PRENATAL TESTOSTERONE EXPOSURE AND DEVELOPMENTAL DIFFERENCES IN RISK FOR DISORDERED EATING

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A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Psychology

2011

ABSTRACT

PRENATAL TESTOSTERONE EXPOSURE AND DEVELOPMENTAL DIFFERENCES IN RISK FOR DISORDERED EATING

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Prenatal testosterone may masculinize (i.e., lower) risk for disordered eating and account for sex differences in prevalence, yet how these effects emerge *and* whether these effects remain static across development is unknown. Opposite-sex (OS) twins provide a natural design to investigate such effects, as OS female twins are thought to be exposed to elevated testosterone in utero from their male co-twin. Although OS female twins have shown masculinized disordered eating relative to other females, findings have been mixed. The current research used a series of studies to investigate whether there are developmental differences in the masculinizing/protective effects of prenatal testosterone exposure in risk for disordered eating.

Study 1 examined whether age moderates the masculinizing effects of prenatal testosterone on disordered eating. OS female twins have shown masculinized disordered eating in early young adulthood, but these effects have not been robustly observed in other time periods, e.g., mid-to-late adolescence or mid-to-late young adulthood. Participants included 764 male and female twins (ages 15-30) and 74 non-twin females (ages 15-23) from the Michigan State University Twin Registry (MSUTR). Two well-validated measures (i.e., Minnesota Eating Behaviors Survey and the Eating Disorder Examination Questionnaire) were used to assess several disordered eating symptoms. Results indicated no evidence for masculinization of disordered eating in OS female twins during mid-to-late adolescence (ages 15-20). In contrast, OS female twins showed substantially masculinized levels of disordered eating across several scales in early young adulthood (ages 21-23). Masculinization of disordered eating in OS female

twins also appeared to be present in mid-to-late young adulthood (ages 24-30), but effects were weaker and more variable across disordered eating scales. These findings suggest developmental windows of expression for the protective effects of prenatal testosterone on disordered eating, with effects strongest under "average" risk periods (i.e., young adulthood) and attenuated under higher risk periods (i.e., mid-to-late adolescence, the peak period for eating disorder onset).

Study 2 was a translational extension of study 1 that aimed to determine if prenatal testosterone's masculinizing effects on disordered eating only become prominent during young adulthood (as observed in study 1), or whether, as predicted by animal data, masculinization effects emerge with puberty. In female animals, early testosterone exposure decreases sensitivity to ovarian hormones during and after puberty. Thus, one potential mechanism for prenatal testosterone's effects on disordered eating may be via decreased sensitivity to the activating effects of ovarian hormones on disordered eating risk. Study 2 examined whether puberty underlies the emergence of prenatal testosterone's masculinization of disordered eating, independent of the confounding effects of several other factors (e.g., adiposity, mood, autonomy, being reared with a brother). Participants included 394 male and female twins and 63 non-twin females (ages 10-15) from the MSUTR. Well-validated measures assessed disordered eating, pubertal status, mood symptoms, and autonomy difficulties. Body mass index was used as a marker of adiposity. Disordered eating did not differ amongst twin types in pre-early puberty, whereas OS female twins fell intermediate to males and SS female twins on levels of disordered eating during mid-late puberty. Masculinization effects in mid-late pubertal OS female twins were not accounted for by adiposity, mood symptoms, autonomy difficulties or being reared with a brother. Taken together, findings indicate that other key factors (e.g., sensitivity to circulating gonadal hormones) likely underlie prenatal testosterone's protective effects on disordered eating.

ACKNOWLEDGEMENTS

I am indebted to the many supportive individuals who have aided in my training and made the completion of this product possible. First and foremost, I am incredibly fortunate to have had the opportunity to be advised and mentored by Dr. Kelly Klump throughout the past nine years of my undergraduate and graduate training. Kelly's enthusiasm for translational biological clinical research turned a student initially intimidated/avoidant of the sciences, into someone passionate and eager to continue in this line of work. I will forever be thankful for the tremendous amount of time, energy, guidance, and support that she has devoted to my personal and professional development. My skills and potential as a clinician and researcher have been greatly enhanced as a result of Kelly's influence as a scholar, teacher, and mentor. I am also fortunate to have had the opportunity to develop my training under the guidance and expertise of my other dedicated and talented committee members, Drs. Alex Burt, Marc Breedlove, and Cheryl Sisk. Each of these mentors offered many valuable training opportunities, feedback, and novel perspectives that contributed to my overall program of research, and specifically, the series of studies discussed herein.

Thank you also to my family for believing in me even when I did not believe in myself, remaining enthusiastic when my enthusiasm wore thin, and continuously reminding me of how blessed I am for the many opportunities I have had throughout my training. Your generosity, support, and patience have allowed me to remain focused and made the completion of this program and project possible. I am grateful to have each of you as a part of my life and love you all very much.

Several sources of funding also made a financially unfeasible project, feasible. I am thankful to the many sources of research support and all of the participants that volunteered their time for study completion. This project was partially supported by:

- a pre-doctoral institutional training fellowship from the National Institute of Mental Health (T32-MH070343)
- a pre-doctoral individual Kirschstein National Research Service Award from the National Institute of Mental Health (1F31-MH084470)
- a Student Research award from the Academy for Eating Disorders (AED)
- a Student Award Program grant from the Blue Cross and Blue Shield of Michigan Foundation (1412.SAP)
- a Dissertation Research award from the American Psychological Association
- the Clarence J. Rosecrans Scholarship from the American Psychological Foundation and Council of Graduate Department of Psychology
- a Graduate Student Research Enhancement award from Michigan State University
- the John Hurley Endowed Fellowship award from the Clinical Psychology program at Michigan State University

Additional funding sources awarded to Dr. Kelly Klump, include:

- a College of Social Science Faculty Initiatives award from Michigan State University
- an Intramural Grant from Michigan State University (71-IRGP-4831)
- Award number 1R21MH63851-01 from the National Institute of Mental Health
- Award number 1R03MH63851-01 from the National Institute of Mental Health

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BD = Body Dissatisfaction

BE/CB = Binge Eating/Compensatory Behavior

BMI = Body Mass Index

EDE-Q = Eating Disorder Examination Questionnaire

EDI = Eating Disorder Inventory

MEBS = Minnesota Eating Behaviors Survey

MLM = Mixed Linear Model

OS = Opposite-sex

SC = Shape Concerns

SS = Same-sex

WC = Weight Concerns

WP = Weight Preoccupation

Chapter 1: Sex Differences and Prenatal Testosterone Effects

Sex differences in eating disorder prevalence are among the most pronounced of any psychiatric disorder. The female-to-male ratio is estimated to range from 4:1 to 10:1 (American Psychiatric Association, 2000; Hudson, Hiripi, Pope, & Kessler, 2007). This sex disparity is often attributed to sociocultural factors that may preferentially increase risk for eating disorders in females, particularly pressures for thinness (Striegel-Moore & Bulik, 2007). Although the influence of sociocultural factors cannot be understated, biological factors may also be important (Culbert, Breedlove, Burt, & Klump, 2008; Klump, Gobrogge, Perkins, Thorne, Sisk, & Breedlove, 2006). Nonetheless, relatively little consideration has been given to the role of biological factors in sexually differentiated risk, resulting in significant gaps in current conceptualizations of eating disorders.

