts was a better fit). We again used the lme4 and lmerTest packages to run this model, and the emmeans package to calculate the EMMs and the pairwise and polynomial contrasts for this model. We were particularly interested in the pairwise contrasts that tested whether at each Time of Measurement, the standardized serum testosterone concentration for men was significantly different from the standardized serum testosterone concentration for women.

3. Results

The data and code used for this analysis can be found at https://osf.io/r7eth/?view_only=d567c0f2fdfe4d11812afd8fdd25f2a6. Nine participants out of 19 (one of the women did not show up for her second session, so she never guessed in which session she received testosterone) correctly guessed the day in which they were given testosterone (the probability of success in guessing the day correctly was 47.37%). This number of correct guesses was not significantly different from the number of correct guesses expected by chance (the *p*-value from a binomial test was 1).

For both women and men, Model 2 was the best fit model for the data (see Table 1). This result suggested that, other than accounting for

Table 1
AIC values for each linear mixed model testing our hypothesized relationships for each sex. The model with the lowest AIC (in bold) for each sex is the best fit model for that sex.

	AIC (women)	AIC (men)
Model 1	103.83	367.68
Model 2	**99.14**	**358.98**
Model 3	123.08	392.21
Model 4	111.77	381.49

random intercepts for each participant, we also had to specify random slopes for Drug Type, indicating that testosterone affected each participant slightly differently. We included the equation for the best fit linear mixed model for women and men (Model 2) below. Model specifications were identical for women and men:

$$serum\ T = \beta_0 + \beta_1 drug_T + \beta_2 time_{15} + \beta_3 time_{30} + \beta_4 time_{60} + \beta_5 time_{120} + \beta_6 drug_T \times time_{15} + \beta_7 drug_T \times time_{30} + \beta_8 drug_T \times time_{60} + \beta_9 drug_T \times time_{120} + b_0 + b_1 drug_T + e$$

where 'β_0' was the intercept, 'β_1–β_9' the regression coefficients (i.e., slopes) for each fixed effect, 'b_0' the error for the intercept at the Participant ID level (i.e., the model term that represented random intercepts for each participant), 'b_1' the error for the slopes of the Drug Type fixed effect at the Participant ID level (i.e., the model term that represented random slopes for Drug Type for each participant), and 'e' the residual error of the overall model at the individual measurement level. In the formula, Drug Type was represented by $drug_T$ (the Testosterone group of this categorical factor) and not $drug_P$ (Placebo), and Time of Measurement by $time_{15}$, $time_{30}$, $time_{60}$, and $time_{120}$, but not $time_{baseline}$, because (as described in the previous section) Placebo and Baseline were the reference categories for these fixed factors (see also Table 2, presenting the output results from this model for women and men).

Results from Model 2 for both women and men showed that the Drug Type and Time of Measurement fixed effect estimates were not statistically significant, but the fixed effect estimates for their interaction (Drug Type × Time of Measurement) were. These results suggested that, for men, testosterone and placebo had a significantly different effect on serum testosterone concentrations 15, 30, 60, and 120 min after gel administration. For women, the results indicated that testosterone and placebo had a significantly different effect on serum testosterone concentrations 15, 30, 60, but not 120 min after gel administration (see Table 2).

Pairwise contrasts between each pair of Times of Measurement for each Drug Type showed that there was no significant difference in serum testosterone concentration over time when women were administered placebo. However, when women were administered testosterone, the serum testosterone concentration at Baseline was significantly lower than the serum testosterone concentrations 30 min (*estimate* = −0.54, *SE* = 0.12, *df* = 68, *t-ratio* = −4.32, $p < 0.001$) and 60 min (*estimate* = −0.49, *SE* = 0.12, *df* = 68, *t-ratio* = −3.97, $p = 0.002$) after drug

Table 2
The results of the best fit model for women (Model 2) and men (Model 2). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Model 2 (women)					
	Fixed effects				
	Estimate	SE	df	*t*	*p*
(Intercept)	0.62	0.11	37.42	5.73	<0.001***
Drug (testosterone)	−0.01	0.15	36.40	−0.05	0.959
Time (15 min)	−0.04	0.13	68.00	−0.32	0.753
Time (30 min)	0.00	0.13	68.00	0.03	0.975
Time (60 min)	−0.01	0.13	68.00	−0.07	0.943
Time (120 min)	0.01	0.13	68.00	0.08	0.933
Drug (testosterone) * Time (15 min)	0.39	0.18	68.00	2.14	0.036*
Drug (testosterone) * Time (30 min)	0.53	0.18	68.00	2.95	0.004**
Drug (testosterone) * Time (60 min)	0.50	0.18	68.00	2.78	0.007**
Drug (testosterone) * Time (120 min)	0.10	0.18	68.00	0.56	0.575

