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Preliminary evidence that gonadal hormones organize and activate disordered eating

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ABSTRACT

Objective. Eating disorders are more common in females than in males. Gender differences may be due to organizational (i.e. prenatal) and activational (i.e. post-natal) gonadal hormone effects that influence sex differences in behavior. This preliminary set of studies examined these effects by investigating relationships between eating disorder symptoms, prenatal testosterone exposure, and adult levels of estrogen in women.

Method. We examined organizational associations by investigating relationships between disordered eating and finger-length ratios, which are known to be somatic markers of prenatal testosterone exposure. Participants included 113 adult female twins drawn from the community. Disordered eating was assessed with the total score from the Minnesota Eating Behavior Survey (MEBS). Finger lengths were hand scored using a ruler and photocopies of both hands. We also investigated activational influences by examining associations between circulating levels of estradiol and disordered eating symptoms. Two independent samples of adult females (n's = 24 and 25) drawn from the community were used for this study. Disordered eating was again assessed with the MEBS total score, while saliva samples were used for assessing estradiol.

Results. Positive associations were found between disordered eating and both finger-length ratios and circulating estradiol levels.

Conclusions. Findings suggest that lower levels of prenatal testosterone exposure and higher adult levels of estradiol are associated with increased eating disorder symptoms. We hypothesize that the relatively low level of testosterone before birth in females permits their brains to respond to estrogens at puberty, when the hormones activate the genes contributing to disordered eating in vulnerable girls.

INTRODUCTION

Several epidemiologic features suggest a role for gonadal hormones in the development of anorexia nervosa (AN) and bulimia nervosa (BN). In addition to being more common in females than in males (APA, 2000), eating disorder symptoms begin at puberty (Hayward *et al.* 1997) and tend to remit by mid-life and the menopausal years (Strober *et al.* 1997; Keel *et al.* 1999). Eating disorder symptoms also show a significant heritability (>50%) (Klump *et al.* 2000), but only in girls who have reached puberty (Klump *et al.* 2003), which also suggests that gonadal hormones may activate the disorders.

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Animal studies extend findings by showing that gonadal hormones have both organizational (i.e. organizing neural circuitry prenatally) and activational (i.e. influencing neural systems and behavior post-natally) influences on core features of eating disorders, including food intake and physical activity. Female rats exposed to testosterone perinatally increase food intake and body weight in adulthood (Madrid *et al.* 1993). In contrast to these organizing effects, circulating estrogens have activational influences that result in decreased food intake and increased physical activity in adult female rats (Dixon *et al.* 2003; Eckel, 2004). Similar effects have been shown across a number of species including hamsters (Morin & Fleming, 1978), guinea-pigs (Butera & Czaja, 1984), sheep (Forbes, 1974), and non-human primates (Bielert & Busse, 1983; Kemnitz *et al.* 1989). Sex differences in food intake and body weight (i.e. males eat more and engage in less physical activity) are also controlled by organizational and activational influences of gonadal hormones in a variety of mammals (Wade, 1972).

Based upon these data, we propose that prenatal testosterone exposure in males, which organizes sex differences in behavior of mammalian model systems (Morris *et al.* 2004), reduces males' likelihood of developing eating disorders, particularly during puberty when disordered eating begins. We further propose that the rise in estrogens during puberty in vulnerable girls activates these symptoms, which are then exacerbated by circulating levels of estrogens in adulthood.

We conducted two studies investigating these hypotheses. We examined overall levels of disordered eating symptoms in menstruating women rather than AN or BN because the neuroendocrine abnormalities (amenorrhea and oligomenorrhea) that result from malnutrition and aberrant eating patterns in these disorders make it difficult to examine etiologic effects of circulating gonadal hormones *versus* disease sequelae. The disordered eating symptoms examined have been found to: (1) show significant sex differences (Anderson & Bulik, 2004); (2) be genetically associated with puberty (Klump *et al.* 2003); and (3) be significant risk factors for the development of AN and BN (Jacobi *et al.* 2004). Although eating disorder symptoms in non-clinical populations could conceivably cause ovarian hormone disruptions, rather than the reverse, our focus on regularly menstruating women (see Method section) significantly decreases this possibility.