Sex steroids are one set of biological factors that are particularly promising candidates for understanding sex differences in risk for eating disorders (Culbert et al., 2008; Klump et al., 2006). Testosterone is critical for the development of sexually-dimorphic characteristics. Exposure to testosterone early in life (i.e., prenatal period in primates; prenatal/perinatal periods in rodents) results in organizational changes to brain structure/function and behavior (Breedlove, 1994; Collaer & Hines, 1995). Organizational effects of hormones are those that change brain structure and function during critical developmental periods (Breedlove, 1994; Collaer & Hines, 1995). These organizational changes are thought to be permanent and persist beyond initial hormone exposure. The prenatal period has been recognized as the traditional organizational period in humans since much of somatic and neural sexual differentiation is driven by testosterone (Breedlove, 1994; Collaer & Hines, 1995). Indeed, if testosterone is present early in

life, male-development will emerge. Female-typical development arises in the absence of testosterone exposure early in life (Breedlove, 1994; Collaer & Hines, 1995).

Animal studies have shown that testosterone's organization of the central nervous system during early development underlies the masculinization (i.e., male-like pattern) of several sexually differentiated phenotypes. Male and female rodents positioned next to males in utero are exposed to elevated levels of prenatal testosterone, and consequently, later show masculinization on several characteristics (Ryan & Vandenbergh, 2002). For example, female rodents that developed in utero between two males display masculinized anatomy (e.g., longer anogenital distance) as well as physiological (e.g., greater sensitivity to circulating testosterone, later pubertal onset, longer estrous cycles) and behavioral (e.g., higher levels of aggression, higher frequency of mounting other females) phenotypes (Ryan & Vandenbergh, 2002). Importantly, the degree of masculinization appears to be related to the degree of intrauterine testosterone exposure, as female rodents positioned between two males are more masculinized than females positioned between a male and a female (Ryan & Vandenbergh, 2002). These masculinization effects are blocked when mothers are treated with antiandrogens, highlighting the critical role of testosterone (Clemens, Gladue, & Coniglio, 1978).

The organizational effects of early testosterone exposure have also been observed for food intake, a key behavior disrupted in eating disorders. Female rats exogenously treated with testosterone during neonatal development display masculinized (i.e., elevated) food intake as adults (Bell & Zucker, 1971; Madrid, Lopez-Bote, & Martin, 1993; Wade, 1972). Castration of male rats on the day of birth results in female-like patterns of feeding behavior, in that permanent decreases in food intake are observed (Wade, 1972). Taken together, findings demonstrate that

alterations of testosterone exposure during critical periods of development produce long-lasting effects that shape sex-typical characteristics, including feeding behaviors.

The masculinizing effects of prenatal testosterone may extend to eating pathology, and thus, have important implications for sex differences in eating disorder risk. Since prenatal testosterone exposure cannot be directly manipulated in humans, two non-invasive methods have been employed – studies of: 1) finger-length ratios [index finger (2D)/ring finger (4D)], and 2) opposite-sex (OS) twin pairs.

Finger-length ratios are sexually dimorphic (i.e., lower 2D:4D in males; Manning, Scutt, Wilson, & Lewis-Jones, 1998) as early as fetal development (Malas, Dogan, Evcil, & Desdicioglu, 2006) and are considered a marker of the prenatal testosterone:estradiol ratio. Lower 2D:4D ratios have been associated with higher levels of prenatal testosterone relative to prenatal estradiol (Lutchmaya, Baron-Cohen, Raggatt, Knickmeyer, & Manning, 2004). Further evidence for the 2D:4D ratio as a proxy of prenatal testosterone exposure is suggested by masculinized (i.e., lower) 2D:4D in individuals exposed to high levels of androgens prenatally (e.g., males and females with congenital adrenal hyperplasia; Brown, Hines, Fane, & Breedlove, 2002; Okten, Kalyoncu, & Yari, 2002) and feminized (i.e., higher) 2D:4D in individuals with XY androgen insensitivity syndrome (Berenbaum, Bryk, Nowak, Quigley, & Moffat, 2009). Studies have also linked lower 2D:4D to several sex-differentiated phenotypes (e.g., attention deficit hyperactivity disorder, autism, psychopathy, schizophrenia; Blanchard & Lyons, 2010; Collinson, Lim, Chaw, Verma, Sim, Rapisarda, & Chong, 2010; De Bruin, Verheij, Wiegman, Ferdinand, 2006; Martel, Gobrogge, Breedlove, & Nigg, 2008), including eating disorders (Quinton, Smith, & Joiner, in press) and disordered eating symptoms (Klump et al., 2006; Smith, Hawkeswood, & Joiner, 2010). For example, lower (i.e., more masculine) 2D:4D finger-length

ratios were associated with lower levels of disordered eating in young adult males (Smith et al., 2010) and females (Klump et al., 2006). These findings suggest that higher levels of prenatal testosterone exposure may be protective against the development of eating pathology, and thus, play a role in sex-differentiated risk.

