Eating Disorders, Psychopathology, and Temperament in Opposite Sex and Same Sex Twins

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ABSTRACT

The purpose of this study was to examine the hypothesis that prenatal exposure to sex hormones affects the risk for developing eating disorder symptoms, full syndrome eating disorders, other psychiatric disorders with known sex differences, and temperament traits in members of opposite sex (OS) versus same sex (SS) twin pairs. Female (N=9433) and male (N=7025) twins from the Swedish Twin Registry with known co-twin sex and zygosity completed questionnaires assessing eating disorder attitudes and behaviors, lifetime history of other psychiatric disorders (including alcohol abuse, substance use, mood disorders, anxiety disorders, and autism/Aspergers), and temperament traits. Females were analyzed separately using logistic regressions and analysis of variance with generalized estimating equations applied to control for the correlated nature of the data. All comparisons were across monozygotic SS twins, dizygotic SS twins, and dizygotic OS twins. Additional analyses were conducted with the full sample to assess whether a linear trend existed across female and male OS and SS twins. After controlling for multiple comparisons, no significant differences were found. Presumed prenatal exposure to sex hormones was not associated with risk or protection for developing eating disorder behaviors, full syndromes, other psychiatric disorders, or temperament traits in this sample.

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LIST OF ABBREVIATIONS

ADHD: Attention deficit hyperactivity disorder

AN: Anorexia nervosa

BMI: Body mass index

BN: Bulimia nervosa

DZ: Dizygotic

FDR: False discovery date

GAD: Generalized anxiety disorder

GEE: Generalized estimating equations

MDD: Major depressive disorder

MZ: Monozygotic

OCD: Obsessive-compulsive disorder

OS: Opposite sex

SS: Same sex

STR: Swedish Twin Registry

Introduction

Anorexia nervosa (AN) and bulimia nervosa (BN) are serious psychiatric disorders. AN is marked by low weight, restrictive eating, overvaluation of weight and shape, and in some subtypes, binge eating and purging (American Psychiatric Association, 2000). BN is marked by binge eating, purging, and the overvaluation of weight and shape in the absence of low weight (American Psychiatric Association, 2000). A significant sex difference exists in both AN and BN with population-based prevalence estimates three to four times higher in females than males (Bulik, et al., 2006; Hudson, Hiripi, Pope, & Kessler, 2007). These estimates are lower than the previously reported ratio of 10:1 female to male (American Psychiatric Association, 2000), as the initial estimates were from clinical and case registry studies and reflect sex differences in treatment-seeking samples rather than actual sex differences in population prevalence (Fairburn & Beglin, 1990; Hoek & van Hoeken, 2003). Even the more conservative estimates, however, indicate a considerable sex difference. Despite the magnitude and consistency of this observation, we know little about factors that contribute to this sex difference. Several theories have been posited, including socio-cultural factors such as the pervasive thin ideal for women (Rolls, Fedoroff, & Guthrie, 1991), the influence of traditional female gender roles (Bruch, 1978; Steiner-Adair, 1986), genetic factors (Strober, Freeman, Lampert, Diamond, &

Kaye, 2001), and hormonal factors (Favaro, Tenconi, & Santonastaso, 2006; Klump, et al., 2006).

To further explore one possible contribution to the observed sex difference, my thesis focuses on the hypothesis that hormonal factors exert an effect on lifetime risk of eating disorder symptomatology as well as full syndrome eating disorders. Specifically, I address the hypothesis that prenatal exposure to testosterone acts as a protective factor in females for developing eating disorder symptoms and full syndromes. To address this hypothesis, I use twin methodology as a proxy measure of prenatal hormone exposure. I also extrapolate the prenatal hormone exposure theory to other comorbid psychiatric disorders and common temperamental and personality traits in eating disorders that also exhibit a sex difference in the population.

Developmental psychopathology of eating disorders

Eating disorder symptoms typically begin around the time of puberty (Hayward & Sanborn, 2002) and, although pre-pubertal cases have been documented, they are far less common (Bryant-Waugh & Lask, 1995; Stein, Chalhoub, & Hodes, 1998). Puberty is a temporal marker for the onset of weight preoccupation, body dissatisfaction, and binge eating (Bulik, 2002; Klump, McGue, & Iacono, 2003). Population based studies indicate that early menarche is a risk factor for both weight control behaviors and eating disorder symptoms (Attie & Brooks-Gunn, 1989; Welch, Doll, & Fairburn, 1997). In a study of 971 middle school-aged girls, Killen and colleagues found that sexual maturation, as measured by the Tanner questionnaire, was positively associated with meeting diagnostic criteria for BN (Killen, et al., 1992). Eating disorder symptoms have been shown to increase linearly across the

pubertal transition (Bulik, 2002; Graber, Brooks-Gunn, Paikoff, & Warren, 1994).

Other studies indicate that eating disorder symptoms plateau at mid-puberty, defined

as the fourth stage of the Tanner scale, or approximately age 13 (Hayward &

Sanborn, 2002; Killen, et al., 1992). Although consensus on the trajectory of

symptoms across the pubertal transition has not yet been reached, observations concur

that puberty is a high-risk time for the onset of eating disorder symptoms.