Model 2 (women)			
	Random effects		
		Variance	SD
ID	(Intercept)	0.03	0.17
	Drug (testosterone)	0.07	0.26
Residual		0.08	0.28

Model 2 (men)					
	Fixed effects				
	Estimate	SE	df	*t*	*p*
(Intercept)	4.20	0.74	13.30	5.64	<0.001***
Drug (testosterone)	0.22	0.65	28.61	0.34	0.735
Time (15 min)	−0.04	0.50	68.22	−0.07	0.944
Time (30 min)	0.02	0.52	68.67	0.03	0.975
Time (60 min)	0.17	0.52	68.67	0.33	0.746
Time (120 min)	0.06	0.52	68.51	0.11	0.912
Drug (testosterone) * Time (15 min)	2.56	0.72	68.33	3.56	<0.001***
Drug (testosterone) * Time (30 min)	3.36	0.72	68.45	4.67	<0.001***
Drug (testosterone) * Time (60 min)	3.23	0.72	68.45	4.48	<0.001***
Drug (testosterone) * Time (120 min)	1.99	0.72	68.37	2.77	0.007**

Model 2 (men)			
	Random effects		
		Variance	SD
ID	(Intercept)	4.29	2.07
	Drug (testosterone)	1.76	1.33
Residual		1.26	1.12

administration; also, the serum testosterone concentrations 30 min (*estimate* = 0.42, *SE* = 0.12, *df* = 68, *t-ratio* = 3.41, $p = 0.009$) and 60 min (*estimate* = 0.38, *SE* = 0.12, *df* = 68, *t-ratio* = 3.06, $p = 0.025$) after drug administration were both significantly higher than the serum testosterone concentration 120 min after drug administration. Polynomial contrasts for the dose-response curve for women indicated that no contrasts were significant for the placebo curve, but for the testosterone curve, the quadratic contrast was significant (*estimate* = −1.68, *SE* = 0.33, *df* = 68, *t-ratio* = −5.13, $p < 0.001$), suggesting that the curve follows a negative quadratic trajectory (see Fig. 2).

Pairwise contrasts between the two Drug Types for each Time of Measurement showed that serum testosterone concentrations for women were significantly lower when women were given placebo rather than testosterone 15 min (*estimate* = −0.38, *SE* = 0.15, *df* = 36.8, *t-ratio* = −2.46, $p = 0.019$), 30 min (*estimate* = −0.52, *SE* = 0.15, *df* = 36.8, *t-ratio* = −3.41, $p = 0.002$), and 60 min (*estimate* = −0.49, *SE* = 0.15, *df* = 36.8, *t-ratio* = −3.22, $p = 0.003$), but not 120 min (*estimate* = −0.09, *SE* = 0.15, *df* = 36.8, *t-ratio* = −0.61, $p = 0.546$), after drug administration (and not at Baseline; *estimate* = 0.01, *SE* = 0.15, *df* = 36.8, *t-ratio* = 0.05, $p = 0.959$).

Pairwise contrasts between each pair of Times of Measurement for each Drug Type showed that there was no significant difference in serum testosterone concentration over time when men were administered placebo. However, when men were administered testosterone, the serum testosterone concentration at Baseline was significantly lower than the serum testosterone concentrations 15 min (*estimate* = −2.53, *SE* = 0.52, *df* = 68.2, *t-ratio* = −4.88, $p < 0.001$), 30 min (*estimate* = −3.38, *SE* = 0.50, *df* = 68, *t-ratio* = −6.75, $p < 0.001$), 60 min (*estimate* = −3.40, *SE* = 0.50, *df* = 68, *t-ratio* = −6.78, $p < 0.001$), and 120 min (*estimate* = −2.05, *SE* = 0.50, *df* = 68, *t-ratio* = −4.09, $p = 0.001$) after drug administration. Polynomial contrasts for the dose-response curve for men indicated that no contrasts were significant for the placebo curve, but for the testosterone curve, the linear (*estimate* = 4.97, *SE* = 1.13, *df* = 68.1, *t-ratio* = 4.40, $p < 0.001$) and quadratic (*estimate* = −8.58, *SE* = 1.33, *df* = 68, *t-ratio* = −6.44, $p < 0.001$) contrasts were significant, suggesting that both a positive linear and a negative quadratic trajectory can describe the curve (see Fig. 3).