Opposite-sex (OS) twin pairs have also been used to examine the role of prenatal testosterone exposure in the development of sexually dimorphic characteristics. Similar to intrauterine effects in animals, OS female twins are thought to be exposed to higher levels of testosterone prenatally due to developing in utero with a male co-twin (Miller, 1994). Consistent with this notion, OS female twins have been shown to be masculinized on sexually-dimorphic traits relative to same-sex (SS) female twins, such as increased sensation seeking (Resnick, Gottesman, & McGue, 1993; Slutske, Bascom, Meier, Medland, & Martin, in press), increased aggressive behavior (Cohen-Bendahan, Buitelaar, van Goozen, Orlebeke, Cohen-Kettenis, 2005), lower anxiety symptoms (Culbert et al., 2008), higher body mass index (Alexanderson, Henningsson, Lichtenstein, Holmang, & Eriksson, in press), and greater masculine attitudes relative to feminine attitudes (Miller & Martin, 1995). However, a main criticism of the OS versus SS twin paradigm is that the masculinization of OS female twins might be due to being raised with a male co-twin. While plausible, socialization effects from being reared with a brother do not appear to completely account for the masculinization of OS female twins. OS female twins have been shown to be masculinized on several sexually-dimorphic physical characteristics unlikely to be influenced by social factors, including larger total brain and cerebellum volumes (Peper, Brouwer, van Baal, Schnack, van Leeuwen, Boomsma, et al., 2009), decreased fertility (Lummaa, Pettay, & Russell, 2007), fewer spontaneous otoacoustic emissions (i.e., number of spontaneous otoacoutisc emissions on par with males; McFadden, 1993), male-

like cerebral lateralization (i.e., left-hemisphere dominance) when processing verbal-auditory stimuli (Cohen-Bendahan, Buitelaar, van Goozen, Cohen-Kettenis, 2004), higher birth weight (Glinianaia, Magnus, Harris, & Tambs, 1998), greater respiratory morbidity (Shinwell, Reichman, Lerner-Geva, Boyko, Blickstein, & Israel Neonatal Network, 2007), increased myopia (i.e., visual deficits; Miller, 1995), lower prevalence of left-handedness (Vuoksimaa, Eriksson, Pulkkinen, Rose, & Kaprio, 2010), greater spatial abilities (e.g., mental rotation, Heil, Kavsek, Rolke, Beste, & Jansen, 2011; Vuoksimaa, Kaprio, Kremen, Hokkanen, Viken, Tuulio-Henriksson, & Rose, 2010), and increased tooth crown size (Dempsey, Townsend, & Richards, 1999). These findings highlight the multitude of sexually dimorphic physical and behavioral characteristics that have been shown to be masculinized in OS female twins.

Importantly, masculinization effects in OS female twins have also been found for disordered eating. Young adult OS female twins have displayed masculinized (i.e., lower) levels of disordered eating relative to SS female twins and non-twin females reared with a brother (Culbert et al., 2008). Lower levels of disordered eating in OS female twins compared to non-twin females reared with a brother suggests that the masculinization of disordered eating is unlikely to be accounted for by socialization effects from growing up with a male sibling, and instead, suggests a particular role for prenatal hormones. Taken together, findings indicate that organizational effects of early testosterone exposure play a significant role in a wide range of sexually dimorphic phenotypes, including eating behavior in animals and disordered eating symptoms in humans. Elevated prenatal testosterone exposure may thus be an important biological mechanism underlying sex differences in risk for eating disorders.

Previous research has typically assumed that prenatal testosterone's masculinizing effects on sex differentiated phenotypes would remain static across development. The current project

challenges this assumption using a developmental psychobiological perspective. Specifically, a series of two studies were conducted to examine whether there are developmental differences in the masculinizing or protective effects of prenatal testosterone exposure in risk for disordered eating. Study 1 investigated whether age may moderate prenatal testosterone's masculinizing effects on disordered eating in OS female twins during mid-to-late adolescence and young adulthood. Study 2 examined whether prenatal testosterone's protective/masculinizing effects on disordered eating are present during earlier adolescence, and even more, whether these effects emerge with puberty as would be predicted by animal data. These empirical studies are the first to investigate the role of development in the expression of prenatal testosterone's protective effects on disordered eating. Findings have the potential to expand current conceptualizations of the etiology of disordered eating and result in new biological etiologic paradigms by highlighting prenatal testosterone exposure as a biological mechanism contributing to developmental and sex differentiated risk.

Chapter 2: Prenatal Testosterone and Age Differences in Risk for Disordered Eating

Evidence from 2D:4D finger-length ratio (Klump et al., 2006; Smith et al., 2010; Quinton, Smith, & Joiner, in press) and OS twin (Culbert et al., 2008) studies suggest that prenatal testosterone exposure may underlie risk for eating pathology. However, most of these studies have examined subjects in young adulthood (M ages ~ 19-21, SD = 1.52-2.35; Culbert et al., 2008; Klump et al., 2006; Smith et al., 2010), and new data with other age groups have produced less consistent results. Raevuouri et al. (2008) investigated the effects of prenatal testosterone on risk for eating pathology in a slightly older sample of young adult twins (M age = 24.4, SD = 0.90). Evidence suggested a lack of masculinization in OS female twins for several disordered eating symptoms, although OS female twins showed trends towards lower rates of anorexia nervosa (p = .10) and intentional weight loss (p = .06) relative to dizygotic SS female twins (Raevuouri, Kaprio, Hoek, Sihvolva, Rissanen, & Keski-Rahkonen, 2008). Baker et al. (2009) found no significant differences in levels of disordered eating between OS and SS female twins in mid-to-late adolescence (15-17 years old).

Mixed findings across studies could arise from a number of factors. First, it may be difficult to detect masculinization effects for categorical phenotypes like eating disorder diagnoses given the relatively low prevalence of these conditions (Hudson et al., 2007) and the necessity of large sample sizes for ample statistical power. Nonetheless, Raevuouri et al. (2008) is the only study that examined masculinization of OS female twins using eating disorder diagnoses. Although a larger sample may have resulted in significant (rather than trend-level) masculinization effects for anorexia nervosa, the examination of eating disorder diagnoses cannot entirely explain mixed results since other studies (i.e., Baker, Lichtenstein, & Kendler, 2009) failed to find masculinization effects with continuous measures of disordered eating.

Second, mixed findings could be due to the use of different disordered eating questionnaires, and thus, slight differences in the constructs examined. Culbert et al (2008) detected masculinization of disordered eating in OS female twins using the total score from the Minnesota Eating Behavior Survey (i.e., a composite score of body dissatisfaction, weight preoccupation, binge eating, and compensatory behavior items; von Ranson, Klump, Iacono, & McGue, 2005), whereas Baker et al. (2008) and Raevuouri et al. (2008) failed to detect masculinization of disordered eating in OS female twins using the Eating Disorder Inventory (i.e., body dissatisfaction, drive for thinness, and bulimia subscales and a composite total score across items; Garner, 1991). Nonetheless, items assessed on the MEBS and EDI are quite similar since the MEBS was initially developed from the EDI (Klump, McGue, & Iacono, 2000; von Ranson, Klump, Iacono, & McGue, 2005). It therefore seems unlikely that construct differences would completely account for discrepant results.

