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ORIGINAL ARTICLE

Preliminary evidence that estradiol moderates genetic influences on disordered eating attitudes and behaviors during puberty

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Background. Puberty moderates genetic influences on disordered eating attitudes and behaviors, with little genetic influence before puberty but large (⩾50%) genetic effects during and after puberty. To date, however, nothing is known about the mechanisms that underlie these effects. Estradiol is a particularly promising candidate, as estrogens become elevated at puberty and regulate gene transcription within neurotransmitter systems important for eating-related phenotypes. The aim of this pilot study was to examine whether estradiol levels moderate genetic influences on disordered eating during puberty.

Method. Participants included 198 female twins (ages 10–15 years) from the Michigan State University Twin Registry. Disordered eating attitudes and behaviors were assessed with the total score, weight preoccupation, body dissatisfaction and binge eating/compensatory behavior subscales of the Minnesota Eating Behavior Survey (MEBS). Afternoon saliva samples were assayed for estradiol levels. Moderation of genetic effects was examined by comparing twin correlations in low *versus* high estradiol groups.

Results. In the low estradiol group, monozygotic (MZ) and dizygotic (DZ) twin correlations for all MEBS scales were similar, suggesting little genetic influence. In the high estradiol group, the MZ twin correlation was more than double the DZ twin correlation, indicating the presence of genetic effects. Findings could not be accounted for by age, body mass index or the physical changes of puberty.

Conclusions. Estradiol may be one important moderator of genetic effects on disordered eating during puberty. Larger twin studies are needed to replicate this pilot work and quantify the extent of genetic moderation.

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Introduction

Developmental twin studies suggest that puberty moderates genetic effects on disordered eating attitudes and behaviors (Klump *et al*. 2003, 2007*a*, *b*). Using continuous measures of general disordered eating (i.e. sum scores of weight preoccupation, body dissatisfaction, binge eating and the use of compensatory behaviors), little-to-no genetic influence was found for disordered eating in pre-pubertal twins (∼0%), but significant genetic effects (∼50%) were observed in twins who had begun puberty. Follow-up studies have replicated these pubertal effects in two separate twin samples (Klump *et al*. 2007*b*; Culbert *et al*. 2009) and have shown that genetic effects emerge during early to mid-puberty rather than late puberty (Culbert *et al*. 2009). Overall, these findings are significant in identifying the differential influence of genes *versus* environment across development. Greater understanding of these developmental shifts may better inform prevention efforts by identifying developmental windows of increased risk and psychosocial and biological factors that are most relevant to the development of eating disorders.

Despite the potential significance of the work, little is known about the mechanisms underlying shifts in genetic effects. One of the primary hypotheses has centered on the initiation of ovarian hormone secretion during puberty (Klump *et al*. 2003, 2007*b*; Culbert *et al*. 2009). Ovarian hormones (estrogen, progesterone) become prominent in girls during puberty and have been found to predict changes in food intake in both animals and humans (e.g. Asarian and

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Geary, 2006; Edler *et al*. 2007; Klump *et al*. 2008). Among ovarian hormones, estradiol is elevated early in puberty and circulating levels increase linearly throughout the pubertal period. This trajectory closely mimics the linear increases in genetic effects observed for disordered eating across puberty (i.e. 0–44%) (Klump *et al*. 2007*b*). Thus, increases in estradiol may account for increases in genetic effects during puberty. Estradiol is a steroid hormone that is known to be a potent regulator of gene transcription within the central nervous system (CNS; Ostlund *et al*. 2003). The direct genomic effects of estradiol highlight its potential role as a moderator of genetic effects on disordered eating during puberty.

The purpose of this pilot study was to directly examine the effects of estradiol in a sample of same-sex female twins. We examined whether levels of estradiol moderate genetic influences on disordered eating during puberty, such that the heritability of these characteristics is greater in twins with higher estradiol levels as compared with twins with lower estradiol levels. We controlled for several potential confounding variables in analyses. Previous research has indicated significant age differences in genetic and environmental influences on disordered eating across adolescence using cross-sectional (Klump *et al*. 2000, 2009) and longitudinal (Klump *et al*. 2007*a*) designs. In addition, body weight is influenced by estradiol levels (Asarian and Geary, 2006), tends to increase at puberty in girls (Grumbach & Styne, 1998) and is influenced by genetic factors (Allison *et al*. 1994). Finally, the physical changes of puberty are correlated with estradiol levels and have been proposed to contribute to increased mean levels of disordered eating (Slap *et al*. 1994). Given the potential importance of these variables, we controlled for age, body mass index (BMI) and the physical changes of puberty in analyses to ensure that they do not account for estradiol's moderation of genetic effects for disordered eating attitudes and behaviors.