Pairwise contrasts between the two Drug Types for each Time of Measurement showed that serum testosterone concentrations for men were significantly lower when men were given placebo rather than testosterone 15 min (*estimate* = −2.79, *SE* = 0.67, *df* = 30.2, *t-ratio* = −4.18, $p < 0.001$), 30 min (*estimate* = −3.59, *SE* = 0.67, *df* = 30.2, *t-ratio* = −5.37, $p < 0.001$), 60 min (*estimate* = −3.45, *SE* = 0.67, *df* = 30.2, *t-ratio* = −5.17, $p < 0.001$), and 120 min (*estimate* = −2.22, *SE* = 0.67, *df* = 30.2, *t-ratio* = −3.32, $p = 0.002$) after drug administration (but not at Baseline; *estimate* = −0.22, *SE* = 0.65, *df* = 28.4, *t-ratio* = −0.34, $p = 0.735$).

We provide complete outputs for the pairwise and polynomial contrasts for both women and men in the Supplementary materials (Part A). As mentioned in the previous section, to make sure these results were robust to the time of the day when the testosterone measurements were taken (morning or afternoon), we re-ran these analyses for women and men adding Time of Day as a random effect in the linear mixed models. These additional analyses did not significantly alter the findings, see Supplementary materials (Part B).

Table 3 shows the results of the best fit linear mixed model that tested for significant differences in the effects of testosterone in women vs. men, after we standardized the serum testosterone concentrations within sex at each time of measurement (as detailed in the previous section). Below, we reported the equation for this model:

$$\begin{aligned} standardized\ serum\ T = {} & \beta_0 + \beta_1 sex_{male} + \beta_2 time_{15} + \beta_3 time_{30} + \beta_4 time_{60} \\ & + \beta_5 time_{120} + \beta_6 sex_{male} \times time_{15} + \beta_7 sex_{male} \\ & \times time_{30} + \beta_8 sex_{male} \times time_{60} + \beta_9 sex_{male} \\ & \times time_{120} + b_0 + e \end{aligned}$$

where 'β_0' was the intercept, 'β_1–β_9' the regression coefficients (i.e., slopes) for each fixed effect, 'b_0' the error for the intercept at the Participant ID level (i.e., the model term that represented random intercepts for each participant), and 'e' the residual error of the overall model at the individual measurement level. Participant Sex was represented by sex_{male} and not sex_{female}, and Time of Measurement by $time_{15}$, $time_{30}$, $time_{60}$, and $time_{120}$, but not $time_{baseline}$, because Female and Baseline were the reference categories for these fixed factors (see also Table 3, presenting the output results from this model).

The results for the fixed effect estimates of this model show that the

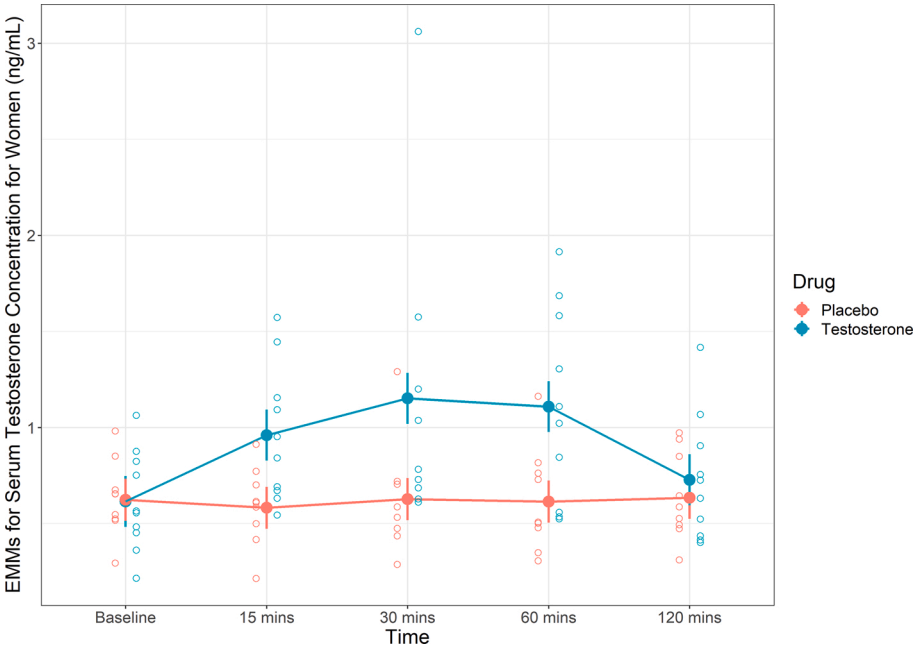

Fig. 2. The estimated marginal means (filled dots) and their standard errors (error bars) for serum T concentrations based on the linear mixed model for women. Empty dots represent each individual serum T concentration measurement for each woman at each Time for either Drug (raw data). The *x*-axis shows each Time of Measurement and the colors represent each Drug Type.