We investigated organizational effects of androgens by examining associations between finger-length ratios and disordered eating in women. Finger-length ratios [index finger (2D)/ring finger (4D)] are sexually dimorphic traits (Manning *et al.* 1998) that: (1) develop as early as the thirteenth week of gestation (Garn *et al.* 1975); (2) correlate with prenatal levels of testosterone obtained by amniocentesis (Lutchmaya *et al.* 2004); (3) are more masculinized (i.e. lower ratios) in men and women with congenital adrenal hyperplasia (CAH) who have high prenatal levels of androgens (Brown *et al.* 2002; Okten *et al.* 2002); and (4) are associated with other behavioral phenotypes that show significant sex differences (e.g. aggression) (Manning *et al.* 2000; Manning, 2002; Bailey & Hurd, 2005). Given the difficulty of obtaining prospective prenatal measures of hormone exposure in humans, finger-length ratios are considered one of the most robust measures of prenatal androgen effects (Manning, 2002; Lutchmaya *et al.* 2004).

We also examined activational influences by investigating relationships between circulating levels of estradiol and the same disordered eating symptoms in two independent samples of adult women.

METHOD

Written informed consent was obtained from all participants after study procedures were explained. Samples in both studies were recruited from the community; thus, subjects were not screened for the presence or absence of an eating disorder or any other form of psychopathology.

Study 1: Organizational influences

Participants

Participants were 113 female twin individuals (mean age = 20·18 years, S.D. = 2·12, range = 18–26 years) who were of mainly Caucasian ethnicity (87%). Subjects were participating in the Michigan State Twin Study and were recruited through a university registrar's office, State of Michigan birth records, and several

forms of advertisement (e.g. newspaper advertisements, flyers).

Assessments

Disordered eating. The total score from the Minnesota Eating Behaviors Survey (MEBS; Klump *et al*. 2000)† was used to assess overall levels of disordered eating symptoms. The MEBS assesses levels of body dissatisfaction, weight preoccupation, binge eating, and compensatory behaviors. Higher scores indicate more disordered eating. The MEBS shows good psychometric properties (Klump *et al*. 2000) and successfully differentiates between women with AN and BN and controls.

Finger-length ratios. Finger-length ratios were calculated from measurements made with a standard ruler (in centimeters) of photocopies of the hands. A template was used for photocopies to ensure standard hand position for all subjects. One research assistant conducted all of the initial measurements of 2D and 4D. A second rater scored a subsample of copies ($n = 36$ copies of both hands) and achieved excellent inter-rater reliability on the 2D and 4D measurements with the initial rater (all intra-class r's$>0{\cdot}97$). In addition, measurements of fingers from hand photocopies showed excellent convergence with in-person measurements of finger lengths (all r's$>0{\cdot}90$; $n = 15$) and with X-rays of hands in previous research (Manning *et al*. 2000). Lower 2D : 4D ratios suggest greater prenatal androgen exposure (Manning *et al*. 1998; Brown *et al*. 2002).

Statistical analyses

Associations between disordered eating and finger-length ratios were examined using Pearson correlations. Given previous research showing ethnic differences in finger-length ratios (Manning *et al*. 2004), correlations were conducted within the entire sample as well as within Caucasian subjects only. Results were essentially identical (data not shown), and thus only correlations within the full sample are reported.

† The Minnesota Eating Behavior Survey [MEBS; previously known as the Minnesota Eating Disorder Inventory (M-EDI)] 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, © 1983 by Psychological Assessment Resources, Inc. Further reproduction of the MEBS is prohibited without prior permission from Psychological Assessment Resources, Inc.

Study 2: Activational influences

Participants

Sample 1 included 24 females (mean age = 19·52 years, S.D. = 0·88, range = 18–21 years) recruited through a volunteer research pool at a large university. Sample 2 included 25 adult female twin individuals (mean age = 21·43 years, S.D. = 1·79, range = 18–25 years) who were participating in a Michigan State University twin study that was unrelated to that used for Study 1. Participants in both samples were mainly of Caucasian ancestry (>70%).