Methods

Participants

Participants included 258 same-sex, adolescent female twins [129 pairs; 63 monozygotic (MZ), 66 dizygotic (DZ)] between the ages of 10 and 15 years (mean = 11.98 years, S.D. = 1.40) drawn from the Michigan State University Twin Registry (MSUTR; Klump & Burt, 2006). The MSUTR is a population-based twin registry that examines developmental differences in genetic and environmental influences for several forms of psychopathology. Recruitment methods for the MSUTR have been described in detail elsewhere (Klump & Burt, 2006). In brief, our adolescent sample was recruited entirely through birth records in Michigan in collaboration with the Michigan Department of Community Health (MDCH). The MDCH randomly selected same-sex female twins meeting our age requirements and living within 90 min of our Michigan State University laboratories. Twins were recruited for study participation until our target sample size for this pilot project was reached. These recruitment methods result in MSUTR samples that are broadly representative of the population of Michigan (Culbert *et al*. 2008).

Zygosity determination

Zygosity was established through a physical similarity questionnaire (Peeters *et al*. 1998) completed by one parent (typically the mother). Indeterminable zygosity scores were resolved by a project principle investigator (K.L.K. or S.A.B.) through a review of item endorsements and/or twin photographs. These methods have been found to be over 95% accurate in determining twin zygosity (Peeters *et al*. 1998) and are used by many population-based twin registries (Iacono *et al*. 1999).

Measures

Demographic information

Twin age, twin ethnicity and parental income were assessed using a standard demographic questionnaire completed by one parent.

Disordered eating attitudes and behaviors

Disordered eating was assessed using the Minnesota Eating Behavior Survey† (MEBS; von Ranson *et al*. 2005). The MEBS was developed from the Eating Disorders Inventory (EDI; Garner *et al*. 1983); item rewordings and additions were made to make the measure appropriate for use with children as young as 9 years old (Klump *et al*. 2000). The MEBS has been employed in previous developmental twin studies of eating disorders (Klump *et al*. 2000, 2003, 2007*a*, *b*; Culbert *et al*. 2008). Our use of the same, developmentally appropriate measure of disordered eating

† The Minnesota Eating Behavior Survey (previously known as the Minnesota Eating Disorder Inventory) was adapted and reproduced by special permission of Psychological Assessment Resources, Inc., 16204 North Florida Avenue, Lutz, Florida 33549, from the Eating Disorder Inventory (collectively, EDI and EDI-2) by Garner, Olmstead, Polivy, Copyright 1983 by Psychological Assessment Resources, Inc. Further reproduction of the MEBS is prohibited without prior permission from Psychological Assessment Resources, Inc.

allows us to explore whether estradiol accounts for previously observed pubertal differences in genetic effects.

The MEBS includes 30 true/false items that assess overall levels of disordered eating via a total score as well as specific disordered symptoms using the weight preoccupation (eight items assessing preoccupation with dieting, weight and the pursuit of thinness), body dissatisfaction (six items assessing dissatisfaction with the size and/or shape of one's body), binge eating (seven items assessing the tendency to engage in binge eating and to think about binge eating) and compensatory behaviors (six items assessing the tendency to use or to contemplate using inappropriate compensatory behaviours, such as self-induced vomiting and laxatives to control weight) subscales. Psychometric properties of the MEBS total score, weight preoccupation and body dissatisfaction subscales are excellent in pre- and early adolescent subjects. For example, the original factor analysis of the MEBS (see Klump *et al*. 2000) and follow-up analyses (see von Ranson *et al*. 2005) support factor congruence for these scales in pre-adolescent and adolescent girls (i.e. factor congruence coefficients >0.88). These scales also exhibit good internal consistency (α values >0.80 in the literature as well as the current sample) and they correlate highly ($r \geqslant 0.70$) with similar measures of disordered eating (e.g. the Eating Disorder Examination Questionnaire; von Ranson *et al*. 2005). Finally, all three scales successfully discriminate between adolescents with anorexia nervosa (AN) or bulimia nervosa (BN) *versus* control participants (von Ranson *et al*. 2005).