Third, since previous studies differed in terms of the age-ranges assessed, discrepant findings across studies may be due to developmental differences in effects. It has been presumed that the masculinizing or protective effects of prenatal testosterone on disordered eating would be static over the lifetime (e.g., Baker et al., 2008). However, the influence of prenatal testosterone on disordered eating could vary across development, particularly since disordered eating is a developmentally-moderated phenotype that increases during adolescence, becomes relatively stable in early-young adulthood, and then declines in late-young adulthood (e.g., Attie & Brooks-Gunn, 1989; Bulik, 2002; Culbert et al., in preparation; Heatherton, Mahamedi, Striepe, Field, & Keel, 1997; Keel, Baxter, Heatherton, & Joiner, 2007; Ohzeki, Otahara, Hanaki, Motozumi, & Shiraki, 1993; Stice, Killen, Hayward, & Taylor, 1998; Stice, Ng, & Shaw, 2010). Disordered eating is also influenced by several factors that show differential risk

effects across development (e.g., dieting; Jacobi, Hayward, de Zwaan, Kraemer, & Stewart, 2004). For example, dieting increases during adolescence and exhibits strong risk effects on disordered eating (Patton, Selzer, Coffey, Carlin, & Wolfe, 1999), whereas decreases in dieting have been associated with decreases in disordered eating symptoms in young adulthood (Heatherton et al., 2007; Keel et al., 2007). The effects of prenatal testosterone as a risk factor for disordered eating may also vary across development, and thus, account for discrepant results observed across ages.

The current study aimed to reconcile discrepant findings by directly examining whether the masculinizing effects of prenatal testosterone on disordered eating varied across late adolescence and into young adulthood. This possibility was examined by comparing levels of disordered eating in females from OS twin pairs, females from SS twin pairs, and males from both SS and OS twin pairs who were between the ages of 15-30. To ensure that any masculinization effects observed in OS female twins were not merely due to socialization effects from growing up with a male co-twin, levels of disordered eating were also compared between OS female twins and non-twin females reared with at least one brother. It was hypothesized that females from OS twin pairs would have significantly lower (i.e., more masculinized) levels of disordered eating than non-twin females, but like the twin comparisons, these masculinization effects were expected to be moderated by age. Notably, multiple, well-validated measures of disordered eating were examined in analyses in order to evaluate the robustness of the findings and potential phenotypic specificity of effects.

METHODS

Participants

Twin participants included a cross-sectional sample of 764 OS and SS twins (i.e., 129 OS male twins; 129 OS female twins; 322 SS female twins; 184 SS male twins) ages 15-30 from the Michigan State University Twin Registry (MSUTR; Klump & Burt, 2006). The MSUTR is a population-based registry of twins recruited across lower Michigan (Klump & Burt, 2006). A sample of 74 non-twin females ages 15-23 who were reared with at least one biological brother within 4 years of their own age were also included in this study. Non-twin females ages 24-30 were not included in the study due to inadequate sample size (n = 5). Although the majority (92.4%) of SS twin and non-twin participants were included in the previous Culbert et al. (2008) study, the OS twin sample contained a substantial number of new twins (n = 71, ~55% of the total OS twin sample) who were recruited after the publication of the previous report. The comparisons of the OS female twins to a previously examined group of SS twin and non-twins allowed us to examine possible masculinization effects using twins drawn from the same population. Moreover, without the comparison groups of SS twins and non-twins, this study could not statistically examine masculinization of disordered eating in OS female twins. Analyses were also conducted with the full OS twin sample as well as only the newly recruited OS twins to ensure that findings replicate.

Participants were divided into three age groups based on sample size considerations and previous research. Participants in mid-to-late adolescence (ages 15-20 years old) were grouped together given that this age range overlapped with the age range investigated in the Baker et al. (2009) study. The remaining participants were divided into two young adult age groups spanning early young adulthood (i.e., ages 21-23) and mid-to-late young adulthood (i.e., ages 24-

30). These young adulthood age groupings were created to overlap with the mean age of the twin samples examined by Culbert et al. (2008) and Raevouri et al. (2008).

A variety of recruitment methods were used for both the twins and non-twins. A sub-sample of twins (20.9%) and non-twins (13.5%) were recruited through birth records in collaboration with the Michigan Department of Community Health (MDCH) and the Michigan Bureau of Integration, Information, and Planning Services (MBIIP) (see Klump & Burt, 2006, for additional recruitment details). However, the majority of twins (79.1%) were recruited through newspaper advertisements, flyers, and university registrar offices. The remaining non-twins (86.5%) were recruited in undergraduate courses and a volunteer research pool at a large Midwestern university. There were no significant differences in levels of disordered eating for twin or non-twin participants recruited through birth records versus participants recruited via other methods (*p*'s = .52-.90).

The majority of participants were Caucasian (83.3% of the total sample) and largely in the middle-to-upper level of socioeconomic status (94.3% of the total sample) based on the Hollingshead index of social position (Hollingshead, 1975). There were no significant differences in ethnicity [χ^2 (12) = 8.54, p = .74] or socioeconomic status [F (3, 760) = 0.23, p = .88] between OS and SS twins. The OS female and non-twin females were also similar in terms of socioeconomic background [F (1, 183) = 0.27, p = .60]. However, the non-twin female sample showed a trend towards being more ethnically diverse than the OS female twins [χ^2 (5) = 9.10, p = .11], and thus, ethnicity was included in analyses comparing female OS twins and non-twins. Importantly, the MSUTR twin and non-twin participants appear to be representative of the recruitment region in terms of racial distributions (Culbert et al., 2008).

The majority of participants completed assessments in the MSUTR laboratory (83.4%). However, if participants were unable to travel to the laboratory, assessments were completed in their home. Levels of disordered eating symptoms were similar between participants who completed assessments in the laboratory and those that completed home assessments (*p*'s = .32-.55).

Measures

Disordered Eating Symptoms

Disordered eating was assessed with the Minnesota Eating Behaviors Survey (MEBS[1]; von Ranson et al., 2005) and the Eating Disorder Examination Questionnaire (EDE-Q; Fairburn & Beglin, 1994). These were the only two available measures of disordered eating in the MSUTR twin sample.

The MEBS is a 30-item true/false self-report questionnaire that consists of a total score and four subscales: binge eating (the tendency to think about and/or engage in binge eating), body dissatisfaction (dissatisfaction with one's body size and/or shape), compensatory behavior (the tendency to use or contemplate use of inappropriate compensatory behaviors, such as self-induced vomiting), and weight preoccupation (the tendency to be preoccupied with dieting, weight, and the pursuit of thinness). Higher scores suggest more pathological eating attitudes and behaviors.

