ORIGINAL ARTICLE

Prenatal Hormone Exposure and Risk for Eating Disorders

A Comparison of Opposite-Sex and Same-Sex Twins

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Context: Although the sex difference in eating disorder prevalence has typically been attributed to psychosocial factors, biological factors may also play a role. Prenatal testosterone exposure is a promising candidate, since it masculinizes behavior in animals and humans via its permanent effects on the central nervous system.

Objective: To examine whether in utero testosterone exposure has masculinizing effects on disordered eating (DE) by comparing opposite-sex (OS) and same-sex (SS) twins. Twin type (SS vs OS) is considered a proxy measure of prenatal hormone exposure, since females from OS pairs are exposed to more testosterone in utero than females from SS pairs. A linear trend in mean levels of DE was predicted based on expected prenatal testosterone exposure, with SS female twins exhibiting the highest levels of DE followed by OS female twins, OS male twins, and SS male twins.

Design: A twin study comparison of OS vs SS twins.

Setting: Michigan State University Twin Registry.

Participants: Participants included 304 SS female twins, 59 OS female twins, 54 OS male twins, and 165 SS male twins.

Main Outcome Measure: Overall levels of DE were assessed with the Minnesota Eating Behavior Survey.

Results: Confirming hypotheses, DE exhibited significant linear trends, with SS female twins exhibiting the highest levels of DE followed by OS female twins, OS male twins, and SS male twins. This linear trend could not be accounted for by levels of anxiety or socialization effects. Indeed, OS female twins exhibited lower levels of DE compared with an independent sample of undergraduate women (n=69) who were raised with 1 or more brothers.

Conclusions: The masculinization of DE in OS female twins is unlikely to be due to socialization effects alone. Biological factors, such as the masculinization of the central nervous system by prenatal testosterone exposure, may also contribute to sex differences in DE prevalence.

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CLEAR SEX DIFFERENCES IN anorexia nervosa and bulimia nervosa exist, in which females outnumber males by about 10:1.[1] Sociocultural differences, such as pressure for thinness in women, have typically been used to explain this sex difference.[2,3] However, biological factors may also play a significant role.[4]

Gonadal hormones (ie, androgens, estrogens, and progestins) are 1 set of biological factors that may be particularly important given their influence on sexually differentiated behavior.[5,6] Indeed, much of human sexual differentiation is thought to occur from organizational effects of the primary androgen, testosterone.[7,8] During prenatal development, testosterone (either directly or through its metabolites dihydrotestosterone and 17 β-estradiol) masculinizes cells throughout the body and the brain.[5] Male genital development is dependent on the presence of testosterone, whereas female genital development occurs in the absence of testosterone.[7] Likewise, while testosterone masculinizes the developing central nervous system (CNS) in males, it is the absence of testosterone that averts such masculinization in females.[8,9]

Animal studies confirm that testosterone's masculinization of the CNS translates into several sexually dimorphic behaviors, including eating behaviors. Animal studies have consistently shown that female rodents adjacent to males in utero later exhibit masculinized physiology (eg, they have later vaginal opening, display greater sensitivity to testosterone), morphology (eg, they have longer anogenital distance), and patterns of behavior (eg, they mate and impregnate later, they are more likely to mount other females and exhibit increased aggression).[10] The magnitude of the masculinization depends on many factors, with fe-

male rodents adjacent to males tending to be intermediate between other female and male rodents on a given trait, rather than completely masculinized.[10] Male rodents adjacent to other males show similar effects (eg, they display greater sensitivity to testosterone, longer anogenital distance, and increased aggression), but the effects are less pronounced than those in females. These masculinized patterns of behavior have been attributed to the prenatal transfer of testosterone from the male pup to the sibling through the mother's bloodstream[11] or amniotic fluid[12,13] because the masculinizing influence of adjacent male fetuses is blocked when mothers are treated with antiandrogens.[14] These findings suggest that variations in testosterone exposure during critical periods of development can permanently alter wide-ranging sexually dimorphic traits.