The MEBS binge eating and compensatory behavior subscales tend to be less internally consistent (i.e. α's <0.65) in early adolescent girls (von Ranson *et al*. 2005). This was also true in the current sample (α's <0.65), particularly in the pre-adolescent girls. However, given that the original EDI from which the MEBS was developed includes items tapping binge eating and compensatory behaviors on the same bulimia scale (Garner *et al*. 1983), we explored whether a combined binge eating/compensatory behavior scale would exhibit acceptable internal consistency. The internal consistency of the combined scale was well within the acceptable range ($\alpha = 0.70$). Consequently, in the current study, we focused our analyses on the MEBS total score, weight preoccupation, body dissatisfaction and the combined binge eating/compensatory behavior subscale. This approach allowed us to examine a range of disordered eating characteristics and attitudes while maintaining the integrity of the twin analyses, which tend to be quite sensitive to measurement issues and error (Plomin *et al*. 1990).

Salivary estradiol

Salivary samples were used to obtain estradiol levels. Monitoring biomarkers in saliva has distinct advantages over doing so in other biological fluids (i.e. urine, serum, plasma) (Shirtcliff *et al*. 2000). For example, saliva sampling is a less invasive method that assesses the unbound hormone, which provides a more accurate indicator of active forms of estradiol. Saliva sampling has also been associated with higher compliance and more robust hormone–behavior associations than bloodspot samples (Edler *et al*. 2007).

Salivary samples were collected during the laboratory visit. Twins were asked to refrain from eating or drinking for 4 h prior to the visit and to refrain from smoking, brushing their teeth or chewing gum for 30 min prior to the saliva collection. Twins then passively drooled down a straw into a cryovial until $\geqslant 4$ ml was produced. All saliva collections occurred between 14:00 and 17:00 hours. We chose to collect during these times because afternoon–early evening diurnal variations in ovarian hormones during puberty tend to be minimal (Grumbach & Styne, 1998; Angold *et al*. 1999) and most families were available for assessments during this time.

Salivary specimens were frozen immediately and stored until they were shipped for analysis to Salimetrics, Inc. (USA). On the day of testing, samples were centrifuged at 3000 rpm for 10 min to remove mucins. Clear samples were pipetted into testing wells to screen for problems with pH. Samples testing outside of the pH range of 4–9 were diluted in phosphate buffer solution to correct pH prior to testing. Samples were then assayed in duplicate using an enzyme immunoassay developed by Salimetrics, Inc. The assay has a minimum detection limit of <1 pg/ml and average intra- and inter-assay coefficients of variation $<7.13\%$ and $<7.45\%$, respectively. Method accuracy, determined by spike recovery and linearity, are 104.2% and 99.9% (acceptable ranges 80–110%). Estradiol values from matched serum and saliva samples show a strong linear relationship for females ($r = 0.80$) (Shirtcliff *et al*. 2000).

Body mass index

BMI [weight (kg)/ height^2 (m^2)] was calculated using laboratory measures of body weight and height. MSUTR research assistants measured twin weight using a digital scale and twin height using a wall-mounted ruler.

Physical changes of puberty

Physical changes of puberty were assessed using the Pubertal Development Scale (PDS; (Petersen *et al*.

1988). The PDS assesses growth spurts, skin changes, body hair growth, breast development and the initiation of menses. Twins self-rated their development in these areas on a 4-point scale: (1) development has not yet begun; (2) development has barely started; (3) development is definitely underway; (4) development seems completed. Menstruation was rated dichotomously as absent (1) or present (4). Similar to previous research (Petersen *et al*. 1988; Klump *et al*. 2003, 2007*b*), average scores (i.e. range 1–4) across all five items were used in analyses. Previous studies of the PDS psychometric properties have supported its reliability (median $\alpha = 0.77$) (Petersen *et al*. 1988) and validity, including showing high correlations with clinician ratings of secondary sex characteristic development (r's = 0.61–0.67) (Petersen *et al*. 1988).