The Biology and Psychology of Puberty

Puberty is a period of enormous hormonal change for both females and males

and typically begins between ages 10-12 (DiVall & Radovick, 2008; Lewis & Lee,

2009). The beginning of puberty is marked by the release of gonadotropin-releasing

hormone (GnRH) by the hypothalamus. The release of GnRH subsequently stimulates

the gonads to release luteinizing hormone and follicle-stimulating hormone (DiVall &

Radovick, 2008). During puberty, female gonads begin producing higher levels of

estrogens (including estradiol) and progesterone, and, concurrently, adrenal glands

begin secreting androgens (including testosterone) (DiVall & Radovick, 2008). The

observable biological changes in females include breast development, pubertal hair

growth, increased adipose tissue, body odor, facial acne, and menarche (DiVall &

Radovick, 2008). In males, the primary sex hormone in puberty is testosterone, and

the physical changes include growth of body, facial and pubic hair, larynx growth

(with associated vocal tone deepening), body odor, acne, and increased muscularity

(Lewis & Lee, 2009).

These hormone changes carry not only biological implications but also related

psychosocial challenges. The psychosocial impact of the physical changes that

females undergo during puberty is considerable (Marino & King, 1980). The most notable physical change in females due to puberty is the increase in adipose tissue, or body fat. This increase in body fat has been associated with increased body dissatisfaction, decreased self-esteem, increased shame, and low mood (Attie & Brooks-Gunn, 1989). The increased body dissatisfaction and negative affect can result in girls attempting to control their weight and shape through extreme weight control behaviors (Graber, et al., 1994; Marino & King, 1980). Females who experience puberty earlier than their peers receive more negative attention and teasing due to their weight and breast development and report embarrassment and anger due to the teasing (Brooks-Gunn & Graber, 1994). These females may be less prepared emotionally and cognitively to deal with the physical and social changes associated with puberty (Brooks-Gunn & Graber, 1994). Females who experience early puberty have been shown to engage in more behaviors to try to lose weight and body fat than their peers who experience puberty later (Attie & Brooks-Gunn, 1989; Welch, et al., 1997).

The psychological response to puberty-related changes in girls is no doubt clinically significant and an important area to target for prevention and treatment; however, the elevated risk of eating disorders at puberty could also be due to a more direct biological effect of the changing hormonal milieu during puberty. Across the life span, as we have noted, eating disorder symptoms tend to emerge around puberty (Hayward & Sanborn, 2002), often become exacerbated during the premenstrual period (Klump, et al., 2003), and tend to remit around the menopausal years (Keel, Mitchell, Miller, Davis, & Crow, 1999; Strober, Freeman, & Morrell, 1997).

Although psychosocial theories could potentially explain this pattern of onset, exacerbation, and remission, a more direct biological effect should also be considered with the most likely culprit being gonadal hormones.

Estrogen and progesterone are the two main classes of hormones that increase during female puberty, as described above, and retreat during menopause (DiVall & Radovick, 2008). A relation between gonadal hormone levels and eating disorder symptoms exists, with both threshold BN and binge-eating symptoms associated with decreases in estradiol and increases in progesterone (Edler, Lipson, & Keel, 2007; Sundblad, Bergman, & Eriksson, 1994) in women. Although considerable research has been conducted on the impact of gonadal hormones in the onset of eating disorder symptoms in females, the effect of gonadal hormones on the onset of eating disorders in males has been understudied.

Prenatal Masculinization

While gonadal hormones may activate behaviors during puberty, prenatal hormone exposure has the potential to cause permanent effects on the body and brain (Navara & Nelson, 2009). Sexually differentiated behaviors are thought to result from effects of testosterone on the cells, body, and brain – a process called masculinization (Becker, et al., 2005). Animal studies indicate that masculinization of the central nervous system, through exposure to testosterone, can lead to the development of sexually dimorphic behaviors, including eating behaviors (Ryan & Vandenbergh, 2002). Early exposure to testosterone, even if not resulting in full masculinization at the cellular or organ level, limits the responsiveness of the brain to estrogen later in life (Phoenix, Goy, Gerall, & Young, 1959).

In rodents, the close proximity of a female to a male in-utero can trigger the masculinization process; females who were close to males in the womb exhibited more male behaviors such as mounting and increased aggression, adult body mass index, and total food intake (Ryan & Vandenbergh, 2002). This masculinization may result from the transfer of testosterone through amniotic fluid (Even, Dhar, & vom Saal, 1992) or the mother's bloodstream (Meulenberg & Hofman, 1991). Findings from the animal literature indicate that prenatal exposure to testosterone may have long-term effects on sexually dimorphic behaviors traits.

Applying Twin Methodology to the Study of Prenatal Masculinization

Manipulating testosterone exposure in humans is unethical, and longitudinal studies tracking changing hormone levels and associated eating disorder symptoms would be logistically prohibitive due to time, cost, and the low base rate of eating disorders. However, twins provide an excellent proxy through which to study the effects of prenatal hormone exposure in humans (Culbert, Breedlove, Burt, & Klump, 2008). Female members of opposite-sex (OS) twin pairs share their prenatal environment with a male and, therefore, similar to the animal studies described above, a female twin of an OS pair will be exposed to higher levels of testosterone prenatally than a female twin from a same-sex (SS) pair or a singleton female. Female twins from OS pairs do show some traditionally masculine personality characteristics, including higher sensation seeking and more aggressive behavior than females from SS pairs (Cohen-Bendahan, Buitelaar, van Goozen, Orlebeke, & Cohen-Kettenis, 2005). These temperament characteristics are not associated with pubertal status or the effects of circulating testosterone (Cohen-Bendahan, et al., 2005).