All participants were required to have regular menses (defined as no skipped periods in the past 6 months and no more than two total skipped cycles in the 6 months preceding) and to be free from oral contraceptive use, hormonal treatment, cigarette use, steroid medication use, and medical conditions known to influence steroid hormone functioning.

Assessments

Disordered eating. The MEBS total score was used to assess disordered eating.

Body mass index. Body mass index [BMI; weight (kg)/height^2 (m)] was calculated using height and weight measurements made with a wall-mounted metric ruler and digital scale, respectively.

Estradiol. Salivary estradiol samples were collected using salivettes at 08.30 hours after an overnight fast (i.e. no food or drink) in the follicular phase of the menstrual cycle (i.e. days 1–3 after the cessation of menses). Samples were frozen and subsequently analyzed by Salimetrics, Inc. (State College, PA, USA). Radioimmunoassay (RIA) techniques were used for analyzing specimens from sample 1. The RIA test (Diagnostic Systems Laboratory, Webster, TX, USA) uses 300 μl of saliva sample per tube. The lower limit of sensitivity is 0·25 pg/ml, range of standard curve from 0·375 pg/ml to 7·5 pg/ml and average intra- and interassay coefficients of variation of less than 6·45% and 9·0% respectively. Method accuracy, determined by spike recovery, and

linearity, determined by serial dilution, are 100·6% and 91·2% respectively. Values from matched serum and saliva samples show the expected strong linear relationship for females ($r = 0{\cdot}80$) (Shirtcliff *et al.* 2000).

By contrast, an enzyme immunoassay (EIA) was used for analyzing sample 2 as this technique requires lower sample volumes. Saliva samples (150 μl) were diluted 1 : 2 in assay diluent and well mixed. A 100 μl aliquot of diluted sample was then pipetted into individual wells of a 96-well microtiter plate. The estradiol lower limit of sensitivity is 1 pg/ml, range of standard curve from 2 pg/ml to 64 pg/ml, and average intra- and interassay coefficients of variation of 5·75% and 6·87% respectively. Method accuracy, determined by spike recovery, and linearity, determined by serial dilution, averaged 103·9% and 103·5% respectively. Estradiol results determined using the EIA protocol are highly correlated [$r(50) = 0{\cdot}97$, $p < 0{\cdot}0001$] with those returned using the RIA method described above.

Statistical analyses

Estradiol values are reported in picograms/milliliter and were log transformed due to positive skew. Because body weight has been strongly and consistently shown to correlate with estradiol levels (Wade, 1972), partial correlations were used to examine relationships between estradiol levels and disordered eating, partialling out BMI. We also examined whether symptoms of depression (assessed with the Beck Depression Inventory; Beck & Steer, 1987) and anxiety (assessed with the Spielberger State Trait Anxiety Inventory; Spielberger *et al.* 1970) influence associations. Overall, partial correlations indicated that neither depression nor anxiety consistently influenced estradiol/disordered eating relationships (data not shown). Thus, we focus on partial correlations that account for BMI only in the results reported here.

RESULTS

Table 1 includes descriptive statistics for MEBS total scores, finger-length ratios, estradiol levels, and BMIs for study subjects. The MEBS total scores were in the mild to moderate range across all samples, although the scope of scores (0–25 in Study 1; 2–23 and 0–14 in samples 1 and 2 respectively from Study 2) was broad and included scores in the severe range. Indeed, 13–26% of the subjects across studies scored above the mean for eating disorder subjects (mean = 12·00) (Klump *et al.* 2000). Means of other variables were either similar to previous research (i.e. finger-length ratios; Manning, 2002) or similar across samples (e.g. BMI).