Statistical analyses

The MEBS total scores were prorated for twins who were missing $\leqslant 10\%$ of the items. Scores were coded as missing for twins missing more than 10% of items. Given the relatively small number of items on the weight preoccupation, body dissatisfaction and binge eating/compensatory behavior subscales (range 6–13 items), subscale scores of twins with one or more missing items were not prorated, but were instead coded as missing. Sample sizes therefore vary somewhat across subscale analyses. The MEBS subscale scores were log transformed to account for positive skew, which resulted in appropriate skew values between -1 and 1 for all scales. Finally, age, BMI, and PDS scores were regressed out of all MEBS subscale scores prior to analyses to ensure that estradiol's effects are not due to these potentially confounding variables.

Ideally, we would have examined differences in the heritability of these scores by estradiol levels using twin moderation models (Purcell, 2002). However, our sample size was too small for these analyses, which typically require several hundred pairs to detect moderation (Purcell, 2002). Instead, in this preliminary study, we divided our twins into 'low' *versus* 'high' estradiol groups using the 50th percentile for estradiol levels (i.e. 5.79 pg/ml) and compared twin correlations in each group. The use of a 50th percentile split allowed us to maximize sample sizes in each group while still examining potentially meaningful differences in hormone effects.

Because MZ twins share approximately 100% of their segregating genes while DZ twins share, on average, 50%, significantly greater MZ relative to DZ twin correlations indicate the presence of genetic effects. By contrast, MZ and DZ twin correlations that are similar in magnitude and are significantly greater than 0 signify a lack of genetic effects, but significant shared environmental influences (i.e. environmental factors that are common to siblings growing up in the same family and contribute to their behavioral similarity). Finally, MZ twin correlations <1.00 indicate the presence of non-shared environmental factors (i.e. factors that are unique to siblings growing up in the same family and contribute to behavioral differences) and measurement error.

Importantly, because our analyses focus on comparing twin pairs with high *versus* low estradiol levels, we could only include pairs if the co-twins were concordant for either low or high estradiol status (Klump *et al*. 2003; Culbert *et al*. 2009). This resulted in the exclusion of 30 pairs from analyses ($n = 14$ MZ, 16 DZ) and a final sample of 99 pairs (49 MZ, 50 DZ). However, there were no significant mean or variance differences in MEBS total scores between twins from concordant *versus* discordant pairs (data not shown), suggesting that this exclusion did not unduly influence our results.

Results

A range of disordered eating attitudes and behaviors was present in our sample. A total of 8.2% of twins scored above the clinical cut-off for the total score (cut-off score = 15.55) (von Ranson *et al*. 2005). Thus, our findings should speak to a variety of disordered eating attitudes and behaviors across the spectrum of severity.

Table 1 includes comparisons of demographic characteristics and study variables for the low *versus* high estradiol groups. There were no significant differences in ethnicity or parental income between groups (all p's >0.05). However, and as would be expected given our categorizations, twins in the high estradiol group were significantly older, they were at later stages of pubertal development and they exhibited higher mean levels of estradiol than twins in the low estradiol group. The two groups did not differ in BMI, mean levels or variability (p's >0.05 for Levene's Test for Equality of Variances) of MEBS scores or the proportion of twins scoring above the MEBS clinical cut-off for the MEBS total score [8.6% *v.* 9.6% in low and high estradiol groups, $\chi^2(1) = 0.22$, $p = 0.64$]. These findings confirm that differences in levels of disordered eating cannot account for potential differences in etiologic effects across groups. Importantly, however, the lack of differences in disordered eating by estradiol levels does not negate a moderating effect of estradiol on genetic effects, as the presence of genetic moderation would be expected to attenuate phenotypic associations. This pattern of results has been observed previously, where puberty

Table 1. *Mean differences in study variables by estradiol group* (n = *198 twins*)