Although informative, applying twin methodology to this research question does not allow one to parse out the effects of socialization (i.e., being raised with a male co-twin) from the impact of prenatal exposure to testosterone. For example, the greater aggression seen in female members of OS pairs could be due to their prenatal exposure to testosterone or factors in their post-natal environment such as engaging in more rough-and-tumble play with their same-age brother. However, the effects of masculinization have been found in biological indicators that are less likely to be impacted by socialization. Female OS twins, compared with female SS twins, have more masculine cerebral lateralization (Cohen-Bendahan, Buitelaar, van Goozen, & Cohen-Kettenis, 2004), finger-length ratio (Manning, Scutt, Wilson, & Lewis-Jones, 1998), tooth size (Dempsey, Townsend, & Richards, 1999), and dental asymmetry (Boklage, 1985). Therefore, it appears as though prenatal exposure to testosterone for female twins of OS twin pairs may result in masculinization of some traits, and this process may be at least somewhat independent of socialization factors (Culbert, et al., 2008).

Prenatal Masculinization and Eating Disorders

The extent to which prenatal masculinization influences eating disorders and eating disorder symptoms is unclear. Three groups have applied twin methodology in four samples to investigate whether prenatal testosterone exposure impacts the liability to develop eating disorders by comparing prevalence estimates of eating disorder symptoms between SS and OS twins (Culbert, et al., 2008; Lydecker, et al., under review; Raevuori, et al., 2008). Twin studies can be used to address this question by comparing prevalence estimates: if one finds dissimilar prevalence

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estimates of eating disorder symptoms or syndromes in female OS versus SS twins, or male OS versus SS twins, then the effects of prenatal hormone exposure may play a role in liability to develop these disorders and symptoms. If one does not find a difference in prevalence between female SS versus OS twins or male SS versus OS twins, then the prenatal hormone exposure theory will not be supported. Using the Minnesota State Twin Registry, Culbert, Breedlove, Burt and Klump (2008) reported significant linear trends of disordered eating based on twin sex status; female SS pairs had the highest levels of disorder eating, followed by females from OS pairs, followed by males from OS pairs, and males from SS pairs (Culbert, et al., 2008). To test whether the effects of socialization rather than prenatal hormone exposure could account for their findings, Culbert and colleagues also compared OS female twins to non-twin females who lived with a brother. The females from OS twin pairs reported lower levels of disordered eating than the females living with a non-twin brother, possibly indicating that prenatal exposure to testosterone, rather than just growing up with a male sibling, is a protective factor for developing disordered eating (Culbert, et al., 2008). Although using females with a non-twin brother as a comparison group did allow the researchers to examine the effects of a male non-twin sibling in the house, they did not account for other potential confounds such as sibling age, birth order, or multiple siblings within a house, all of which may be related to risk for developing eating disorder symptoms.

Raevuori, Kaprio, Hoek, Sihvola and Keski-Rahkonen (2008) used an OS twin design with data from the Finnish Twin Registry to test the prenatal hormone exposure hypothesis. In addition to comparing rates of symptoms of disordered eating

between OS and SS twins, they also compared broad and narrow diagnoses of AN and BN. No significant differences were observed for eating disorder behaviors, broad, or narrow diagnoses of AN or BN across monozygotic (MZ) SS twins, dizygotic (DZ) SS twins, or OS twins (Raevuori, et al., 2008). Given the compelling research indicating genetic factors in the liability to develop both full syndrome eating disorders as well as eating disorder symptoms (Bulik & Tozzi, 2004; Mazzeo, Mitchell, Bulik, Reichborn-Kjennerud, et al., 2009), separating MZ and DZ twins in analyses is a methodological strength of this study. Analyzing MZ and DZ twins separately did not have any effect on their findings; the authors found no support for the prenatal hormone exposure hypothesis.

Lydecker, Mitchell, Kendler, Reichborn-Kjennerud, Bulik, and Mazzeo (under review) used data from the Mid-Atlantic Twin Registry and the Norwegian Twin Registry in two separate studies. In both studies, Lydecker and colleagues compared MZ SS twins, DZ SS twins, and OS twins on variables of eating disorder symptoms and full diagnoses. Neither sample provided any support for the prenatal hormone exposure hypothesis.

Given that the three studies that used the OS paradigm have had conflicting observations, my thesis aims to clarify and extend the uncertain findings of the prenatal hormone exposure literature on eating disorder symptoms and full diagnoses in an independent cohort of twins. As diagnoses for AN and BN are not stable across the lifespan (Eddy, et al., 2008; Tozzi, et al., 2005), contain overlapping and heritable diagnostic criteria (American Psychiatric Association, 2000; Mazzeo, Mitchell, Bulik, Reichborn-Kjennerud, et al., 2009), and have shared genetic factors (Bulik, et al.,

2009), I will compare the effects of prenatal hormone exposure both on individual eating disorders symptoms as well as threshold diagnostic syndromes. Acknowledging the limitations of this application of twin methodology, notably the inability to rule out socialization factors, I will use the OS twin design as a proxy measure of prenatal testosterone exposure to add to the literature on its impact on the emergence of eating disorders symptoms.

Impact of Prenatal Masculinization on Other Psychiatric disorders

Although eating disorders have one of the highest sex differences of any psychiatric disorder, many psychiatric disorders are more prevalent in one sex. Specifically, depression and anxiety disorders are more prevalent in women, whereas of the prevalence of autism spectrum disorders and alcohol and substance disorders are higher in men (Kessler, Chiu, Demler, Merikangas, & Walters, 2005; Kessler, et al., 1994). Genetic and psychosocial factors are often cited as the mechanism contributing to observed sex differences. Although neuroendocrinologists have long studied the effects of hormones on the organization of the brain and the relation of brain organization to behavior, these findings are just now being translated into the field of psychopathology (Martel, Klump, Nigg, Breedlove, & Sisk, 2009). Testosterone affects dopaminergic circuits that could result in males being more liable to develop disorders of inattention, such as attention deficit hyperactivity disorder (ADHD), or disorders of behavioral regulation (Martel, et al., 2009). Females, on the other hand, experience high levels of estradiol, which impacts the amygdala and serotonergic pathways rendering them more liable to develop mood based disorders such as depression or anxiety (Martel, et al., 2009).