The masculinizing effects of early testosterone exposure extend to food intake in male and female mammals. In gonadally intact mammals, testosterone increases food intake, which is considered a "masculinized" effect. For example, female rats neonatally treated with testosterone show increased (ie, masculinized) food intake[15,16] and body weight[17] as adults. Ovariectomized female rats treated with androgens show increased food intake and greater sensitivity to the effects of testosterone on feeding.[16] Similarly, male rats castrated on the day of birth show permanent decreases in body weight and decreases in responsiveness to the effects of testosterone on food intake.[16]

Prenatal exposure to testosterone in humans cannot be readily examined or manipulated. Consequently, researchers have studied opposite-sex (OS) twins as a proxy for the masculinizing effects of testosterone on human behavior. Females from OS twin pairs share a prenatal environment with a male co-twin and are thought to be exposed to increased levels of prenatal testosterone.[10] Consistent with expectations, these females exhibit more masculine personality characteristics (eg, increased sensation seeking[18,19] and aggressive behavior[19]) than females from same-sex (SS) twin pairs. Importantly, these differences have not been systematically related to pubertal stage, menarcheal status, or the activational effects of circulating testosterone.[19] Postnatal socialization (ie, being raised with a male co-twin) could conceivably influence these masculinized patterns; however, masculinization has been observed for traits that are unlikely to be socially influenced. For example, females from OS twin pairs exhibit more masculine cerebral lateralization (ie, left-hemisphere dominance when processing verbal-auditory stimuli[20]), spatial ability,[21] otoacoustic emissions,[22] tooth size,[23] and dental asymmetry.[24] Thus, prenatal testosterone exposure likely alters a variety of sexually dimorphic traits in females from OS twin pairs, and importantly, the masculinization of these traits is at least partially independent of psychosocial factors.

As noted earlier, animal studies consistently support the masculinizing effects of prenatal testosterone exposure on feeding behaviors. Given the central role of food intake in all forms of eating disorders, prenatal testosterone seems likely to also influence DE symptoms. Indeed, a recent study found significant associations between increased prenatal testosterone exposure (ie, measured using 2D:4D [second digit:fourth digit] finger-length ratios[25]) and lower levels of DE in adult women.[4] These findings were significant in suggesting that prenatal testosterone exposure may masculinize brain circuits that affect DE in women and serve as a protective factor against eating disorder development. Nonetheless, these data cannot speak to whether prenatal testosterone exposure accounts for sex differences in DE. Studies comparing OS with SS twins might clarify whether sex differences in prenatal testosterone exposure explain sex differences in eating disorder prevalence.

The current study aimed to investigate the potential masculinizing effects of prenatal testosterone exposure on sex differences in DE using the OS twin study design. Disordered eating was compared across 4 groups of twins (ie, females from SS twin pairs, females from OS twin pairs, males from OS twin pairs, males from SS twin pairs) to determine whether levels of DE differed by expected levels of prenatal testosterone exposure. A linear trend for DE was hypothesized, such that females from SS twin pairs would show the highest levels of DE, followed by females from OS twin pairs, males from OS twin pairs, and males from SS twin pairs. Importantly, we also examined potential confounds to these predicted linear relationships. First, we compared levels of anxiety across OS and SS twins. Anxiety is a significant risk factor for DE[26] that has been shown to be influenced by the organizational effects of testosterone.[27] Given these links, we investigated whether our proxy for prenatal testosterone exposure (ie, twin type) demonstrated effects on DE that were independent of its effects on anxiety. In addition, we sought to rule out psychosocial explanations (ie, socialization from growing up with a male co-twin) for masculinization of DE by comparing females from OS twin pairs with nontwin females raised with 1 or more brothers. Females from OS twin pairs were expected to exhibit more masculinized levels of DE than nontwin females who were raised with a male sibling.

METHODS

This study was approved by the Michigan State University (MSU) institutional review board. Written informed consent was obtained after study procedures were explained to all participants.

PARTICIPANTS

Twins

The sample included 582 twins participating in the community-based Michigan State University Twin Registry (MSUTR).[28] Sample sizes by twin type were as follows: 304 (172 monozygotic [MZ] [identical]; 132 dizygotic [DZ] [fraternal]) SS female twins, 59 DZ OS female twins, 54 DZ OS male twins, and 165 (103 MZ; 62 DZ) SS male twins. Sample sizes vary slightly in statistical analyses because of missing data (see "Data Preparation" subsection). Further, the sample size for OS male twins was smaller than that of OS female twins because 5 OS male twins did not complete their assessments.