Variable	Low estradiol (*n* = 92–94 twins)	High estradiol (*n* = 102–104 twins)	*t* (193–196)	*p*
Demographic variable				
Ethnicity				
Caucasian	84 (89%)	90 (87%)	–	–
Black	8 (9%)	10 (9%)	–	–
Asian/Pacific Rim	2 (2%)	0 (0%)	–	–
Other	0 (0%)	4 (4%)	–	–
Parental income				
< $20 000	4 (5%)	6 (6%)	–	–
$20 000–$40 000	17 (17%)	12 (12%)	–	–
$40 000–$60 000	34 (36%)	24 (23%)	–	–
$60 000–$100 000	20 (21%)	36 (35%)	–	–
> $100000	20 (21%)	24 (23%)	–	–
Missing	0 (0%)	2 (1%)		
MEBS scales				
Total score	6.13 (5.24)	6.43 (5.74)	−0.42	0.68
Weight preoccupation	2.39 (2.21)	2.66 (2.41)	−1.04	0.30
Body dissatisfaction	1.23 (1.69)	1.49 (1.79)	0.03	0.98
Binge eating/compulsive behaviors	1.67 (1.88)	1.52 (1.90)	0.85	0.40
Hormones				
Estradiol levels	3.20 (1.47)	13.86 (8.03)	−12.67	< 0.001
Covariates				
Age	11.70 (1.41)	12.23 (1.35)	−2.70	0.007
BMI	19.94 (4.41)	20.69 (3.78)	−1.29	0.20
Standardized BMI	−0.09 (1.07)	0.08 (0.92)	–	–
PDS score	2.39 (0.84)	2.67 (0.86)	−2.24	0.03

MEBS, Minnesota Eating Behaviors Survey; BMI, body mass index; PDS, Pubertal Development Scale.

Values for demographic variables are the number of pairs (percent of pairs). Values for all other variables are means (S.D.). Estradiol levels were measured in pg/ml. Although log-transformed MEBS total scores were used in analyses, raw scores are provided in the table for descriptive purposes. Likewise, standardized BMI scores are included in the table for descriptive purposes only; all analyses were conducted using raw BMI scores only.

was found to be a significant moderator of genetic effects despite negligible phenotypic associations with disordered eating (Klump *et al*. 2003; Culbert *et al*. 2009).

Twin correlations for the MEBS scales are depicted in Table 2. Differences between MZ and DZ twin correlations varied by estradiol status for all three MEBS scales. In the low estradiol group, the MZ and DZ twin correlations were approximately equal, suggesting a lack of genetic effects. In these twins, it would appear that shared and non-shared environmental influences predominate. By contrast, in the high estradiol group, the MZ twin correlations were more than double the DZ twin correlations using all MEBS subscale scores. Notably, the MZ/DZ twin differences in the high estradiol group reached or approached traditional thresholds for statistical significance (i.e. *p*'s = 0.03–0.12). Moreover, effect sizes for all twin correlations in the high estradiol group were in the moderate-to-large range (i.e. *q*'s = 0.37–0.59), suggesting clinically significant differences in MZ and DZ twin similarity for disordered eating.

Nonetheless, we were interested in exploring whether statistically significant differences in twin correlations would emerge in the high estradiol group if we examined a more extreme group. We consequently reconstituted our high estradiol twin group into those falling in the highest tertile of estradiol levels (estradiol level = 8.13 pg/ml, $n = 29$ pairs, 14 MZ, 15 DZ). The difference in twin correlations now reached statistical significance for total score ($r_{MZ} = 0.88$, $r_{DZ} = 0.17$, $Z = 2.88$, $p = 0.002$), weight preoccupation ($r_{MZ} = 0.81$, $r_{DZ} = -0.06$, $Z = 3.44$, $p = 0.0003$) and body dissatisfaction ($r_{MZ} = 0.69$, $r_{DZ} = -0.14$, $Z = 2.37$, $p = 0.008$) and approached significance for the binge eating/compensatory behaviors subscale

Table 2. *Twin correlations for disordered eating attitudes and behaviors by low and high estradiol levels* (n = *99 pairs*)