The MEBS scales have demonstrated good psychometric properties in male and female samples (Mardersian, Wu, Culbert, Burt, Nigg, & Klump, in preparation; von Ranson et al.,

[1] The Minnesota Eating Behavior Survey (MEBS; previously known as the Minnesota Eatng 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, Copyright 1983 by Psychological Assessment Resources, Inc. Further reproduction of the MEBS is prohibited without prior permission from Psychological Assessment Resources, Inc.

2005). The factor structure of the MEBS has also been replicated across males and females (von Ranson et al., 2005). Scores on the MEBS show robust sex differences in adolescents and adults (Culbert et al., 2008; von Ranson et al., 2005) as well as associations with organizational (Culbert et al., 2008; Klump et al., 2006) effects of gonadal hormones. The MEBS total score has also exhibited expected correlations with external correlates, such as depressive symptoms and body mass index (Klump, Culbert, Slane, Burt, Sisk, & Nigg, submitted). Similar associations were also observed in the current study between the MEBS subscales and external correlates: depressive symptoms (male r's = .20-.28, female r's = .30-.45; all *p*'s <.01) and body mass index (male r's = .19-.46, female r's = .13-.46; all *p*'s <.01). In addition, the MEBS has been shown to successfully discriminate between individuals with an eating disorder versus controls (von Ranson et al., 2005).

Only the MEBS total score, body dissatisfaction, and weight preoccupation scales were included in this study, as these scales showed good internal consistency in the full sample (α's = .81-.90), both sexes (α's = .72-.90), and each age group (α's = .79-.90). The binge eating and compensatory behavior subscales could not be examined separately due to low internal consistency (α's = .10-.54), primarily in the male twins. However, similar to previous research (e.g., Klump et al., 2010), a composite score that summed the binge eating and compensatory behavior items exhibited adequate internal consistency in the full sample (α = .74), both sexes (α' = .65-.76), and each age group (α's = .71-.75). Thus, this composite score of binge eating and compensatory behaviors was included in analyses.

The EDE-Q is a 36-item self-report questionnaire that assesses disordered eating over the past 28 days, in terms of shape concerns (dissatisfaction and discomfort with one's body shape), weight concerns (preoccupation with weight and a desire to lose weight), eating concerns

(preoccupation with food, eating in secret, and guilt about eating), and dietary restraint (restraint over eating and avoidance of eating). A total score is comprised of items across all subscales. Higher scores on the EDE-Q scales suggest higher levels of disordered eating symptoms.

The EDE-Q has demonstrated excellent psychometric properties in previous studies of males and females (Lavendar, De Young, & Anderson, 2010; Peterson, Crosby, Wonderlich, Joiner, Crow, Mitchell, et al., 2007; Zehr, Culbert, Sisk, & Klump, 2007), including good long-term test-retest reliability (Mond, Hay, Rodgers, Owen, & Beamont, 2004a). The EDE-Q has also demonstrated high correlations with scores attained via the Eating Disorder Examination interview (Binford, Le Grange, & Jellar, 2005; Carter, Aime, & Mills, 2001; Mond, Hay, Rodgers, Owen, & Beamont, 2004b). Similar to the MEBS, expected correlations between the EDE-Q scales and external correlates, i.e., depressive symptoms (male r's = .25-.45, female r's = .24-.53; all *p*'s <.01) and body mass index male r's = .37-.40, female r's = .22-.38; all *p*'s <.01), were observed in the current study.

The EDE-Q total score, shape concerns, and weight concern scales showed excellent internal consistency in this study (i.e., full sample, α's = .84-.95; both sexes, α's = .77-.95; each age group, α's = .83-.95). Internal consistency for the dietary restraint subscale was also generally adequate in the full sample (α = .77), both sexes (male α = .67; female α = .79), and each age group (α's = .70-.80). Thus, the EDE-Q total score, shape concerns, weight concerns, and dietary restraint scales were examined in analyses. The EDE-Q eating concerns subscale was not examined in analyses since internal consistency for this scale was well below the acceptable range in males (α = .37).

The MEBS and EDE-Q scales were highly correlated in the current sample of male and female twins: MEBS and EDE-Q total scores (males, r = .85; females, r = .88; all *p*'s <.001),

MEBS weight preoccupation and EDE-Q weight concerns subscales (males, r = .69; females, r = .72; all *p*'s <.001), and MEBS body dissatisfaction and EDE-Q shape concerns subscales (males, r = .73; females, r = .76; all *p*'s <.001). The moderate to high correlations across measurement scales allow the current study to examine the replicability of masculinization effects across measures as well as the unique effects for each scale.

Statistical Analyses

The MEBS and EDE-Q total scores were prorated if participants were missing 10% or fewer of the items. Scores were coded as missing for a small number of participants missing more than 10% of the total or subscale items on the MEBS (1.7-2.4% of the total sample; MEBS total score, n = 14; body dissatisfaction, n = 18; weight preoccupation, n = 20; binge eating/compensatory behaviors, n = 18) and EDE-Q (3.8-8.09% of the total sample; EDE-Q total score, n = 64, dietary restraint, n = 32; shape concerns, n = 68; weight concerns, n = 62). Sample sizes therefore vary slightly across analyses. The larger proportion of missing data for the EDE-Q, relative to the MEBS, was because the EDE-Q was not fully administered to a small subset of twins (6.28% of the total twin sample, n = 48) due to changes in the MSUTR assessment protocol. There were no significant differences in MEBS scores between twins with versus without EDE-Q data (*p*'s = .32-.99). Thus, participants with available EDE-Q data appear to be representative of the full sample in terms of levels of disordered eating.

Skewness and kurtosis were examined for all disordered eating variables. The MEBS binge eating/compensatory behavior composite score and the EDE-Q dietary restraint score were log transformed ($\log_{10} X + 1$) prior to analyses due to positive skew.

Twin Type Comparisons

Mixed linear models (MLM) were used to examine whether the masculinizing effects of prenatal testosterone on disordered eating vary across development. MLM is an ideal statistical method since the non-independence of dyadic twin data is accounted for by nesting the lower-level unit (i.e., twin identification number) within an upper-level unit (i.e., twin pair identification number that is shared by co-twins). Mean differences on the MEBS and EDE-Q scales were examined as a function of twin type (i.e., all male, OS female, and SS female twins) and age group (i.e., ages 15-20, 21-23, and 24-30). SS and OS male twins did not significantly differ on levels of disordered eating in any age group (all MEBS and EDE-Q variables: *p*'s = .42-.99), and thus, were combined in analyses to maximize sample sizes[2].