Since rates of DE vary across ethnic groups,[29] ethnicity was assessed using a demographic questionnaire to ensure that it did not account for differences in DE across groups. The majority of twins self-identified as white (84.4%) and were largely in the middle to upper levels of socioeconomic status (**Table 1**) based on the Hollingshead Index of Social Position.[30] There were

Table 1. Demographic Information for Twin and Nontwin Participants

Demographic Variable	No. (%)		
	All Twins (N=582)	Opposite-Sex Female Twins Only (n=59)	Nontwin Controls (n=69)
Age, y, mean (SD) [range]	20.83 (2.35) [18-29]	20.66 (1.73) [18-28]	20.73 (1.82) [18-27]
Ethnicity			
White	491 (84.4)	53 (89.8)	50 (72.5)
African American	59 (10.1)	4 (6.8)	8 (11.6)
Hispanic	13 (2.2)	1 (1.7)	2 (2.9)
Native American	5 (0.9)	0	0
Asian/Pacific Islander	4 (0.7)	0	5 (7.2)
Other	10 (1.7)	1 (1.7)	4 (5.8)
Socioeconomic status, level[a]			
1	190 (32.6)	20 (33.9)	26 (37.7)
2	254 (43.6)	24 (40.7)	29 (42.0)
3	94 (16.1)	9 (15.2)	12 (17.4)
4	28 (4.8)	5 (8.5)	2 (2.9)
5	17 (2.9)	1 (1.7)	0

[a] Level 1 = major business and professional workers; level 2 = medium business, minor professional, technical workers; level 3 = skilled craftsmen, clerical, sales workers; level 4 = machine operators, semiskilled workers; and level 5 = unskilled laborers, menial service workers.

Table 2. Racial Categories for MSUTR Twins and Individuals From the Recruitment Region[a]

Racial Category	MSUTR Female Twins, %	Females Aged 18-30 y, %	χ^2_1	MSUTR Male Twins, %	Males Aged 18-30 y, %	χ^2_1
White	84.0	81.7	0.14	85.1	79.2	1.22
Black	10.8	16.0	1.07	9.0	16.7	2.83
Asian/Pacific Islander	1.1	1.9	0.34	0	3.4	3.05
Native American	0	0.4	1.01	2.2	0.7	0.34

Abbreviation: MSUTR, Michigan State University Twin Registry.

[a] The recruitment region includes all counties within a 2-hour radius of Michigan State Universities.[28] Data for males and females from the general population come from Michigan census data (see http://www.michigan.gov/mdch). Because Michigan census data only report race and not ethnicity, rates of Hispanic or "other" ancestry could not be obtained. Consequently, MSUTR twins who identified themselves as Hispanic or "other" are not included in the table totals. All P's $>.05$.

no significant differences in ethnicity ($\chi^2_{12}=14.02$; $P=.30$), age ($F_{3,78}=0.86$; $P=.46$), or socioeconomic status ($\chi^2_{12}=15.21$; $P=.23$) between twin types.

The MSUTR used a variety of recruitment methods,[28] including newspaper and e-mail listserve advertisements, recruitment flyers, and recruitment mailings sent to twin pairs identified by university registrars' offices. The majority of the sample (94.2%) was recruited using these methods. Additional twin pairs (5.8%) were recruited later in the study through the use of birth records obtained from the Michigan Department of Community Health. Importantly, MSUTR twins appear to be representative of men and women from the general population of Michigan in terms of racial categorization (**Table 2**). Indeed, there were no significant differences in participant race between MSUTR twins and individuals from the recruitment region (ie, within a 2-hour drive of MSU).

Most twin participants (86.2%) completed the assessments in our MSU laboratory. However, if a twin could not travel to our MSU laboratory, a questionnaire packet was mailed to their home (14% of the sample). Twins who completed mailed assessments had significantly higher levels of DE (mean [SD], 7.90 [6.03]) than those assessed in the laboratory (mean [SD], 6.13 [5.79]; $t_{1,579}=2.46$; $P=.01$). Thus, assessment method was included as a covariate in DE analyses (see "Comparisons of OS and SS Twins" subsection).

Nontwin Controls

The nontwin control sample included 69 undergraduate women with at least 1 brother with whom they were raised. These women were recruited in undergraduate classes and a volunteer research pool at a large Midwestern university. Like the twin sample, the majority of nontwin participants were white (72.5%) and in the middle to upper level income class range (Table 1). Importantly, OS female twins and nontwin participants did not significantly differ in terms of age ($t_{1,125}=0.21$; $P=.84$) or socioeconomic status ($\chi^2_4=3.28$; $P=.51$). However, the nontwin participants showed a trend toward being significantly more ethnically diverse than the OS twin sample ($\chi^2_4=7.61$; $P=.11$). Consequently, ethnicity was included as a covariate in analyses comparing OS twin and nontwin women.