	Low estradiol group ($N=47$ pairs)					High estradiol group ($N=52$ pairs)				
MEBS Scales	MZ ($n=22$–25)	DZ ($n=18$–22)	Z	p	q	MZ ($n=21$–25)	DZ ($n=24$–27)	Z	p	q
Total score	0.46* (0.05 to 0.75)	0.54* (0.10 to 0.81)	−0.31	0.38	0.10	0.47* (0.05 to 0.76)	0.04 (−0.38 to 0.44)	1.46	0.07	0.47
Weight preoccupation	0.46* (0.05 to 0.75)	0.57** (0.20 to 0.80)	−0.53	0.30	0.15	0.58** (0.24 to 0.80)	0.21 (−0.19 to 0.55)	1.52	0.06	0.45
Body dissatisfaction	0.49** (0.12 to 0.75)	0.53** (0.14 to 0.78)	−0.17	0.43	0.06	0.37* (−0.03 to 0.67)	0.04 (−0.38 to 0.44)	1.18	0.12	0.35
Combined Binge Eating/Compensatory Behavior Scale	0.23 (−0.20 to 0.59)	0.20 (−0.25 to 0.58)	0.10	0.46	0.03	0.65** (0.35 to 0.84)	0.18 (−0.22 to 0.53)	1.96	0.03	0.59

MEBS, Minnesota Eating Behaviors Survey; Z, test of equality that examines differences between monozygotic (MZ) and dizygotic (DZ) twin correlations; *q*, effect size for corresponding *Z* value.

Sample sizes are for pairs of twins. Variations in sample size are due to missing data for the confounding variables. The 95% confidence intervals for each correlation are included in parentheses. One-tailed *p* values were used for comparisons between MZ and DZ twins.

* $p<0.05$, ** $p<0.01$, two tailed. The twin correlation is significantly different from zero.

($r_{MZ}=0.78$, $r_{DZ}=0.34$, $Z=1.40$, $p=0.08$). Taken together, these findings suggest the presence of genetic effects on disordered eating in the high estradiol group. They further indicate a lack of shared environmental influence, but significant non-shared environmental effects, on disordered eating at high estradiol levels.[1]‡

Discussion

Findings provide preliminary support for our hypothesis that levels of estradiol moderate genetic effects on several types of disordered eating attitudes and behaviors. Using a pilot sample of twins, disordered eating exhibited little-to-no genetic influence in twins with low estradiol levels during puberty, but moderate-to-substantial genetic effects in twins with higher estradiol levels. These effects were present even when controlling for age, BMI and the physical changes of puberty. Taken together, the results provide initial support for a role for estradiol in the genetic diathesis of disordered eating attitudes and behaviors during puberty.

The unique effects of estradiol levels above and beyond the influence of the confounding variables deserves note. These findings suggest that estradiol's effects are unlikely to be artifacts of age, increased body weight or the physical changes during puberty that have been hypothesized to influence disordered eating risk in girls. Results also indicate that age differences in genetic effects observed previously (i.e. increases in heritability between ages 11 and 14 years; no changes in heritability from ⩾14 years) (Klump *et al*. 2000, 2007*b*, 2009) may be due to increases in estradiol that characterize puberty and typically occur between the ages of 11 and 14 years (Kaltiala-Heino *et al*. 2003).

Moving forward, it will be important to replicate these findings using larger sample sizes and explore mechanisms underlying estradiol's effects. A critical function of estradiol during development is the regulation of gene transcription within the CNS (Ostlund *et al*. 2003). When activated by hormones, intracellular steroid hormone receptors act as transcription factors to regulate mRNA expression in neurobiological systems. Increased genetic effects on disordered eating during puberty may therefore result from increases in estradiol and its genomic effects on the structure and/or function of the CNS through the production of neurotransmitters, their receptors or their signal transduction mechanisms. Animal data show that genes and gonadal hormones influence the pattern and timing of changes in brain structure and function during puberty (Primus & Kellogg, 1991; Spear, 2000; Nunez *et al*. 2002; Zehr *et al*. 2006; Ahmed *et al*. 2008). Emerging data suggest that these same effects may be present in humans. Levels of estradiol are inversely correlated with changes in neural structures in girls during puberty, and these neural changes appear to be influenced by genetic factors (Lenroot *et al*. 2009; Peper *et al*. 2009*a*, *b*). Taken together, data across disparate sources suggest that increases in estradiol

‡ The notes appear after the main text.

during puberty may increase genetic influences on disordered eating through differential organization of neural circuitry underlying risk.