The majority of twin research has examined the extent to which these psychiatric disorders are due to genetic and environmental factors by comparing concordance rates (i.e., both twins having the illness) of MZ and DZ twins in structural equation modeling to parse the variance in liability into additive genetic effects, shared environmental effects, or unique environmental effects (Bulik, Sullivan, Wade, & Kendler, 2000). However, little research using the OS and SS paradigm has been conducted for psychiatric disorders other than eating disorders. Given the hypothesis that prenatal hormone exposure affects brain organization and may be related to the development of sexually different illnesses, applying this methodology to other illnesses would be the logical next step. Therefore, my thesis builds on the prenatal hormone exposure hypothesis that has been tested in eating disorders by investigating whether other psychiatric disorders that are either comorbid with eating disorders or have a significant sex difference have different prevalence estimates in OS versus SS twins.

Impact of Prenatal Masculinization on Temperament and Personality

One of the most studied effects of masculinization in animals is aggressive behavior. Although temperamental and personality traits differ between the sexes and would be a logical extension of the prenatal hormone exposure theory, temperament and personality, other than aggression, has received little attention in either the animal or human literature. A recent meta-analysis showed that women tend to be higher in reward dependence and harm avoidance as measured by Cloninger's Temperament and Character Inventory (Cloninger, Svrakic, & Przybeck, 1993). Another meta-analysis found that, across 30 countries, females have higher scores on neuroticism,

and males have higher scores of extraversion on the Eysenck Personality Questionnaire (Lynn & Martin, 1997). However, no significant sex differences were found for novelty seeking, persistence (Miettunen, Veijola, Lauronen, Kantojarvi, & Joukamaa, 2007), or perfectionism as measured by the Multidimensional Perfectionism Scale (Parker & Adkins, 1995). Therefore, my thesis also further extends the prenatal hormone exposure hypothesis by examining differences in temperamental and personality traits between OS and SS twins.

Summary

Female sex is a risk factor for eating disorders, and given that eating disorders tend to emerge with puberty and remit with menopause, hormones may play a role in vulnerability to developing these disorders and symptoms. Animal studies show that prenatal hormone exposure has lasting effects on brain development as well as physical and behavioral characteristics. The prenatal hormone exposure hypothesis for eating disorders builds on the animal literature and posits that being an OS twin may be a protective factor for eating disorder liability for females and a risk factor for OS male twins. Further, we can extrapolate the prenatal hormone exposure hypothesis as a protective or risk factor to other disorders and temperaments and personality traits that have known sex differences and genetic vulnerabilities.

Hypotheses

I hypothesized that, due to masculinization, prenatal exposure to an OS twin will impact the prevalence of specific disorders, symptoms, and temperamental and personality traits. Hypotheses were based on behaviors, disorders, and temperament and personality traits with known sex discrepancies or, where no clear sex

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discrepancy exists, common comorbidity with eating disorders. Specifically, I hypothesized that:

1. *Eating disorders and behaviors:* The prevalence of eating disorders and behaviors will be lower in female members of OS twin pairs than female members of MZ or DZ SS twin pairs and higher in male members of OS twin pairs than male members of MZ or DZ SS twin pairs. Females from MZ and DZ SS twin pairs will have the highest rates, followed by females from OS twin pairs, followed by males from OS twin pairs, followed by males from MZ and DZ SS twin pairs.

2. *Other Psychiatric disorders:* The prevalence of autism/Aspergers, attention deficit hyperactivity disorder (ADHD), alcohol problems (abuse and/or dependence) and drug use (10 times or more in one month) will be higher in female members of OS pairs than female members of MZ and DZ SS pairs and lower in male members of OS pairs than male members of MZ or DZ SS pairs. For these disorders, males from MZ and DZ SS twin pairs will have the highest rates, followed by males from OS twin pairs, followed by females from OS twin pairs, followed by females from MZ and DZ SS twin pairs. The prevalence of major depressive disorder (MDD), generalized anxiety disorder (GAD), specific phobias, obsessive/compulsive disorder (OCD) and panic disorder will be higher in female members of MZ and DZ SS pairs than female members of OS pairs and lower in male members of MZ and DZ SS pairs than male members of OS pairs. For these disorders, females from MZ and DZ SS twin pairs will have the highest prevalence, followed by females

from OS twin pairs, followed by males from OS twin pairs, followed by males from MZ and DZ SS twin pairs.

3. *Temperament and personality traits:* Female members of OS twin pairs will have lower neuroticism and higher extraversion scores than female members of MZ and DZ SS twin pairs. Male members of OS twin pairs will have higher neuroticism and lower extraversion than male members MZ and DZ SS twin pairs. For neuroticism, females from MZ and DZ SS twin pairs will have the highest scores, followed by females from OS twin pairs, followed by males from OS twin pairs, followed by males from MZ and DZ SS twin pairs. For extraversion, males from MZ and DZ SS twin pairs will have the highest scores, followed by males from OS twin pairs, followed by females from OS twin pairs, followed by females from MZ and DZ SS twin pairs. I hypothesized that there will be no difference between members of OS and MZ or DZ SS twin pairs for females or males on levels of self-directedness, concern over mistakes, personal standards and doubt about actions as there are no consistent findings of sex differences in these traits.