MEASURES

Disordered Eating

The total score from the Minnesota Eating Behavior Survey[2,31,32] (MEBS) was used to assess overall DE, including levels of body dissatisfaction (ie, dissatisfaction with the size and/or shape of one's body), weight preoccupation (ie, preoccupation with dieting, weight, and the pursuit of thinness), binge eating (ie, the tendency to engage in episodes of overeating as well as having attitudes conducive to binge eating), and compensatory behavior (ie, the tendency to use or contemplate using behaviors such as self-induced vomiting and laxatives to control weight). Higher total scores indicate higher levels of pathological eating attitudes and behaviors. Importantly, these types of DE symptoms have shown robust sex differences,[32] with females exhibiting significantly more of these symptoms than males. Previous research has supported

the reliability and validity of this measure in both sexes.[32] The MEBS total score successfully discriminates between girls with clinical eating disorders (ie, anorexia nervosa and bulimia nervosa) and age-matched controls without an eating disorder.[32] In addition, the MEBS factor structure has been replicated across male and female samples.[32]

In the current study, the MEBS total score internal consistency was excellent for females ($\alpha = .90$) and males ($\alpha = .84$). There was also ample variability in MEBS scores, with a significant proportion of SS male twins (1.8%; n = 3), OS male twins (5.6%; n = 3), OS female twins (12.1%; n = 7), SS female twins (18.6%; n = 57), and nontwin females (18.8%; n = 13) scoring higher than the MEBS total score mean (score = 15) for young adult women with anorexia nervosa or bulimia nervosa (K.L.K, M. McGue, PhD, and W. G. Iacono, Minnesota Twin Family Study, unpublished data, 2007).

Anxiety

The State-Trait Anxiety Inventory[33] (STAI) was used to assess trait levels of anxiety (ie, stable or enduring anxiety) in the twin sample only. The trait subscale exhibits expected sex differences, with higher levels of trait anxiety in females.[27] Previous research has also documented its reliability and validity in both sexes.[33] Internal consistency of this subscale was excellent in the current sample (ie, male twins, $\alpha = .88$; female twins, $\alpha = .94$).

Body Mass Index

Body mass index (BMI) (calculated as weight in kilograms divided by height in meters squared) was calculated and used as an indication of adiposity. In the twin sample, height was measured with a wall-mounted scale, and weight was measured with a digital scale. For the nontwins, height and weight were assessed via self-report. Previous research suggests that self-reported height and weight are valid and reliable measures of actual height and weight.[34-36] There were no differences in BMI between OS and SS twins of the same sex. However, because males had higher BMIs than females and BMI was related to the MEBS total score in both sexes (r's = 0.12-0.68; all P's $< .001$), BMI was included as a covariate in all DE analyses.

STATISTICAL ANALYSES

Data Preparation

Missing data were addressed using a prorating system. If 10% or less of the MEBS total score or STAI scale items were missing, then scores were prorated. If more than 10% of the items were missing, the scores were coded as missing. In total, MEBS data were coded as missing for 1 OS female twin (1.7%), and STAI data were coded as missing for 2 OS male twins (3.7%) and 3 SS female twins (1%). The MEBS score exhibited a positively skewed distribution and was therefore log transformed prior to analyses. A P value of .05 was used for all analyses.

Comparisons of OS and SS Twins

Hierarchical linear models (HLMs) were used to examine mean differences in DE and anxiety across OS and SS twins. Hierarchical linear models are an extension of the general linear model where factors are assumed to have a linear relationship with the dependent variable. Hierarchical linear models are ideal for examining the hypotheses in this study because the nonindependence of the twin data can be accounted for by nesting a level 1 variable within a level 2 unit. In the case of twins, the individual twins are the level 1 unit, while the twin pair (identified by a "family" variable that is shared by co-twins) is the level 2 unit.