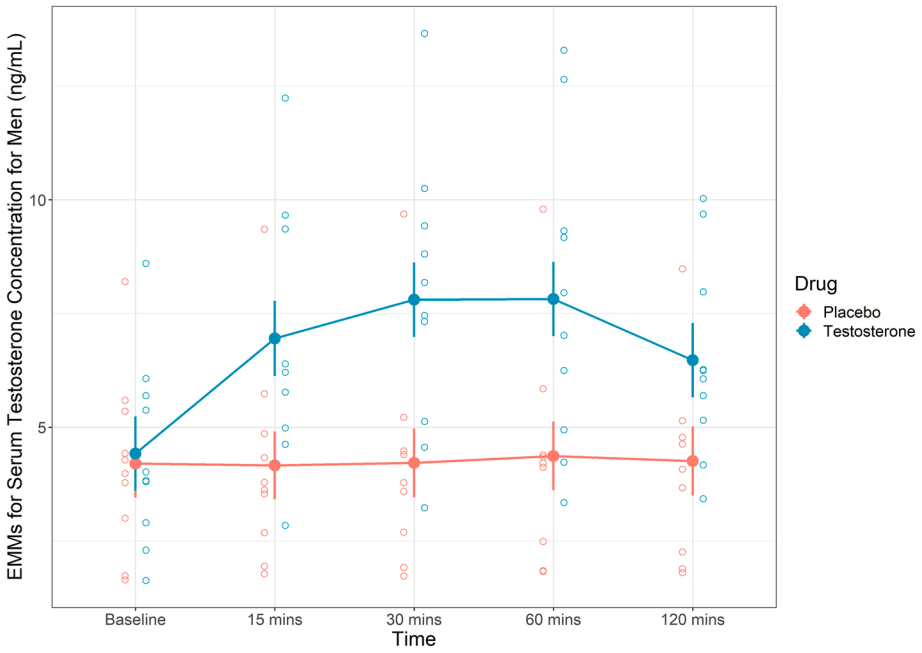

Fig. 3. The estimated marginal means (filled dots) and their standard errors (error bars) for serum T concentrations based on the linear mixed model for men. Empty dots represent each individual serum T concentration measurement for each man at each Time for either Drug (raw data). The *x*-axis shows each Time of Measurement and the colors represent each Drug Type.

Table 3
The results of the best fit linear mixed model that tested for sex differences in the effects of testosterone (comparing the dose-response curves of women and men). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

	Fixed effects				
	Estimate	SE	df	*t*	*p*
(Intercept)	0.00	0.50	44.93	0.00	1.000
Sex (male)	−0.00	0.71	44.93	0.00	1.000
Time (15 min)	1.33	0.50	71.08	2.64	0.010*
Time (30 min)	2.07	0.50	71.08	4.11	<0.001***
Time (60 min)	1.90	0.50	71.08	3.78	<0.001***
Time (120 min)	0.43	0.50	71.08	0.86	0.391
Sex (male) * Time (15 min)	−0.11	0.72	71.26	−0.15	0.881
Sex (male) * Time (30 min)	−0.43	0.71	71.08	−0.60	0.550
Sex (male) * Time (60 min)	−0.25	0.71	71.08	−0.36	0.722
Sex (male) * Time (120 min)	0.56	0.71	71.08	0.79	0.434

	Random effects		
		Variance	SD
ID	(Intercept)	1.27	1.13
Residual		1.27	1.13

only significant estimates were those of Time, suggesting that for women (the reference category), serum testosterone concentrations 15, 30, and 60 min after testosterone administration were significantly higher than the serum testosterone concentration at Baseline. The fact that the estimates of the Sex × Time interaction were not significant suggested that the effect of testosterone over time was not significantly different for men and women.

Fig. 4 shows the EMMs and SEs for standardized serum testosterone concentrations for women and men after testosterone administration based on this linear mixed model. Like the fixed effect estimates from the linear mixed model, pairwise contrasts showed that at each Time of Measurement, the standardized serum testosterone concentrations of women and men were not significantly different from each other (e.g., 60 min after testosterone administration, the standardized serum testosterone concentration of women was not significantly higher than that of men: *estimate* = 0.25, *SE* = 0.71, *df* = 44.8, *t-ratio* = 0.36, $p = 0.723$; see the Supplementary materials, Part C, for a complete output of all contrasts).