Methods

Participants

Participants in the current study are from the Swedish Twin study of Adults:

Genes and Environment (STAGE) study of the Swedish Twin Registry (STR;

http://ki.se/ki/jsp/polopoly.jsp?d=9610&l=en), a large population-based prospective

sample of Swedish twins born 1959-1985 (Lichtenstein, et al., 2006). The STAGE

data are a subset of the larger STR database which consists of approximately 85,000

twin pairs. Data were collected in 2005 using web-base questionnaires.

Approximately 25,000 individuals responded from the total sample of 43,000

individuals, resulting in a response rate of 59.6%. The ages of the twins ranged from

20-47 years. The questions spanned 34 sections, including health, physiology,

biology, socio-demographics, socioeconomics, life habits and behaviors. The

Regional Ethics Committee at the Karolinksa Institutet and the Biomedical

Institutional Review Board at the University of North Carolina at Chapel Hill

approved this study. A detailed description of the study design is described elsewhere

(Lichtenstein, et al., 2002; Pedersen, Lichtenstein, & Svedberg, 2002).

Zygosity

We determined zygosity based on the responses to the following questions:

(1) During childhood, were you and your twin partner as like as 'two peas in a pod' or

no more alike than siblings in general? and (2) How often did strangers have

difficulty in distinguishing between you and your twin partner when you were children? Twin pairs were classified MZ if they *both* responded 'as alike as two peas in a pod' for Q1 and 'almost always' or 'often' for Q2. If *both* twins responded 'not alike' for Q1 and 'seldom,' 'almost never' or 'never' for Q2, they were classified DZ. All other twins were classified as 'not determined.' This algorithm was validated previously with a panel of 47 SNPs in a random sample of 198 twin pairs and ninety five percent (N=188) were correctly classified (Lichtenstein, et al., 2002).

Measures

Lifetime history of narrowly and broadly defined AN and BN were assessed using an expanded, on-line Structured Clinical Interview for DSM-IV Disorders (SCID)-based instrument. Full Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) criteria were used to develop algorithms for broad and narrow definitions of AN and BN (American Psychiatric Association, 2000). Criteria and responses required to meet diagnostic criteria are listed in Tables 1 (AN) and 2 (BN). Specifically, lifetime lowest adult body mass index (BMI; calculated as weight in kilograms divided by height in meters squared); lowest BMI during the period of active eating disorder illness; amenorrhea; binge eating with loss of control, frequency, and duration; and recurrent inappropriate compensatory behaviors were assessed. Individuals were asked: 1) whether there was a period of time that they "weighed much less than other people thought you ought to weigh;" 2) how much weight and shape influenced self-evaluation; 3) whether they felt fat during periods of low weight; and 4) how afraid they were about gaining weight or becoming fat during periods of low weight for the AN diagnosis. For the BN diagnosis they were asked: 1)

whether they had ever had eating binges when they ate "what most people would regard as an unusually large amount of food in a short period of time;" 2) whether they felt out of control during these binges; 3) whether they engaged in any purging behaviors; 4) the frequency and duration of the behaviors; and 5) whether "weight and shape are important things that affect how I feel about myself" or are "the most important things that affect how I feel about myself."

Lifetime prevalence of major depressive disorder and generalized anxiety disorder were assessed using the SCID-based instrument and full DSM-IV diagnostic criteria were used. Lifetime prevalence of specific phobias, panic disorder, OCD, autism/Aspergers and ADHD were assessed with the question "have you ever had any of the following problems." Response options were 'yes' and 'no.' Alcohol abuse and/or dependence were assessed using the SCID based instrument and used full DSM IV diagnostic criteria (American Psychiatric Association, 2000). Drug use was measured as using marijuana/hash, opioids, stimulants, hallucinogens, sedatives and/or hormones 10 times or more in one month which is Criteria 1 of drug dependence (American Psychiatric Association, 2000).

The temperament and personality variables were assessed using a Swedish language version of several questionnaires. Self-directedness was assessed using ten questions from the Temperament and Character Inventory (TCI) (Cloninger, Przybeck, Svrakic, & Wetzel, 1994). Chronbach's alphas for the SD subscale in a normative Swedish population is .81 (Brandstrom, et al., 1998). Concern over mistakes, personal standards, and doubt about actions were each assessed using four items from the subscales from the Frost Multidimensional Perfectionism Scale (Frost,

Marten, Lahart, & Rosenblate, 1990). Subscales from the Eysenck Personality

Questionnaire were used to assess extraversion (9 items) and neuroticism (18 items)

(Eysenck & Eysenck, 1975).

Statistical Analyses

Analyses were conducted using PROC GENMOD in SAS version 9.1 (SAS

Institute Inc., 2004). All continuous variables were standardized to a mean of zero

and a variance of one prior to analysis.

In the first set of analyses, males and females were analyzed separately and

comparisons were across MZ, DZ SS, and OS twins. To examine if differences in

prevalence of AN, BN, disordered eating behaviors, autism/Aspergers, mood

disorders, substance use disorders, or various anxiety disorders existed across the

zygosity groups, logistic regression analyses using generalized estimating equations

(GEE) were conducted. For the continuous measures, including lifetime lowest and

highest BMI and measures of temperament and personality, analysis of variance

(ANOVA) with GEE were applied to compare across zygosity groups. GEE accounts

for the non-independence of the data due to the use of relatives in the sample and

generates several statistics. Type 3 score statistics were used for testing the

significance of each independent variable in the model. GEE generates means

adjusted for cluster relationships as well as model covariates. If we randomly sampled

twins from a population, then the prevalence should be similar for all groups.