MLM models initially examined a twin type main effect, age group main effect, and the interaction between twin type and age group on levels of disordered eating. The interaction between twin type and age was of primary interest in this study since a significant interaction would suggest that the influence of twin type on disordered varies by age. However, MLM models assume a linear interaction effect, yet findings from previous data suggest that age may non-linearly moderate the masculinization of disordered eating in OS female twins (i.e., no effect in mid-to-late adolescence, significant effects in early young adulthood, smaller effects in mid-late young adulthood). If age nonlinearly moderates twin type effects on disordered eating, then the MLM twin type by age interaction would be attenuated (or even non-significant). Consequently, analyses were conducted two ways to examine possible linear or nonlinear moderation of twin type: 1) twin type by age group interaction effects for each disordered eating

[2] A lack of significant mean differences between SS and OS male twins is consistent with previous studies examining disordered eating (Baker et al., 2009; Raevuori et al., 2008) and studies that have examined other sex-differentiated characteristics (e.g., sensation seeking, Resnick et al., 1993).

score (linear interaction effects), and 2) the main effect of twin type on each disordered eating score, conducted separately for the three age groups (non-linear interaction effects).

MLM simple main effect models (i.e., non-linear models) examined twin type as a predictor of disordered eating separately for each age group (i.e., 15-20 years, 21-23 years, 24-30 years). The simple main effect models allowed for the examination of non-linear age moderation effects as well as a comprehensive investigation of pair-wise twin type differences on disordered eating (i.e., identification of which twin types differ) within each age group. Main effect models were initially run using OS female twins as the reference group to obtain pair-wise comparisons between OS female twins versus males and OS female twins versus SS female twins. Models were then re-run using SS female twins as the reference group to attain the pair-wise comparison between SS female twins versus males, which would indicate whether sex differences on levels of disordered eating were present. The confirmation of sex difference effects was important since masculinization of OS female twins would only be expected/detectable in the presence of sex difference effects.

Zygosity was effect coded (monozygotic = 1; dizygotic = -1) and included as a covariate in all MLM models. OS twin paris are dizygotic, whereas SS male and female twin pairs are either dizygotic or monozygotic. If SS male and female twins show increased concordance for low or high levels of disordered eating, respectively, then mean differences on disordered eating between twin groups could potentially be inflated. Although there were no differences in mean levels of disordered eating between SS monozygotic and dizygotic twins (male twins: *p*'s = .46-.70; female twins: *p*'s = .24-.95), models were adjusted for zygosity to ensure that possible non-significant differences did not unduly influence results.

Female OS Twin and Non-Twin Comparisons

Analysis of covariance (ANCOVA) was used to investigate mean differences in levels of disordered eating in OS female twins and non-twin females, controlling for ethnicity. Consistent with the twin type analyses, ANCOVA models tested main effects of participant type (i.e., female OS twins vs. female non-twins reared with a brother), main effects of age group (i.e., ages 15-20 and 21-23), and the interaction of participant type by age group on levels of disordered eating. If OS female twins exhibit significantly lower (i.e., more masculinized) levels of disordered eating than non-twin females, findings would suggest that being reared with a male sibling could not account for masculinization patterns of disordered eating in OS female twins. Differential masculinization effects between age groups would be indicative of possible developmental shifts in the protective effects of prenatal testosterone exposure on disordered eating risk.

RESULTS

Descriptive Statistics

Twin and non-twin participants endorsed a range of disordered eating attitudes and behaviors within each age group (see Tables 1a and 2a). A number of participants also scored above the clinical mean of the MEBS total score (i.e., eating disorder sample $M = 15.55$, von Ranson et al., 2005). These descriptive statistics suggest that there was sufficient variability in disordered eating scores to examine possible differences between twin types and non-twin females.

Twin Type Comparisons

Results largely supported hypotheses and suggested that the magnitude of masculinization of disordered eating in OS female twins is moderated by age. Table 2a displays

the raw means and standard deviations for twin type in each age group and indicated the possible presence of non-linear age-moderation of masculinized disordered eating in OS female twins. For example, OS female twins appeared to show similar levels of disordered eating in the 15-20 year old age group, whereas mean differences on disordered eating between OS and SS female twins appeared to be somewhat larger in the 21-23 year old age group relative to the 24-30 year old age group (see Table 2a). Indeed, in the MLM interaction models, twin type exhibited a significant main effect, but twin type by age interactions ranged from trend-level to non-significant for all disordered eating scores (see Table 3a). By contrast, simple main effect models suggested that the lack of significant twin type by age interactions were likely due to the presence of non-linear age moderation effects (see Table 4a; Figures 1a-3a). Findings indicated masculinization of OS female twins, with the strongest masculinization effects observed in early young adulthood.

Specifically, replicating findings by Baker et al. (2009), the masculinization of disordered eating was not present in OS female twins during mid-to-late adolescence (i.e., 15-20 year-old age group). Although significant main effects of twin type were observed for all MEBS and EDE-Q variables (see Table 4a), these results appeared to be driven by sex differences in these scale scores (see Table 4a). That is, OS and SS female twins exhibited similar levels of disordered eating in the 15-20 year old age group and had significantly higher levels of disordered eating than males (see pair-wise comparisons, Table 4a; see Figure 1a). These findings suggest that prenatal testosterone's protective/masculinizing effects on disordered eating in OS female twins are negligible from ages 15-20.

In contrast, findings indicated substantial masculinization of disordered eating in OS female twins ages 21-23 (see Table 4a & Figure 2a). Twin type exhibited significant main effects

on all MEBS and EDE-Q disordered eating scales (see Table 3). However, unlike ages 15-20, pair-wise comparisons indicated masculinization of disordered eating in OS female twins ages 21-23 (see Table 4a), as levels of disordered eating in OS female twins more closely resembled males than SS female twins (see Table 4a). The masculinization effects on disordered eating in the 21-23 year old age group of OS female twins appeared to be small in magnitude for dietary restraint (see Figure 2a), but medium-to-large in magnitude for all other disordered eating symptoms (see Figure 2a). These masculinization effects are largely consistent with those previously reported by Culbert et al. (2008), which is not particularly surprising given that a large proportion of the current sample were included in the previous analyses. To ensure that results of the 21-23 year old age group were not unduly affected by the inclusion of this previous OS twin sample, analyses were re-run including only the newly recruited 21-23 year old OS twins (n = 18 OS twin pairs). Findings were largely consistent with the full sample despite the reduction in sample size (see Table 5a). The magnitude of masculinization effects were also on par with the full sample in that small effects were observed for dietary restraint (Males vs. OS Female Twins, Cohen's d = .05; SS Female vs. OS Female Twins, Cohen's d = .22), whereas medium-to-large effects were observed across the other disordered eating symptoms (males vs. OS female twins, Cohen's d = .10-.28; SS female vs. OS female twins, Cohen's d = .40-.62).