Notably, our findings may appear to conflict with those showing that lower levels of estradiol are associated with increased levels of binge eating over the course of the menstrual cycle in women with BN (Edler *et al*. 2007) and women from the community (Klump *et al*. 2008). However, the current study focused on a different level of analysis (i.e. within-twin pair similarity *versus* within-person associations) than previous work. Thus, the current work examines the potential influence of estradiol on heritability of disordered eating over puberty, whereas previous work has examined the potential influence of estradiol on levels of disordered eating in adult women. The effects of estradiol on disordered eating may differ by developmental stage, such that inverse phenotypic associations are observed in adulthood, whereas no phenotypic (but significant genetic) associations are present during puberty (Klump & Keel, unpublished observations). These differential effects fit nicely with the organizational/activational hypothesis of gonadal hormone action, which predict: (1) gonadal hormones organize risk for disorder during puberty through changes in gene transcription and resulting changes in brain structure/function; (2) these changes organize the brain to respond to circulating levels of hormones in adulthood, which activate and/or influence the expression of behavior (Sisk & Zehr, 2005). When hormone organization/activational effects are present, modest phenotypic associations between gonadal hormones and behavior are observed during puberty, whereas significant phenotypic associations are present in adulthood (Sisk & Zehr, 2005). This pattern of phenotypic associations fits with that observed for disordered eating and could help explain seemingly disparate findings for estradiol during puberty *versus* adulthood (Klump & Keel, unpublished observations).

Much more research is needed to confirm and replicate our pilot study results and examine speculative hypotheses regarding mechanisms of estradiol's effects. Ideally, future work would address limitations of our pilot study. Our sample sizes were small and limited our ability to conduct biometric model fitting to quantify the degree of genetic moderation. We examined a community sample of twins whose findings may not generalize to a clinically diagnosed sample. Given the very low prevalence of AN and BN in pre- and early adolescence (Bulik, 2002), it would be difficult, if not impossible, to examine our hypotheses in a purely clinical sample of twins. Our sample exhibited a wide range of disordered eating attitudes and behaviors, including clinical levels of these characteristics. Thus, our findings likely speak to the range of disordered eating present in the population at large. Nonetheless, additional work in larger twin samples is needed to determine the generalizability of the findings to AN and BN. Studies in larger samples may also track estradiol's role in moderating genetic effects on disordered eating in specific age groups.

Finally, we collected salivary hormone and disordered eating data on a single day. It is preferable to obtain multiple measures on different days to obtain more stable estimates of these phenotypes, particularly estimates of estradiol levels. Future research should obtain multiple measures of hormones, eating disorder phenotypes (i.e. self-report, interview) and covariates in larger samples of twins to confirm and extend this pilot work.

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Declaration of Interest

None.

Notes

[1] Because some of our twins were post-menses at the time of study, we re-ran all of our correlations excluding twins who had experienced at least one menstrual cycle ($n=90$ twins). Despite the large decrease in sample size, the pattern of results remained unchanged, with minimal genetic effects in the low estradiol group for total score ($r_{MZ}=0.59$, $r_{DZ}=0.46$, $Z=0.38$, $p=0.35$), weight preoccupation ($r_{MZ}=0.41$, $r_{DZ}=0.49$, $Z=-0.21$, $p=0.42$), body dissatisfaction ($r_{MZ}=0.54$, $r_{DZ}=0.34$, $Z=0.51$, $p=0.31$) and binge eating/compensatory behaviors ($r_{MZ}=-0.09$, $r_{DZ}=-0.01$, $Z=-0.16$, $p=0.44$), but indications of genetic effects in the high estradiol group, where the MZ twin correlation was more than double the DZ twin correlation for total score ($r_{MZ}=0.34$, $r_{DZ}=0.06$, $Z=0.53$, $p=0.29$), weight preoccupation ($r_{MZ}=0.48$, $r_{DZ}=0.19$, $Z=0.68$, $p=0.24$), body dissatisfaction ($r_{MZ}=0.41$, $r_{DZ}=0.11$, $Z=0.38$, $p=0.35$) and binge eating/compensatory behavior ($r_{MZ}=0.41$, $r_{DZ}=0.15$, $Z=0.49$, $p=0.31$) subscales.

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