However, because pairs of twins were selected and genetic effects contribute to these

traits, then prevalence appears to be higher in MZ twins than DZ twins as MZ twins

share 100% of their genes. Therefore, it is important to use GEE to correct for the

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relatedness of twins. Post hoc contrasts, which use these adjusted means, were requested to assess pairwise group differences. The score statistics will be presented as χ^2 for all analyses. Age at interview and birth weight were entered as covariates in all models, except when the model could not run with both covariates due to low prevalence, in which case only age was entered as a covariate. Although I had directional hypotheses, all significance tests were two-tailed as χ^2 do not have normal distributions and cannot support one-tailed hypothesis testing. P-values were adjusted for multiple testing using the method of false discovery rate (Benjamini, Drai, Elmer, Kafkafi, & Golani, 2001), which controls for the expected proportion of Type I errors (i.e., rejecting the null hypothesis when it is true).

Analysis 1: MZ, DZ SS, and OS twins were compared on the prevalence of broad and narrow AN and BN and disordered eating behaviors (binging, vomiting, laxative use, diuretic use, excessive exercise and fasting), and on means of lifetime highest and lifetime lowest BMI. Males and females were analyzed separately.

Analysis 2: The prevalence of autism/Aspergers, alcohol problems, substance use, depression, and the anxiety disorder variables was compared across female members of MZ, DZ SS, and OS pairs and male members of MZ, DZ SS, and OS pairs.

Analysis 3: MZ, DZ SS, and OS twins scores on concern over mistakes, doubts about actions, personal standards, neuroticism, extraversion and self-directedness were compared in females and in males.

For the second set of analyses, females and males were analyzed in the same models. These analyses aimed to determine whether the prevalence of AN, BN,

disordered eating behaviors, autism/Aspergers, mood disorders, anxiety disorders or substance use disorders differed by sex, zygosity, and the interaction of sex and zygosity in the full sample. To examine if differences in prevalence of the disorders and the behaviors existed across sex and zygosity groups, logistic regression analysis using generalized estimating equations (GEE) were conducted. Analysis of variance (ANOVA) with GEE was used to compare across sex and zygosity groups on the continuous measures: lifetime lowest and highest BMI and measures of temperament and personality. The models for these analyses included the zygosity of the twin (MZ, DZ SS, or OS) the sex of the twin and the interaction of zygosity and sex as main effects. If the interaction of zygosity and sex was not significant, the model with only zygosity and sex as main effects was presented as it is the most parsimonious model. Age at interview and birth-weight were entered into all models as covariates.

Analysis 4: The prevalence of broad and narrow AN, BN and disordered eating behaviors (binging, vomiting, laxative use, diuretic use, excessive exercise and fasting) and means of lifetime highest and lifetime lowest BMI was compared across zygosity and sex.

Analysis 5: Males and females as well as zygosity groups were compared on the prevalence of autism/Aspergers, alcohol problems, substance use, depression, and the anxiety disorder variables.

Analysis 6: Scores on concern over mistakes, doubt about actions, personal standards, neuroticism, extraversion and self-directedness were compared across zygosity and sex.

Results

Eating Disorder Diagnoses and Behaviors: Females

Table 3 presents the lifetime prevalence of the eating disorder diagnoses (narrow and broad) and eating disordered behaviors and the means of lifetime lowest and highest BMI for the different female twin types (MZ, DZ SS, and OS). Lifetime prevalence of eating disorders, eating disordered behaviors and mean low and high BMI did not differ significantly across MZ, DZ SS, and OS twins after controlling for multiple comparisons.

Eating Disorder Diagnoses and Behaviors: Males

The prevalence of the eating disorder diagnoses (narrow and broad) and eating disordered behaviors and the means for lifetime lowest and highest BMI for the different male twin types are presented in Table 4. There were no differences in lifetime prevalence of eating disorder diagnoses or behaviors nor were there differences in lifetime lowest or highest BMI for males across MZ, DZ SS, or OS twins. However, no males met criteria for narrow AN, only eight males met criteria for broad AN (three MZ, two DZ SS and three OS), three met for narrow BN (one in each of the three zygosity groups) and 15 for broad BN (five MZ, three DZ SS and seven OS). Even in this large population based sample, the prevalence of eating disorders in males prohibited analysis of narrow AN, and the other ED diagnosis analyses may be underpowered.

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Other Psychiatric Disorders

Tables 5 and 6 present the lifetime prevalence of the other psychiatric disorders in female and males twins respectively by zygozity. Contrary to the hypothesis, there were no significant differences in prevalence of any of the disorders across zygosity for either females or males. Only five women had autism/Aspergers (three MZ, two DZ SS and zero OS); the low prevalence prevented analysis of this disorder.

Temperament and Personality

The means and standard deviations for the six temperament and personality variables are presented in Table 7 (females) and Table 8 (males). No significant differences across zygosity groups were observed for either females or males on any of the temperament or personality variables.

Analyses of Full Sample: Effects of Sex and Zygosity

The results from analyses including both sexes for the eating disorders, eating disordered behaviors, and the BMI measures are presented in Table 9. All models had a significant main effect of sex; however, none of the models had a significant main effect of zygosity. None of the models had a significant main effect of the interaction of sex and zygosity, therefore the models are presented with only main effects of sex and zygosity.

The results from the models including both sexes for the other psychiatric disorders are presented in Table 10. There was a significant main effect of sex for the models of GAD, OCD, panic disorder, phobias, autism/Aspergers, and alcohol problems but not for ADHD or substance use. There was not a significant main effect

of zygosity for any model. None of the models demonstrated a significant interaction between sex and zygosity; therefore, the models are presented with only main effects of sex and zygosity.