Masculinization of disordered eating in the 24-30 year old OS female were less pronounced (see Table 4a & Figure 3a). Main effects for twin type ranged from trend-level to significant for all disordered eating scores (see Table 4a). Pair-wise comparisons indicated no significant differences between OS female twins and SS female twins on levels of disordered eating, but the lack of significant effects appeared to be due to small sample sizes. OS female twins fell intermediate to male and SS female twins on disordered eating symptoms (see Table

4a & Figure 3a), but in contrast to the 21-23 year old age group, masculinization effects in the 24-30 year old age group were generally small in magnitude (see Figure 3a). In addition, masculinization effects were more variable across measures or constructs of disordered eating symptoms (see Figure 3a, OS female twins vs. SS female twins: MEBS body dissatisfaction, d = .23 vs. EDE-Q shape concerns, d = .11; MEBS weight preoccupation, d = .19 vs. EDE-Q weight concerns, d = .35). These findings are somewhat consistent with results from Raevuori et al. (2008), where small (trend-level) masculinization effects in OS female twins emerged for some disordered eating phenotypes (e.g., anorexia nervosa, intentional weight loss) but not others (e.g., purging). Together these findings suggest that the masculinization of disordered eating in OS female twins may exhibit more phenotypic specificity and may weaken with age[3].

Female OS Twin and Non-twin Comparisons

Comparisons between female OS twins and non-twins were largely consistent with hypotheses and the twin type comparison results described above. Age moderated the masculinization of disordered eating in OS female twins relative to non-twin females reared with at least one brother. Interactions between participant type (OS female twins vs. non-twin females) and age group ranged from trend-level to non-significant (see Table 6a) for all disordered eating variables, but the lack of significant interaction effects was likely due to sample size limitations. Indeed, follow-up simple main effect models suggested clear differences in the magnitude of masculinization in OS female twins between age groups. OS female twins

[3] Although OS and SS female twins did not significantly differ on mean levels of body mass index (BMI) in any age group (*p*'s = .18-.39), significant associations between BMI and disordered eating scores were present in all twin types and across age groups (r's = .16-.38, *p*'s = .05-.001). Thus, post-hoc analyses were conducted to ensure that BMI could not account for differential masculinization of disordered eating in OS female twins. Results from twin type by age interaction models and twin type main effect models that were adjusted for BMI were nearly identical to those presented herein (data not shown), indicating that BMI cannot explain the findings in this study.

and non-twin females did not differ significantly on levels of disordered eating at ages 15-20 (see Tables 7a). Conversely, with the exception of dietary restraint, OS female twins had substantially lower levels of disordered eating symptoms at ages 21-23 (see Tables 7a). Mean differences between non-twin females and OS female twins in the 21-23 year old age group were medium-to-large in effect for all disordered eating scores (Cohen's d = .45-.79) except dietary restraint (Cohen's d = .06). Results provide further evidence to suggest that the masculinization of disordered eating in OS female twins is moderated by age, and importantly, that these effects do not appear to be accounted for by being reared with a male sibling.

Post-hoc analyses were also conducted to investigate whether the magnitude of age differences between non-twin females and their brother unduly influenced results, as age differences could affect the degree of socialization via the amount of time siblings spent together. Using the same methods as Culbert et al. (2008), ANCOVA models were re-run after eliminating non-twin females whose closest-in-age brother was more than 2 years older or younger. Results largely replicated the full sample. Minimal mean differences were observed between OS female twins and non-twin females on levels of disordered eating at ages 15-20 (Cohen's *d* = .05-.19), yet substantial differences were detected at ages 21-23 (disordered eating in OS female twins < non-twin females; Cohen's *d* = .45-.66). Pearson correlations between disordered eating and age differences between non-twin females and their closest-in-age brother were also small and non-significant (r's = .03-.15, *p's* = .23-.79), further suggesting that the magnitude of age differences did not significantly alter the findings of this study.

DISCUSSION

This study was the first to examine whether discrepant findings for prenatal testosterone's masculinizing effects on disordered eating could be due to age-moderation effects. Findings

suggested that discrepant findings in the literature may be primarily accounted for by age differences between samples. Replicating findings of Baker et al (2009), results indicated no evidence for masculinization of disordered eating in OS female twins during mid-to-late adolescence (ages 15-20) across any of the MEBS or EDE-Q scales. In contrast, the protective/masculinizing effects of prenatal testosterone on disordered eating were pronounced across all MEBS and EDE-Q scales during early young adulthood (ages 21-23), such that OS female twins showed substantially lower (i.e., more masculinized) levels of disordered eating than SS female twins and more closely resembled male twins in this age group. These findings were similar to those of Culbert et al. (2008) even when OS twins included in the previous report were excluded from analyses. Results from the 24-30 year old age group paralleled those of Raevuori et al. (2008) as masculinization of disordered eating in OS female twins appeared to be present, but effects were smaller in magnitude and more variable across disordered eating symptoms and measurement constructs. Taken together, findings suggest that discrepant findings in the literature are likely accounted for by developmental windows of expression for the protective effects of prenatal testosterone on disordered eating.

Importantly, socialization of being reared with a brother did not appear to account for the masculinization of disordered eating in OS female twins. OS female twins showed substantially lower levels of disordered eating than non-twin females reared with at least one brother, but similar to twin type comparisons, effects were most prominent in the 21-23 year-old age group. These results remained unchanged when controlling for age differences in the siblings, and further, minimal associations were observed between disordered eating and the magnitude of age differences between non-twin females and their brothers. Overall, these findings highlight that

prenatal testosterone, rather than socialization, may masculinize disordered eating in OS female twins during early young adulthood.

If prenatal testosterone's effects on disordered eating exert developmental influences, how might this occur? While mechanisms underlying these effects are not yet clear, the influence of prenatal testosterone on sexually dimorphic phenotypes likely involves complex interactions between genetic influences, hormonal vulnerabilities, and environmental risk factors. Prenatal testosterone may cause steroid-dependent differentiation of the centeral nervous system early in development, but the subsequent biological and genetic substrates are further modified by environmental factors. Thus, the "environment" may act to exacerbate or suppress prenatal testosterone's masculinization effects on disordered eating.