Two categorical predictors were used in the HLM analyses: twin sex (male or female) and co-twin's sex (male or female). Each level of these predictor variables could have a different linear effect on the dependent variable (eg, DE). A significant effect of prenatal testosterone exposure in these analyses would be suggested if there was a main effect for co-twin's sex (eg, levels of DE differ significantly as a function of having a male or female co-twin). This statistical model has been referred to previously as the Actor-Partner Interdependence Model.[37,38]

Separate HLMs were conducted for DE and anxiety. An additional HLM mediation model, following the Baron and Kenny[39] method, was then run with co-twin's sex as the predictor, trait anxiety as the mediator, and twin sex, BMI, and assessment method as covariates. This model examined whether anxiety mediates the association between prenatal testosterone exposure (measured via co-twin's sex) and DE.

Following recommendations,[37,38] categorical variables (ie, twin sex, co-twin's sex, assessment method) were effect coded (ie, sex = 1 for males, –1 for females; assessment method = 1 for home assessment, –1 for laboratory assessment), and continuous variables (ie, BMI, anxiety) were centered prior to conducting HLM analyses.

Comparisons of OS Twins and Nontwins

An analysis of covariance (accounting for BMI and ethnicity) was used to investigate mean differences in DE between OS female twins and nontwin females. Notably, because having more than 1 biological older brother has previously been associated with increased prenatal testosterone exposure,[40] Pearson correlations were used to examine whether the number of older brothers was significantly associated with DE in the twin and nontwin samples. There were no significant associations between number of biological older brothers and DE (nontwin sample: $r = 0.07$; $P = .16$; twin sample: $r = -0.11$; $P = .41$).

RESULTS

COMPARISONS OF OS AND SS TWINS

Table 3 presents results from the HLM analyses. As expected, a significant main effect of twin sex on DE was observed, with females reporting significantly higher levels of DE than males (Table 3). More importantly, however, findings suggested that the co-twin's sex also influences levels of DE. A significant main effect of co-twin's sex was found for the MEBS total score (Table 3), indicating that in both males and females, decreased (ie, more masculinized) levels of DE are associated with having a male co-twin. Examination of the means revealed the expected linear trend where SS female twins, who purportedly had the lowest level of prenatal testosterone exposure, showed the highest levels of DE, followed by OS female, OS male, and SS male twins (**Figure**). Trend tests confirm the significance of this linear trend ($F_{1,577} = 92.27$; $P < .001$). Importantly, this linear trend cannot be accounted for by increased concordance for high or low levels of DE in MZ female or male twins, respectively. When analyses were rerun including only DZ twins, HLM results remained unchanged (Table 3). The linear trend also remained significant ($F_{1,301} = 49.80$; $P < .001$), with SS female DZ twins having the highest mean level of DE, fol-

Table 3. HLM Results for Disordered Eating and Anxiety Symptoms in Twins[a]

Model	β	Standard Error	*df*	*t* Test	*P* Value
Disordered Eating					
All twins					
Predictors					
Twin sex	–0.13	0.02	534.91	–8.06	<.001
Co-twin's sex	–0.05	0.06	528.46	–3.28	.001
Twin sex × co-twin's sex	0.02	0.02	301.83	0.86	.39
Covariates					
Assessment method (laboratory vs home)	0.04	0.02	406.29	1.76	.08
BMI	0.03	0	535.09	7.28	<.001
DZ twins only					
Predictors					
Twin sex	–0.13	0.02	299.00	–6.33	<.001
Co-twin's sex	–0.06	0.02	298.89	–2.80	.005
Twin sex × co-twin's sex	0.01	0.02	152.31	0.45	.65
Covariates					
Assessment method (laboratory vs home)	0.03	0.02	207.12	1.24	.22
BMI	0.03	0.01	287.53	4.94	<.001
Trait Anxiety					
All twins					
Predictors					
Twin sex	–2.28	0.46	566.80	–4.98	<.001
Co-twin's sex	–1.20	0.46	566.80	–2.61	<.01
Twin sex × co-twin's sex	0.39	0.65	305.73	0.59	.56
DZ twins only					
Predictors					
Twin sex	–2.02	0.56	290.16	–3.59	<.001
Co-twin's sex	–1.06	0.56	290.16	–1.88	.06
Twin sex × co-twin's sex	0.42	0.66	156.51	0.63	.53

Abbreviations: BMI, body mass index (calculated as weight in kilograms divided by height in meters squared); DZ, dizygotic; HLM, hierarchical linear model.