The results from models including both sexes for the temperament and personality variables are presented in Table 11. Significant sex differences were found for concern over mistakes, extraversion, neuroticism, and self-directedness but not for doubts about action or personal standards. No significant zygosity differences were found for any of the temperament and personality variables. None of the models demonstrated a significant interaction between sex and zygosity; therefore, the models are presented with only main effects of sex and zygosity.

Discussion

The purpose of this study was to investigate the validity of the prenatal hormone exposure theory and expand this approach to examine other psychiatric disorders and temperament and personality traits. No support for prenatal hormone exposure acting as either a protective or risk factor for liability to develop full DSM-IV diagnosed eating disorders, eating disordered behaviors, other psychiatric illnesses, or any temperament or personality traits was found. In both the female sample and the male sample, the prevalence of the disorders, eating behaviors, and the means of the temperament and personality variables of interest were similar across MZ, DZ SS and OS twins of the same sex. When the females and males twin samples were examined together, some significant main effects of sex emerged, but there were no significant main effects of zygosity for any behavior, disorder, or temperament or personality variable. These results concur with previous studies reporting limited or no evidence for the prenatal hormone exposure theory as it applies to eating disorders (Lydecker, et al., under review; Raevuori, et al., 2008). This is the first study, to my knowledge, to explore the prenatal hormone exposure theory for other psychiatric disorders, temperaments or personality traits.

The lack of association between zygosity and liability to develop full syndrome eating disorders or eating disordered behaviors in the current sample of Swedish twins does not support a previous finding of a linear trend of prevalence of

disordered eating across female SS and OS twins and male OS and SS twins (Culbert, et al., 2008). Potential explanations for these discrepant results include differences in the variables studied, the nature of the study population, and the analytic approach. Culbert and colleagues examined the effects of having an OS twin on a scale score of disordered eating derived from the Minnesota Eating Behavior Survey, not on threshold eating disorder diagnoses (Culbert, et al., 2008). Their disordered eating measure may capture more transient characteristics that vary with development as only a relatively small proportion of people with disordered eating develop a full syndrome eating disorder (Santonastaso, Friederici, & Favaro, 1999). The relation between their continuous measure and threshold eating disorders is unknown. Furthermore, the cognitive symptoms related to disordered eating, such as weight concern and the importance of weight/shape to self-evaluation, are more influenced by environmental factors and less heritable than eating disorder behaviors, such as amount of weight lost, vomiting, and laxative use (Mazzeo, Mitchell, Bulik, Aggen, et al., 2009; Mazzeo, Mitchell, Bulik, Reichborn-Kjennerud, et al., 2009; Reichborn-Kjennerud, et al., 2004). Therefore, the cognitive symptoms assessed in self-report measures of disordered eating may be less biologically determined than full threshold diagnostic presentations, suggesting that the positive findings from the Culbert and colleagues study might reflect factors associated with twin socialization rather than prenatal exposure to hormones. Although Culbert and colleagues attempted to control for socialization by comparing OS females to females with a non-twin brother, the presence of other siblings, specifically other female siblings was not accounted for, nor were DZ SS females compared to females with a non-twin sister (Culbert, et al.,

2008). Although an intriguing control, this approach only partially addressed the confound of socialization as having an opposite sexed brother of a different age is a markedly different interpersonal experience than having an opposite sex twin.

Another methodological difference that might have contributed to the divergent findings is the difference in sample demographics between the Minnesota Twin Registry and the other three samples. The sample from the Minnesota Twin Registry is a volunteer twin sample and may therefore be subject to sampling bias, whereas the other four samples examined were from population-based registries with good response rates. Given the methodological differences between the current study and the study published by Culbert and colleagues, as well as the negative findings from several population-based twin studies using similar methodology, the findings from the current study suggest that prenatal hormone exposure does not confer substantial effects on liability to develop eating disorders or eating disordered behaviors.

This study is the first, to my knowledge, to apply the opposite sex paradigm to examine liability to develop other psychiatric disorders and to examine temperament and personality traits. Although I had negative findings for an effect of zygosity for all of the psychiatric disorders and temperament and personality traits for both females and males, this study was just an initial investigation. We do not yet have sufficient evidence to conclude definitively that prenatal hormone exposure does not affect liability to develop psychiatric disorders or temperament and personality traits. Given the sex differences that exist in some psychiatric disorders and given the complex effects that testosterone and estrogen have on brain development and

neurotransmission (Martel, et al., 2009), it is important to note that this study relies on a proxy measure of prenatal hormonal exposure on risk for psychiatric disorders. This study was only able to assess whether the prevalence of certain disorders and means of temperament and personality traits are different between SS and OS twins. Alternative designs including animal models that allow direct manipulation of prenatal hormonal exposure may assist with elucidating mechanisms and conditions under which prenatal hormonal exposure could influence risk for the development of certain behaviors, and temperament and personality traits.

Strengths

The strengths of the current study include the large sample size, good participation rate, quality of the eating disorder diagnostic assessment, the inclusion of other psychiatric diagnoses and temperaments, and age of interview older than typical age of onset for eating disorders (Currin, Schmidt, Treasure, & Jick, 2005). This sample is by far the largest, and most adequately powered of all of the studies conducted to date on prenatal hormone exposure in OS and SS twins. The inclusion of other diagnoses—some with male preponderance—is also a strength by applying an intriguing methodology to disorders other than eating disorders.

Limitations

Although a strength for eating disorders, age at interview was a limitation for the analyses of some of the other disorders as some of the twins were as young as 20 years old, which is younger than the age of onset for some of the other disorders such as depression (American Psychiatric Association, 2000).