These results suggested that a 0.3 mg dose of intranasal testosterone increases serum testosterone concentration in women in the same proportion that an 11 mg dose increases serum testosterone concentration in men over the course of 120 min after testosterone administration. In fact, based on the EMMs from the best fit model for women, women's testosterone concentration increased by 56% ([0.961 ng/ml − 0.616 ng/ml] / 0.616 ng/ml * 100), 87% ([1.152 ng/ml − 0.616 ng/ml] / 0.616 ng/ml * 100), 80% ([1.109 ng/ml − 0.616 ng/ml] / 0.616 ng/ml * 100), and 18% ([0.728 ng/ml − 0.616 ng/ml] / 0.616 ng/ml * 100), at 15, 30, 60 and 120 min after testosterone administration, respectively. This percentage increase was very similar to that observed in men. Based on the EMMs from the best fit model for men, men's testosterone concentration increased by 57% ([6.95 ng/ml − 4.42 ng/ml] / 4.42 ng/ml * 100), 76% ([7.80 ng/ml − 4.42 ng/ml] / 4.42 ng/ml * 100), 77% ([7.82 ng/ml − 4.42 ng/ml] / 4.42 ng/ml * 100), and 46% ([6.47 ng/ml − 4.42 ng/ml] / 4.42 ng/ml * 100), at 15, 30, 60 and 120 min after testosterone administration, respectively.

We used the LMERConvenienceFunctions package (version 3.0; Tremblay, 2020) to make plots of the models' residuals and the DHARMa package (version 0.4.0; Hartig, 2021) to test whether the residuals met model assumptions. Despite the presence of some outliers, models' assumptions were largely met. Based on tests from the DHARMa package for the three best fit linear mixed models described here (Tables 2 and 3), the number of residuals' outliers were not significantly higher than expected, the residuals' distribution was not significantly different from that expected from the fitted models, and the residuals were not significantly over- or under-dispersed. Therefore, the dependent variable (raw or standardized serum testosterone concentration) was not transformed for any of these three models.

See the Supplementary materials for equivalence tests run on the standardized serum testosterone EMMs of women and men (Part D).

3.1. Results for mood, heart rate, and blood pressure

We included a detailed report of the method and results for analyses

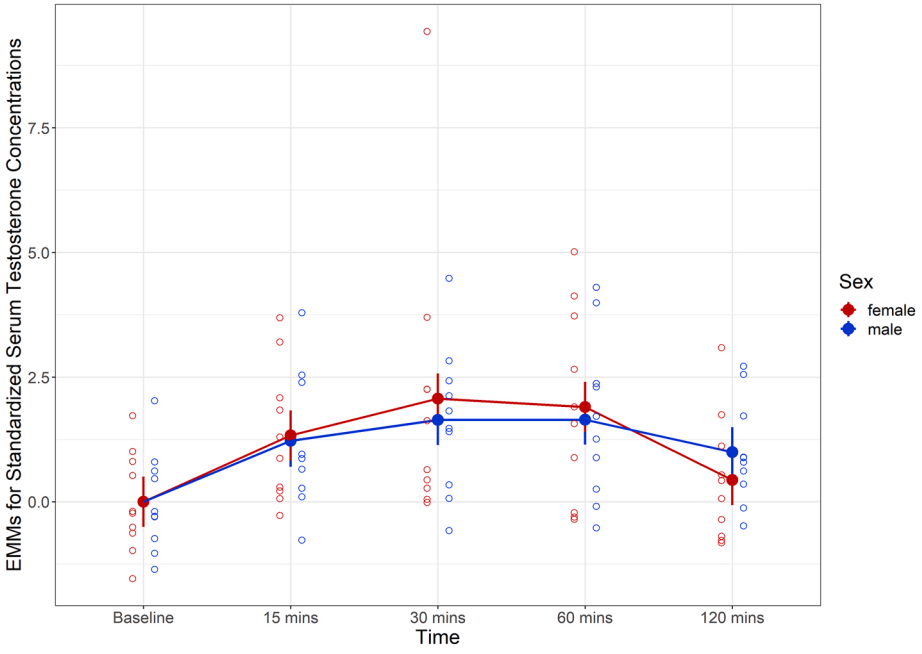

Fig. 4. The estimated marginal means (filled dots) and their standard errors (error bars) for standardized serum T concentrations after T administration based on the linear mixed model comparing men and women. Empty dots represent each individual standardized serum T concentration for each participant at each Time of Measurement for either Sex (raw data). The *x*-axis shows each Time of Measurement and the colors represent each Sex. As an example, a value of 1.33 on the *y*-axis (the EMM for women 15 min after T administration) means that the EMM for women at that Time of Measurement is 1.33 SDs above the mean of the baseline measurements for women on the day T was administered.

testing the effects of testosterone on heart rate, blood pressure, and mood in the Supplementary materials (Parts E–I). Here, we only briefly summarized key results for these dependent variables. Testosterone did not have a significantly different effect than placebo after baseline on heart rate, diastolic blood pressure, positive affect, and negative affect (i.e., there was no significant main effect of Drug Type, nor any significant Drug Type × Time of Measurement interactions for these dependent variables). We instead found a significant Drug Type × Time of Measurement × Participant Sex interaction for systolic blood pressure. Testosterone, compared to placebo, might decrease systolic blood pressure only for men (there was no significant effect of testosterone for women). Specifically, 30 min after drug administration, systolic blood pressure was significantly lower when men were given testosterone than when they were given placebo. We also found a significant main effect of Time of Measurement on diastolic blood pressure and negative affect. Upon examination of pairwise contrasts, we found some evidence that diastolic blood pressure increased over time, and negative affect decreased over time. We did not find convincing evidence that these time effects on diastolic blood pressure and negative affect were significantly different between the testosterone and placebo drug conditions.