[a] Sex variables were effect coded as 1 for males and –1 for females. Assessment method was effect coded as 1 for home assessment and –1 for laboratory assessment.

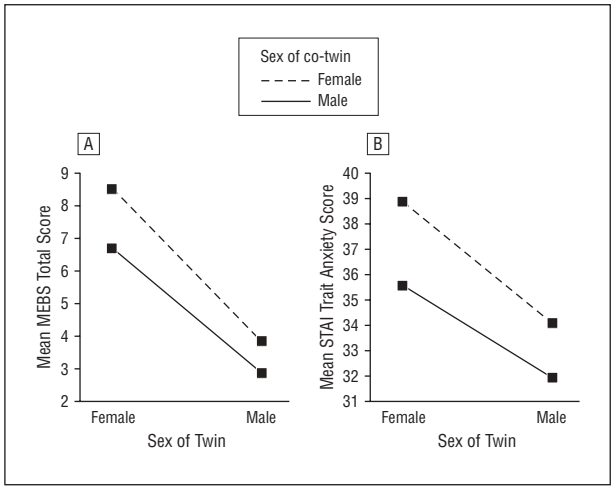

Figure. Mean scores by sex of twin and sex of co-twin in 582 participants. Sample sizes vary across twin type and dependent variables (see text for sample sizes). A, Minnesota Eating Behavior Survey (MEBS) total score. B, State-Trait Anxiety Inventory (STAI) Trait Anxiety score. The sexes of twins are represented along the x-axis and the sexes of co-twins are represented with a dotted (ie, female) or solid (ie, male) line, which is plotted at the corresponding mean disordered eating or anxiety score along the y-axis.

lowed by OS female DZ twins, OS male DZ twins, and SS male DZ twins.

Hierarchical linear model results for anxiety paralleled those for DE. Main effects for twin sex and co-twin's sex were observed (Table 3), as were linear trends in anxiety means across all twins ($F_{1,573}=39.83$; $P<.001$) (Figure) and the DZ twin sample only ($F_{1,297}=17.28$; $P<.001$). Importantly, however, the mediation model in the full twin sample showed that anxiety only partially mediated associations between prenatal testosterone exposure (ie, measured by co-twin's sex) and DE (**Table 4**). Specifically, although the relationship between co-twin's sex and DE (step 1) decreased when anxiety was included in the model (step 4), co-twin's sex remained a significant predictor of DE.

COMPARISONS OF FEMALE OS TWINS AND NONTWINS

Analysis of covariance results indicated a significant main effect for group in which females from OS twin pairs reported significantly lower levels of DE than nontwin females with at least 1 brother (**Table 5**). Thus, socialization from growing up with a male sibling does not appear to account for the masculinization of DE in OS female twins. Nonetheless, since the magnitude of age differences between nontwin female participants and their brothers may have influenced the amount of time spent together, a post hoc analysis of covariance was run after eliminating nontwin females whose closest-in-age brother was greater than 2 years older or younger. Results remained unchanged, as OS female twins continued to have lower levels of DE (mean [SD], 6.59 [5.63]) than nontwin females who had at least 1 brother (mean [SD], 9.01 [4.65]; n=22; $F_{1,73}=5.42$; $P=.02$; $d=0.45$]. In addition, post hoc Pearson correlations indicated no significant association between DE and the magnitude of age difference between nontwin females and their closest-in-age brother ($r=0.05$; $P=.70$).

COMMENT

To our knowledge, this was the first study to use OS and SS twin pairs to explore the masculinizing effects of prenatal testosterone exposure on the development of DE. Overall, our data were consistent with a "free martin" effect (ie, masculinized behavior due to in utero exposure to testosterone[41,42]) and provide evidence that increased levels of prenatal testosterone exposure may masculinize DE. Specifically, a linear trend in mean levels of DE was observed, where SS female twins showed the least masculinized (ie, highest) DE, followed by OS female twins, OS male twins, and SS male twins. Although several factors likely influence sex differences in DE, our findings highlight that biological factors likely play a role in this process. Specifically, masculinization of the CNS by prenatal testosterone exposure may decrease risk for DE in males vs females.