Other limitations include the inability to directly measure prenatal hormone exposure, the absence of data on age of menarche, small sample of males with eating disorders and females with austism/Aspgers, and self-report "yes/no" for some of the psychiatric disorders.

The majority of the work conducted on the prenatal hormone exposure theory has used either animal models or twins as a proxy for prenatal hormone exposure. The present design rests on the assumption that these fetuses are being exposed to the hormonal milieu of their twin, in the absence of direct measures of in utero hormone levels or measures of the effects of prenatal hormone exposure either on circulating levels of hormones in adulthood or on the brain development across the lifespan. The one marker of human perinatal androgen level, digit ratio, remains controversial (McIntyre, 2006) and as mentioned earlier, collecting hormone levels in utero is not feasible. Information on age at menarche was also unavailable. Given that early menarche can be a risk factor for developing an eating disorder (Attie & Brooks-Gunn, 1989; Welch, et al., 1997) and that the age at menarche in western societies is decreasing, those data could have assisted in addressing questions about the impact of prenatal hormonal exposure. Much of the twin literature on age at menarche compares MZ and DZ SS twins. It is unknown whether being a female member of an OS or a SS pair influences age at menarche and how this could then influence risk for eating disorder onset. This analysis would only be valuable had we found differences in prevalence of eating disorders in female members of OS and SS pairs.

Conclusions and Future Directions

Given four concurring results from independent samples and one dissenting report, the weight of the evidence suggests that prenatal hormone exposure does not play a measurable role in affecting liability to develop eating disorders. While it is possible that an effect exists, the effect is either too small to be detected by our methodology or is overshadowed by other factors. Given the limitations of this investigation, including limited "yes/no" self-report diagnoses, additional more sophisticated inquiries into the impact of prenatal hormonal exposure on risk for psychiatric disorders are warranted. Given that age of menarche (Attie & Brooks-Gunn, 1989; Welch, et al., 1997) and circulating levels of hormones do appear to impact liability to develop and the course of eating disorders and disordered eating (Edler, et al., 2007; Sundblad, et al., 1994), future investigations should evaluate the relation between the changing pubertal hormonal milieu and the emergence of mood and eating disorders symptoms in real time in longitudinal studies, rather than continuing to rely on a twin proxy. Similar designs may also shed light on the observed sex discrepancies of the other disorders, temperament and personality traits addressed in this investigation.

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Table 1. Criteria used for Anorexia Nervosa diagnoses

Criteria*/Item	Response to Meet Criteria
Anorexia Nervosa (AN) Narrow	
1a. Had a period of time when you weighed much less than other people thought you ought to weigh? 1b. BMI calculated from lowest weight and height at lowest weight.	1a. Yes AND 1b. BMI < 17.55
2. During the time of low weight, how afraid were you that you might gain weight or become fat? (response on 5 point Likert Scale)	2. (4) Very afraid OR (5) Extremely afraid
3. During the time of low weight, did you feel fat? (response on a 5 point Likert Scale)	3. (4) Very afraid OR (5) Extremely afraid
4a. Before this time, had your periods started? 4b. If yes, did they stop? 4c. For how long did they stop?	4a. If 'No,' then criteria met. OR If 'Yes,' then go to 4b. 4b. Yes 4c. At least 3 months
AN Broad	
1a. Had a period of time when you weighed much less than other people thought you ought to weigh? 1b. BMI calculated from lowest weight and height at lowest weight.	1a. Yes AND 1b. BMI < 18.55
2. During the time of low weight, how afraid were you that you might gain weight or become fat? (response on 5 point Likert Scale)	2. (4) Very afraid OR (5) Extremely afraid
3. During the time of low weight, did you feel fat? (response on a 5 point Likert Scale)	3. (4) Very afraid OR (5) Extremely afraid

Table 2: Criteria used for Bulimia Nervosa Diagnoses

Bulimia Nervosa (BN) Narrow	
1a. Have you even had eating binges when you ate what most people would regard as an unusually large amount of food in a short period of time? 1b. When you were having eating binges, did you feel your eating was out of control? (response on 5 point Likert Scale)	1a. Yes AND 1b.(4) Very much OR (5) Extremely
2. Which of these did you use during the same time that you were binge eating? Making yourself vomit? Laxatives? Diuretics? Diet Pills? Exercise more than 2 hours per day? Fast or not eat? Other methods?	2. A 'Yes' response to any of these items meets criteria.
3a. When you were binging the most, how many binges would you have in a month? 3b. For how long did you have binge eating episodes?	3a. At least 8 times a month AND 3b. At least 3 months
4. Statements regarding weight and shape on a 5 point Likert scale	4. Weight or shape are important things that affect how I feel about myself. OR Weight or shape are the most important things that affect how I feel about myself.
BN Broad	
1a. Have you even had eating binges when you ate what most people would regard as an unusually large amount of food in a short period of time? 1b. When you were having eating binges, did you feel your eating was out of control? (response on 5 point Likert Scale)	1a. Yes AND 1b.(4) Very much OR (5) Extremely
2. Which of these did you use during the same time that you were binge eating? Making yourself vomit? Laxatives? Diuretics? Diet Pills? Exercise more than 2 hours per day? Fast or not eat? Other methods?	2. A 'Yes' response to any of these items meets criteria.
3a. When you were binging the most, how many binges would you have in a month? 3b. For how long did you have binge eating episodes?	3a. At least 4 times a month AND 3b. At least 3 months
4. Statements regarding weight and shape on a 5 point Likert scale	4. Weight or shape are important things that affect how I feel about myself. OR Weight or shape are the most important things that affect how I feel about myself.