Table 1. *Means* (*standard deviations*) *for disordered eating*, *finger-length ratios*, *estradiol and weight variables*

Variables	Mean (S.D.)
Study 1 ($n = 113$)	
MEBS total score	8·01 (6·00)
Right 2D : 4D	0·99 (0·04)
Left 2D : 4D	1·00 (0·05)
BMI	23·12 (4·71)
Study 2	
Sample 1 ($n = 24$)	
MEBS total score	7·58 (5·30)
Estradiol	0·24 (0·12)
BMI	22·35 (4·21)
Sample 2 ($n = 25$)	
MEBS total score	6·68 (4·55)
Estradiol	7·79 (6·07)
BMI	23·38 (4·11)

MEBS, Minnesota Eating Behavior Survey; 2D : 4D, length of index (second digit) finger (in cm) divided by the length of the ring (fourth digit) finger (in cm); BMI, body mass index [weight (kg)/height^2 (m)]; estradiol, salivary estradiol (pg/ml). Differences in means between sample 1 and sample 2 estradiol levels can be accounted for by differences in assay techniques (i.e. radioimmunoassay for sample 1 and enzyme immunoassay for Study 2).

Given our unidirectional hypotheses, one-tailed p values were used for all analyses.

Study 1

Pearson correlations examining relationships between finger-length ratios and disordered eating were significant (right 2D : 4D, $p = 0{\cdot}005$; left 2D : 4D, $p = 0{\cdot}006$) and in the predicted direction (see Fig. 1). These findings indicate that lower levels of prenatal androgen exposure are associated with increased eating disordered symptoms.

Study 2

Partial correlations between disordered eating and estradiol levels were of moderate effect sizes (Cohen, 1988) and showed positive associations between estradiol levels and disordered eating in both samples (sample 1, $p = 0{\cdot}05$; sample 2,

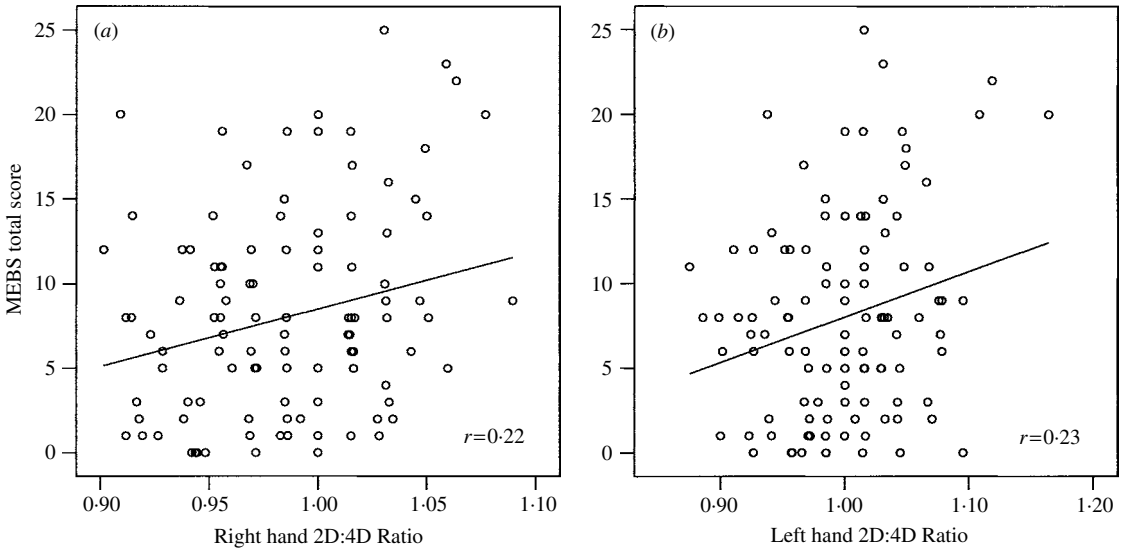

FIG. 1. Pearson correlations between disordered eating symptoms and finger-length ratios: (*a*) right-hand ratios ($n=113$); (*b*) left-hand ratios ($n=113$). Disordered eating was assessed with the total score of the Minnesota Eating Behavior Survey (MEBS).