4. Discussion

In the current study, we aimed to develop a pharmacological challenge paradigm that produced a proportionally similar testosterone response in men and women that was within the normal physiological range for each sex. Results indicated that a lower dose of intranasal testosterone gel (0.3 mg) administered to women produced a rapid and highly similar rise in serum testosterone compared to the rise in serum testosterone caused by a higher dose (11 mg) administered to men. Hypotheses 1–3 were all supported, except that for women, the serum testosterone concentration 120 min post drug administration in the testosterone treatment was not significantly higher than that in the placebo treatment at the same time of measurement. These findings are important as they set the stage for future larger-scale studies examining sex similarities/differences in the effects of testosterone on various psychological and behavioural processes.

Over the past few decades, single-dose testosterone administration paradigms have been developed for use in human experimental work. These paradigms have utilized different modes of administration (e.g., sublingual, transdermal, intramuscular, intranasal), dosages, and time lags from drug administration to assessment of outcome measures (see Carré and Robinson, 2020). Most of the work in women has focused on using the sublingual approach which rapidly increases serum testosterone levels to concentrations that are well above the normal physiological range (Tuiten et al., 2000). In contrast, pharmacological challenge studies in men have primarily utilized transdermal and intranasal delivery approaches that produce relatively rapid rises in serum testosterone levels to the high-normal physiological range.

Research on the effects of testosterone on various outcome measures has typically been conducted in either men or women. At times, some of these separate studies have provided convergent results. For instance, in women, a single dose of sublingual (Hermans et al., 2008) and intranasal (van Wingen et al., 2009) testosterone increased amygdala reactivity to facial signals of threat (e.g., angry and fearful faces). In men, a single dose of transdermal testosterone also increased amygdala responses to angry facial expressions (Goetz et al., 2014). In other work, some effects were found in women, but not men. For instance, van Honk et al. (2011) reported that the administration of testosterone reduced cognitive empathy performance in women. In contrast, no main effects of transdermal or intranasal testosterone on cognitive empathy performance were found in young men (Carré et al., 2015; Nadler et al., 2019).

To our knowledge, only one study has included both men and women in a single-dose testosterone paradigm aimed at investigating the effects of testosterone on economic decision-making. Specifically, Kopsida et al. (2016) administered 60 mg of transdermal testosterone (or placebo) to a sample of men and women (n = 68) and examined whether their manipulation influenced participants' willingness to accept unfair financial offers in the Ultimatum Game. The authors reported a relatively weak (and not statistically significant) effect whereby individuals receiving testosterone were more willing to accept unfair offers, and this effect was not moderated by participant sex. Notably, the small sample utilized in this study was underpowered for detecting statistical interactions. Furthermore, an important limitation of this work is that Kopsida et al.'s (2016) testosterone manipulation produced drastically different testosterone responses in men and women. For men, there was a 120% increase in serum testosterone concentrations, with levels increasing to the high-normal physiological range (10 ng/ml). For women, there was a 5800% increase in testosterone concentrations, with

levels increasing to well beyond the normal physiological range typically observed in women (the testosterone levels increased to >13 ng/ml).

Although paradigms that have increased testosterone to supra-physiological concentrations can clearly establish a causal role of testosterone, such findings can be difficult to interpret given that the concentrations observed are far beyond what a person would experience under naturally relevant conditions. Indeed, as noted by Quispe et al. (2015) on p. 101 "[n]atural and sexual selection can only act on phenotypes that are realized in nature. This means that only hormone manipulations within the existing physiological range can mimic existing conditions on which selection could act on". If the goal is to determine the extent to which context-dependent changes in testosterone play a causal role in modulating ongoing and/or future social behaviour (see Geniole and Carré, 2018), manipulations that yield concentrations within the normal physiological range would be most appropriate.

In addition, testosterone's effects on physiology and behaviour may be non-linear, with testosterone having a dose-dependent positive effect within a physiological range, and showing no effect, or even a negative effect beyond that particular range. For example, in vitro (human endothelial cells) and in vivo (female rats) work indicated that testosterone administration had a positive effect on endothelial function, but only if administered within the normal physiological range (Goglia et al., 2010). Also, Cherrier et al. (2007) reported beneficial cognitive effects of testosterone supplementation, but only for men who had moderate (within the normal physiological range) increases in testosterone. In contrast, no beneficial effects were observed for men whose testosterone levels increased beyond the normal physiological range. Collectively, these findings indicate that testosterone's impacts on certain outcome measures may only emerge when administered within the normal, within-sex, physiological range.