The masculinization of DE does not appear to be completely explained by levels of anxiety or by socialization effects. While anxiety partially mediated the association between prenatal testosterone exposure and DE, supporting some shared transmission,[43] primary conclusions remained unchanged even when controlling for levels of anxi-

Table 4. HLM Examining Anxiety as a Mediator of the Relationship Between Co-Twin's Sex and Disordered Eating in All Twins[a]

Model	β	Standard Error	*df*	*t* Test	*P* Value
Step 1: co-twin's sex→disordered eating	−0.05	0.02	526.48	−3.38	.001
Covariates					
Twin sex	−0.13	0.02	531.72	−8.15	<.001
Assessment method (laboratory vs home)	0.03	0.02	406.52	1.63	.11
BMI	0.03	0	536.07	7.28	<.001
Step 2: co-twin's sex→trait anxiety	−1.26	0.46	563.03	−2.76	<.01
Covariates					
Twin sex	−2.30	0.47	562.97	−4.90	<.001
Assessment method (laboratory vs home)	0.58	0.66	460.05	0.88	.38
BMI	0.13	0.11	568.42	1.22	.22
Step 3: trait anxiety→disordered eating[b]	−0.03	0.02	491.66	−2.15	.03
Covariates					
Twin sex	−0.10	0.02	507.08	−6.52	<.001
Assessment method (laboratory vs home)	0.02	0.02	380.56	1.55	.12
BMI	0.02	0	487.48	7.28	<.001
Step 4: co-twin's sex and trait anxiety→disordered eating[b]	−0.03	0.02	491.66	−2.15	.03
Covariates					
Twin sex	−0.10	0.02	507.08	−6.52	<.001
Assessment method (laboratory vs home)	0.02	0.02	380.56	1.55	.12
BMI	0.02	0	487.48	7.28	<.001

Abbreviations: BMI, body mass index (calculated as weight in kilograms divided by height in meters squared); HLM, hierarchical linear model.
[a] Sex variables were effect coded as 1 for males and −1 for females. Assessment method was effect coded as 1 for home assessment and −1 for laboratory assessment.
[b] Estimates for steps 3 and 4 are identical because these parameters were calculated within the same equation.

Table 5. Mean Differences in Disordered Eating Between Female Opposite-Sex Twins and Nontwins

	Mean (SD)			
Variable	Nontwin Females (n=69)	Opposite-Sex Female Twins (n=58)	$F_{1,123}$	*P* Value
Dependent variable				
Disordered eating	10.00 (5.75)	6.59 (5.63)	12.08	.001
Covariates				
BMI	23.48 (4.38)	23.08 (3.93)	28.03	<.001
Ethnicity[a]			0.34	.56

Abbreviation: BMI, body mass index (calculated as weight in kilograms divided by height in meters squared).
[a] Ethnicity is a categorical variable; thus, a mean value cannot be computed for this variable.

ety. Further, OS female twins had lower levels of DE than nontwin females who were raised with a brother. Admittedly, there may be something unique about having a brother the exact same age that accounts for the significantly lower levels of DE in OS female twins compared with nontwin females. Although plausible, it is unclear how having a same-age brother (rather than a brother who is close in age, but not the same age) could fully account for the lower (ie, masculinized) levels of DE in OS female twins compared with nontwin females. Moreover, findings remained unchanged even after controlling for age differences between nontwin females and their brothers.

The masculinized pattern of DE observed in our OS female twins parallels previous findings in animals (eg, intrauterine position effects[10]) and humans (eg, girls with congenital adrenal hyperplasia[44,45]) that support the masculinization of sexually dimorphic traits by prenatal testosterone exposure. If prenatal testosterone exposure does masculinize DE, as it does with several other sexually dimorphic traits, it is important to understand the mechanisms underlying the effects.

There are at least 2 ways in which prenatal testosterone exposure may influence predispositions to DE: (1) testosterone may organize anatomical and functional differences between the sexes in terms of key DE characteristics (eg, food intake, satiety) that increase risk for eating disorders, and/or (2) testosterone may have organizational effects on sensitivity to circulating ovarian hormones later in life, which then differentially activates predispositions to DE. While definitive data are not available to support either of these specific mechanisms, speculative hypotheses can be proposed on the basis of preliminary animal and human studies.