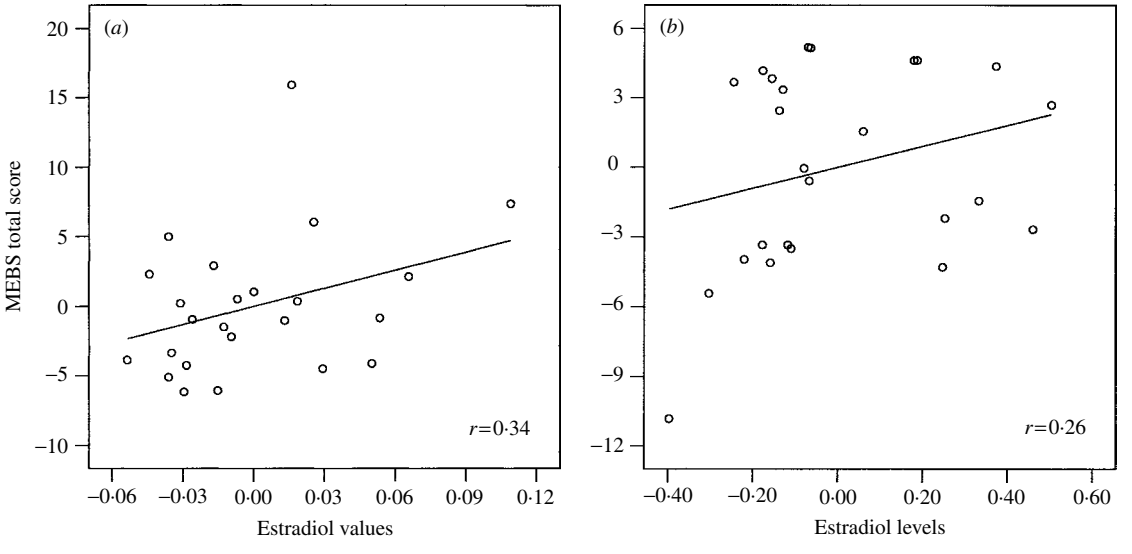

FIG. 2. Partial correlations between disordered eating and circulating estradiol levels in (*a*) sample 1 ($n=24$) and (*b*) sample 2 ($n=25$). Disordered eating was assessed with the total score from the Minnesota Eating Behavior Survey (MEBS). Correlations are partial correlations accounting for the effects of body mass index (BMI). Thus, values for MEBS total score and estradiol levels are residual scores after BMI has been partialled out.

$p=0·07$) (see Fig. 2). The combined probability of this covariation occurring by chance alone in the two samples is 0·005, indicating that higher circulating levels of estrogens increase the probability of disordered eating.

DISCUSSION

Both the organizational and activational effects described above would be expected to make eating disorders more common in females than

in males, suggesting that gonadal hormones may affect disordered eating symptoms known to increase the risk for AN and BN (Jacobi *et al.* 2004). The mechanism of these effects remains unclear; they could be direct (i.e. gonadal hormones directly organize and activate predispositions to disordered eating symptoms) or indirect (i.e. gonadal hormones influence body fat compositions, appetitive characteristics, etc. that then increase risk for engaging in disordered eating practices). However, developmental twin studies (Klump *et al.* 2003) showing the activation of genetic effects on disordered eating during puberty indicate that at least some of the gonadal hormone influences may be genetically mediated. Thus, speculative hypotheses about the nature of the genetic effects are warranted.

In animal models, early androgen exposure characteristic of males makes the brain less responsive to estrogens in adulthood. For example, female rodents and primates exposed perinatally to testosterone are much less likely to display sexual receptivity as adults, even if supplied with exogenous estrogen (Phoenix *et al.* 1959; Thornton & Goy, 1986). Our findings suggest that part of the genetic diathesis for eating disorders may be organized by relatively low prenatal exposure to androgen typical of females and then activated at puberty and maintained in adulthood by circulating estrogens.

The activated genetic influence may be related to genes encoding for the estrogen receptors or neuronal systems that are influenced by circulating estrogens. A significant association between a particular variant of the estrogen receptor beta (ERβ) gene and AN (Eastwood *et al.* 2002) and BN (Nilsson *et al.* 2004) has been reported, although some conflicting findings exist (Rosenkranz *et al.* 1998). The altered function of this variant may be revealed when estrogen levels rise during puberty. ERβ plays a key role in the anorexic effects of estrogen on food intake in rats; selective inhibition of ERβ blocks the ability of exogenous estrogen to reduce food intake (Liang *et al.* 2002). These ERβ functions may operate through the paraventricular nucleus (PVN) of the anterior hypothalamus, which is involved in the estrogen-mediated influences on food intake and body weight. Indeed, ERβ is the predominant estrogen receptor in the PVN, regulating most estrogen-mediated neuroendocrine activities in this region in mice (Zhang *et al.* 2004).