It is also important to note that even within the normal physiological range, effects of testosterone may not be dose-dependent, but may follow a step-function or threshold-function (Adkins-Regan, 2005). That is, once the dose is at or above a particular threshold, the measured behaviour will be similar regardless of any further increase in the hormone. For instance, one meta-analysis revealed that testosterone improved sexual function in hypogonadal men, but not in eugonadal men, for whom testosterone levels were already in the normal physiological range (Isidori et al., 2005). Therefore, planning experiments that manipulate testosterone concentrations within normal physiological levels might also shed new light on the specific testosterone concentrations that influence physiological responses or social behaviours and/or the thresholds above which testosterone effects are no longer observed.

Although we were able to produce a similar testosterone response in men and women, the current study does not address the optimal time point for the assessment of outcome measures after administration of testosterone. Studies that have utilized the sublingual approach have used a 3–4.5 hour time lag from testosterone administration to the assessment of physiological, psychological, and behavioural outcomes. The rationale for using this time lag was based on the seminal work of Tuiten et al. (2000) who repeatedly assessed vaginal pulse amplitude to sexual stimuli in women after testosterone administration. In this work, the authors reported peak testosterone levels within 15 min of drug application, and a return to baseline concentrations within 90 min. Nevertheless, changes in vaginal pulse amplitude were not detected until approximately 3–4.5 h after peak testosterone levels were detected, and when testosterone levels were back to baseline concentrations. Dozens of studies in women using the same sublingual manipulation and time lag have found that testosterone modulates various processes (e.g., risk-taking, threat-related neural function, economic decision-making, see Bos et al., 2012 for a review).

Notably, one study in women found that intranasal testosterone administration rapidly (within 45 min) increased amygdala reactivity to angry and fearful facial expressions (van Wingen et al., 2009). Similarly, Goetz et al. (2014) reported that a single dose of transdermal testosterone (100 mg) to young men increased amygdala responses to angry facial expressions approximately 90 min after drug application. These relatively rapid effects are consistent with work in animal models suggesting that steroid hormones can have relatively rapid, perhaps non-genomic effects on outcome measures. For instance, 30 min after receiving a single dose of testosterone (500 μg), male mice began mounting receptive females quicker than male mice administered placebo (James and Nyby, 2002). Similarly, gonadally intact male rats injected with testosterone (100 μg) demonstrated shortened intromission and ejaculation latencies 60 min after injection (Malmnäs, 1977).

Other work in male goldfish indicates that administration of testosterone rapidly (within 30–45 min) increased approach responses towards visual cues of females, an effect that was blocked by the administration of an aromatase inhibitor, suggesting that testosterone's effects on approach-related behaviour was mediated by an estrogen-related mechanism (Lord et al., 2009). Finally, in other work, Pultorak et al. (2015) examined the extent to which administration of testosterone would modulate ultrasonic vocalizations (USVs) to novel females among male California mice. USVs are used as a metric of sexual interest towards novel females. The authors reported that testosterone rapidly decreased USVs among pair bonded males, suggesting that testosterone may promote fidelity by reducing sexual interest towards novel females in this monogamous species. Such rapid, perhaps non-genomic effects of testosterone on behavioural outcomes may involve a number of cellular mechanisms, including binding to membrane-bound androgen receptors or membrane associated receptors/binding proteins, alterations in membrane flexibility, modulation of intra-cellular calcium, and/or activation of second messenger pathways (see Foradori et al., 2008 for a detailed discussion of the various non-genomic mechanisms of action).

It is not a straightforward task to determine the optimal time lag to use between testosterone administration and assessment of outcome measures of interest. One approach is assessing an outcome measure repeatedly over time to determine the time point at which testosterone has the most robust effect (e.g., Tuiten et al., 2000 approach using vaginal pulse amplitude as the outcome measure). Using a physiological measure (e.g., vaginal pulse amplitude or threat-related brain function) may provide optimal resolution for this purpose. Another approach is to use a time lag that coincides with correlational work in which acute changes in testosterone map onto behaviour. Specifically, in two independent studies, we reported that an acute increase in testosterone during a competitive interaction positively predicted subsequent aggressive behaviour in men, but not women (Carré et al., 2013; Carré et al., 2009). In both studies, saliva samples were collected before and after a competitive interaction, and aggressive behaviour was assessed approximately 10–15 min later. To the extent that acute changes in testosterone were playing a causal role in modulating aggressive behaviour, testosterone appeared to be doing so rapidly. Therefore, in our recent pharmacological challenge studies, we measured aggressive behaviour shortly after peak testosterone concentrations were achieved (see Carré et al., 2017; Geniole et al., 2019). Of course, it is possible that acutely elevating testosterone can have both rapid and/or delayed effects, and that this may ultimately depend upon the particular outcome measure assessed.