Extant animal research indicates that early testosterone exposure organizes food intake and accounts for well-documented sex differences in feeding and body weight (ie, male rats eat and weigh more than females). These effects in animal research are isolated to feeding behaviors (ie, increased and decreased food intake) rather than cognitive components (eg, weight preoccupation, body dissatisfaction) of eating disorders. Nonetheless, biological mechanisms for sex differences in DE may map more

directly onto these core feeding behaviors than the cognitive features of eating disorders. If so, the masculinization of DE in males and OS female twins may partially occur through the organization of brain structures or functions that influence food intake. Structural brain differences between males and females are region specific,[46] with significant sexual dimorphisms in brain areas (eg, anterior cingulate, amygdala, orbital frontal cortex, hypothalamus, insula[46]) involved in food intake and body weight.[47] These areas are also marked by high levels of sex steroid receptors during critical periods of early brain development, suggesting that their structural differentiation may be influenced by prenatal and perinatal testosterone exposure.[46,48,49]

Sex differences in DE may also be due to sex differences in brain function. Few studies have examined this possibility, although 2 studies observed sex differences in brain responses to hunger[50] and satiety[50,51] in several brain regions (eg, dorsolateral prefrontal cortex). Clearly, additional studies examining sex differences in brain function are needed, but initial results are promising in suggesting that males and females may differ in food-related brain processes that are important for eating disorders.

Prenatal testosterone exposure may also affect DE by decreasing CNS sensitivity to gonadal hormones in adulthood. Prenatal testosterone exposure in female rodents decreases their sensitivity to ovarian hormones later in life.[16] These effects may be particularly important for feeding behaviors, since ovarian hormones directly influence food intake and DE in adult women. Specifically, decreased levels of estrogen, and increased levels of progesterone, are associated with increased food intake and binge eating in clinical[52] and nonclinical (K.L.K., P. K. Keel, PhD, K.M.C., C. Edler, MA, unpublished data, 2007) samples of adult women. Indeed, changes in ovarian hormones appear to drive menstrual cycle changes in binge eating (K.L.K., P. K. Keel, PhD, K.M.C., C. Edler, MA, unpublished data, 2007).[52] Therefore, lower rates of DE in adult OS female twins may be due to increased prenatal testosterone exposure, which decreases sensitivity to the activating effects of ovarian hormones on DE. While all of these hypotheses are speculative, they are worthy of additional investigation as they highlight potential pathways between the masculinizing effects of testosterone and correspondingly lower levels of DE symptoms.

Despite the strengths of this study, several limitations must be noted. First, participants were not clinically diagnosed with an eating disorder. Therefore, it is unclear how well the study's findings generalize to clinical populations. Although DE symptoms lie on a continuum,[53-56] show prospective associations with eating disorders,[57] and are considered precursive,[57] it will be important for future studies to replicate our results with clinical populations.

Second, the extent to which our participants are representative of the general population is somewhat unclear. Our twin samples appear to be roughly representative of the surrounding population based on racial categories. Nontwin female participants (who were college students) were comparable with OS female twins in terms of age and socioeconomic status and roughly comparable in ethnicity. Nonetheless, future studies should use larger population-based samples to replicate our results and ensure generalizability of the findings.

Third, we were unable to directly assess levels of prenatal testosterone exposure. The most empirically supported and accepted view[6] is that prenatal testosterone and its metabolites (ie, dihydrotestosterone and 17 β-estradiol) masculinize the brain and sexually dimorphic behavior in males and females[6]; thus, prenatal testosterone exposure seems very likely to play some role in our observed effects. Evidence for the feminizing effects of prenatal estrogen in males is conflicting, with most studies showing no significant effects.[6] Nonetheless, we did not directly measure prenatal gonadal hormone exposure and, thus, cannot definitively rule out possible feminization (or demasculinization) of DE by prenatal estrogen exposure in OS male twins. Future research therefore is needed to confirm that the masculinization effects are due to prenatal testosterone exposure.

Fourth, information on placenta type (ie, monochorionic or dichorionic) was not available for MSUTR participants, but this information would be important to examine in future twin studies of prenatal effects. Finally, future research should also investigate differences in brain processes related to feeding (eg, hunger, food intake, satiety) and differential sensitivity to ovarian hormones in OS vs SS twins. These investigations will likely provide new insight into how prenatal hormone exposure alters biological mechanisms and, ultimately, influences predispositions to DE in males and females.

In conclusion, findings from this study contribute to the literature on sex differences in eating disorder prevalence. Sociocultural factors (eg, pressure for thinness in women) have typically been used to explain the sex difference in eating disorder prevalence. However, our results suggest that the masculinizing effects of prenatal testosterone, characteristic of male development, may also play a significant role.

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