Estrogen may also affect eating pathology by influencing the function of neurotransmitters. Alterations in serotonergic functioning have been repeatedly linked to eating disorders with several studies suggesting a particular association with the 5-HT2A receptor (Frank *et al.* 2002; Bailer *et al.* 2004). This receptor is more sensitive to estrogen regulation than others (Ostlund *et al.* 2003) and shows the strongest association with AN of any candidate gene examined to date (Gorwood *et al.* 2002; Klump & Gobrogge, 2005).

Taken together, our preliminary data suggest a speculative, yet intriguing hypothesis – that the genetic diathesis of disordered eating is organized by prenatal androgens, triggered by the rise in estrogens during puberty in girls, and exacerbated throughout adulthood by circulating estrogens. This genetic effect may be related to estrogen-regulated genes that eventually lead to disordered eating symptoms that increase risk for full syndromal AN and BN. This theory remains largely untested, as our data only provide preliminary support for organizational and activational hormone effects. We hope that future research will examine this hypothesis directly to determine its relevance for the etiology and genetic diathesis of eating disorders.

Several limitations of our studies should be noted. First, sample sizes in our estradiol studies were small. Nonetheless, the correlations represent moderate effect sizes and were replicated across two independent samples of women, suggesting that they are clinically significant and robust. Second, because this was a pilot study, we used single assessments of hormones during one phase of the menstrual cycle. Additional research is needed to determine whether similar phenotypic associations are present across menstrual cycle phases. Preliminary efforts in this realm have been promising (e.g. Lester *et al.* 2003).

Third, we did not examine circulating levels of testosterone in the adult women. Some research has suggested that women with polycystic ovary syndrome (PCOS) might have increased rates of BN in adulthood (Raphael *et al.* 1995; Morgan *et al.* 2002). PCOS is associated with increased circulating levels of testosterone as well as

increased levels of estradiol (Mitwally & Casper, 2004; Carmina *et al*. 2005; Gadducci *et al*. 2005). It is possible that binge eating in these subjects is associated with increased estradiol levels (rather than testosterone) that result from anovulation (Carmina *et al*. 2005) as well as the aromatization of testosterone into estradiol (Geary, 2001; Hirschberg *et al*. 2004; Mitwally & Casper, 2004). Examining associations between testosterone, estradiol and disordered eating in adult women without PCOS would be the first step in examining this possibility. Although some animal research suggests that PCOS might be associated with increased prenatal exposure to testosterone (Abbott *et al*. 2002), and thus BN might also be associated with increased exposure, the PCOS animal model has not been extended to humans (Dumesic *et al*. 2005) and our data did not show this association. In fact, our data confirmed our hypothesis of decreased prenatal androgen exposure correlating with increased levels of disordered eating. More work is needed to replicate these associations and clarify their meaning for relationships between testosterone, PCOS and eating disorders.

A fourth limitation of our study is that we focused on circulating levels of estradiol in adult women rather than girls during the pubertal period. Future longitudinal research is needed to confirm that the rise in estrogens during puberty activates the disordered eating symptoms and their genetic diathesis. Fifth, we examined eating disorder symptoms rather than clinical eating disorders. However, the symptoms investigated are some of the strongest prospective predictors of AN and BN (Jacobi *et al*. 2004), suggesting that they indeed have relevance for the clinical syndromes. In addition, the neuroendocrine abnormalities that result from AN and BN (i.e. attenuated ovarian hormone functioning) make it difficult to examine activational hormone influences in women who are already ill with these conditions. Although it is possible that subsyndromal symptoms may influence hormone levels, the direction of effects would be the opposite (i.e. depressed estradiol levels correlating with eating disorder symptoms) to what we observed.

Finally, our hypothesis of genetic mediation of hormone influences remains to be tested. Additional twin and molecular genetic studies that directly test this hypothesis for a range of eating disorder symptoms and syndromes are needed to confirm our theory of genetic mediation of observed effects.

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DECLARATION OF INTEREST

None.

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