Experiments that acutely increase testosterone concentrations within the high-normal physiological range in both men and women will be crucial to understand whether the causal (rapid or delayed) effects of testosterone on outcome measures of interest are adaptive for both sexes. As suggested by the Fitness Model of Testosterone Dynamics (Geniole and Carré, 2018), it is acute increases in testosterone triggered by social cues of challenge/competition that might affect fitness-relevant behaviours. In a review of the evidence on the rapid effects of testosterone (which included both correlational and experimental studies), Geniole and Carré (2018) summarized that acute increases in testosterone are especially likely to rapidly potentiate social behaviours like aggression, risk-taking and mate-seeking, and intra-sexual

competition in both women and men. In the context of social challenge and competition, these behaviours can all increase reproductive fitness (or social status, which might then in turn increase fitness). It is only by raising testosterone concentrations to a high natural level in both sexes that we can truly replicate correlational observations, compare effects in men and women, and assess whether testosterone has played a key adaptive role in our evolutionary history.

We also explored potential effects of testosterone on mood, and heart rate and blood pressure. We found that testosterone did not affect men's or women's mood. Previous studies testing the effects of a single dose of testosterone (in either men or women) on several outcome measures similarly reported that testosterone had no effects on mood (e.g., Nave et al., 2018; van Wingen et al., 2008; Wu et al., 2017). Testosterone also did not affect heart rate or diastolic blood pressure, but we found that it might rapidly decrease systolic blood pressure (only 30 min after drug administration) in men.

Early studies with male rats have found that long-term testosterone administration to castrated male rats can increase the rats' systolic blood pressure (Chen and Meng, 1991; Fischer and Swain, 1977). Kienitz and Quinkler (2008) reviewed the literature on the relationship between androgens and blood pressure (with a focus on humans) and found contradictory results. They concluded that while longer-term exposure to testosterone may ultimately lead to an increase in blood pressure through various physiological vasoconstricting mechanisms, short-term acute intracoronary exogenous testosterone administration can promote vasodilation in men with coronary artery disease. In support of these findings on the cardiovascular effects of long-term testosterone exposure, researchers have recently argued that long-term testosterone replacement therapy with the goal of preventing Type II diabetes might lead to cardiovascular problems, including an increase in systolic blood pressure (Sattar et al., 2021; Wittert et al., 2021).

Instead, so far studies examining the effects of single-dose testosterone administration on blood pressure have found mixed results. In one study, intravenous testosterone administration in elderly men with coronary artery disease had no significant effects on systolic blood pressure (White et al., 1999). In another recent single-dose administration study with 120 healthy young men (between-subjects design), men given testosterone (150 mg transdermal), compared to men given placebo, experienced a higher increase in systolic blood pressure in response to a somatic stressor, but a lower increase in systolic blood pressure in response to a social stressor (Kutlikova et al., 2020). These results suggested that the effects of testosterone on systolic blood pressure might be contextual and that testosterone might act to reduce social anxiety (Kutlikova et al., 2020).

Our results contribute novel evidence to the study of the relationship between testosterone and blood pressure, as we found that a single dose of intranasal testosterone gel (11 mg) might rapidly and temporarily decrease men's systolic blood pressure, in line with the short-term testosterone administration cardiovascular effects observed by Kienitz and Quinkler (2008). We note, however, that our results for mood, heart rate, and blood pressure should be interpreted with caution given the small sample size of this experiment (the sample size was sufficient to detect the effect of testosterone on serum testosterone concentration with ~87% power, but it might have been too small for the other dependent variables).

5. Conclusions

Over the past few decades, researchers have developed single-dose testosterone administration paradigms for women and men. However, so far these paradigms have increased testosterone within the high-normal physiological range in men, but to supraphysiological levels in women. This limitation makes it difficult to compare the effects of testosterone across the sexes and to draw conclusions about physiologically plausible effects in women. By using a 0.3 mg intranasal dose of testosterone in women and an 11 mg dose in men, we developed a pharmacological challenge paradigm that produces a proportionally similar increase in testosterone concentrations in both sexes (within the high-normal physiological range for both sexes). This paradigm will allow researchers to design studies with mixed-sex samples that test physiologically plausible sex differences/similarities in the causal effects of testosterone. Through this paradigm, researchers will also be able to examine the possible adaptive functions of acute increases in testosterone in both sexes.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.yhbeh.2021.105046.

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