Our results suggest that the masculinization of disordered eating is relatively constant in males (i.e., levels of disordered eating remained low across age groups), whereas prenatal testosterone's masculinizing effects on disordered eating in females are most strongly expressed under the "average" risk environment. The masculinization of disordered eating in OS female twins was completely eliminated during the peak period of risk for the onset of eating disorders (ages 15-20; Lewinsohn, Striegel-Moore, & Seeley, 2000; Steinhausen, Gavez, & Metzke, 2005; Stice, Marti, Shaw, & Jaconis, 2009), most pronounced when eating disorder risk is prominent but not at its peak (i.e., ages 21-23; Lewinsohn et al., 2000), and weaker during mid-to-late young adulthood (i.e., ages 24-30) when risk has been shown to decline (Heatherton et al., 1997; Keel et al., 2007). Individual differences in disordered eating between females during higher or lower (but not "average") risk periods may thus largely result from differences in other contributing factors, rather than differences in prenatal hormone exposure.

A number of sex-specific and developmentally-relevant risk factors are potential candidates for these "other" contributing factors. Female-specific risk factors during mid-to-late adolescence may suppress the expression of prenatal testosterone's masculinizing effects on disordered eating. For example, increases in key risk factors for disordered eating such as perceived pressures for thinness, internalization of the thin ideal, dieting, negative body image, and time spent with peers or dating (Stice, Ng, & Shaw, 2010; Field, Camargo, Barr, Berkey, Roberts, Colditz, 2001; Linville et al., in press) may ***increase*** risk for eating pathology in all females during mid-to-late adolescence, regardless of prenatal testosterone exposure (Stice, Ng, & Shaw, 2010; Field, Camargo, Barr, Berkey, Roberts, Colditz, 2001; Linville et al., in press). These etiologic risk factors may essentially "trump" the small-to-moderate protective effects of prenatal testosterone and subsequently attenuate disordered eating differences between OS and SS female twins.

Although young adulthood is also a risk period for eating pathology, the relative risk for eating disorder onset is lower in young adulthood than in mid-to-late adolescence. Early young adulthood (ages 21-23) may thus represent an "average" risk period for eating pathology that potentiates the expression of prenatal testosterone's masculinization or protective effects on disordered eating. Decreases in the magnitude of masculinization of disordered eating in OS female twins during mid-to-late young adulthood (ages 24-30), relative to early young adulthood (ages 21-23), may be due to changes in etiologic factors that ***decrease*** risk for disordered eating in all adult females. For example, decreases in dieting, increases in positive body image, and changes in life roles (e.g., marriage and motherhood; Heatherton et al., 1997; Keel et al., 2007) have been linked to decreases in disordered eating symptoms from early-to-late young adulthood. If changes in these developmental risk factors are equally relevant for OS and SS

female twins, then an overall "lower risk" for eating pathology could serve to diminish the magnitude of mean disordered eating differences between OS and SS female twins. All of these hypotheses currently remain speculative. Future studies should aim to identify possible mechanisms underlying developmental shifts in the magnitude of masculinized disordered eating in OS female twins.

Several limitations of this study must be acknowledged. Data were cross-sectional, and thus, this study could not confirm that age-related differences in the magnitude of masculinization effects reflect developmental changes in effects. Longitudinal studies are needed to confirm the presence of within-person developmental shifts in prenatal testosterone's protective/masculinizing effects and to identify which putative risk factors may account for such changes. Sample sizes were also small for the non-twin females and twins in the 24-30 year old age group, and consequently, this study had low statistical power in analyses examining these groups. Larger sample sizes will be necessary to confirm the results of this study. A community-based sample of twins was used, and thus, it remains unknown whether findings generalize to clinical populations. Nonetheless, findings from this study likely have etiologic relevance given the wide range of disordered eating symptoms examined and the fact that some of these symptoms (e.g., body dissatisfaction, weight preoccupation) are the strongest precursors to the development of eating disorders (Jacobi et al., 2004). Further, examining developmental differences in the masculinization of OS female twins using clinical populations would be extremely difficult, as it would involve investigating age differences in the onset of eating disorders, when the onset of eating disorders in adulthood is low. Finally, twin type was used as a proxy of prenatal testosterone exposure. Given that direct measures of prenatal testosterone are difficult to obtain, the use of other models of prenatal testosterone exposure (e.g., girls with

congenital adrenal hyperplasia, 2D:4D finger-length ratios) should also be employed to corroborate the results of this study.

Overall, findings from this study are significant in suggesting that prenatal testosterone may be a biological mechanism that underlies sex differences in risk for disordered eating. However, prenatal testosterone's protective/masculinizing effects on disordered eating appear to be moderated by age, and thus, complex hormone-environment interactions likely exist. Moving forward it will be important to identify how prenatal testosterone exerts its masculinzing/protective effects on disordered eating and which specific factors modify the magnitude of these effects. Future studies may also benefit by investigating the masculinization of disordered eating at other developmental periods (i.e., earlier adolescence and later adulthood) to gain an even more comprehensive understanding of the developmental moderation of these effects. Early-to-mid adolescence may be a particularly promising period given animal data suggesting that prenatal testosterone's effects may emerge during this critical developmental stage.

Chapter 3: Prenatal Testosterone and Risk for Disordered Eating during Puberty

Findings from study 1 suggested that the masculinization of disordered eating in OS female twins is moderated by age. While masculinization of disordered eating is not evident in OS female twins during mid-to-late adolescence (see study 1, chapter 2; Baker et al., 2009), prominent effects are observed in early young adulthood (see study 1, chapter 2; Culbert et al., 2008) and small effects appear to be present in mid-to-late young adulthood (see study 1, chapter 2; Raevuori et al., 2008). Nonetheless, a key limitation of previous studies is that twins were studied in later adolescence and young adulthood only. Whether masculinization of disordered eating is present during other stages of development is therefore unknown.

Earlier adolescence may be important to examine given that it corresponds with the pubertal emergence of sex differences in disordered eating. Specifically, males and females show similar levels of disordered eating during childhood and early adolescence (Maloney, McGuire, Daniels, & Specker, 1989; Ohzeki et al., 1993; Stice, Agras, & Hammer, 1999). Substantial sex differences in levels of disordered eating are present in mid-adolescence (Culbert et al., in preparation; Ohzeki et al., 1993). Importantly, puberty appears to account for the emergence of sex differences in disordered eating during adolescence (Culbert et al., in preparation). Sex differences in disordered eating are negligible before puberty, whereas females exhibit substantially higher levels of disordered eating than males after puberty, independent of age (Culbert et al., in preparation). The emergence of this sex difference appears to be due to increases in levels of disordered eating in girls during puberty, as levels of disordered eating in males have been shown to remain relatively constant across puberty (Culbert et al., in preparation). Investigating whether the masculinization of disordered eating in OS female twins (relative to SS female twins) also becomes evident during puberty has the potential to identify

prenatal testosterone as an etiologic mechanism underlying sex-differentiated risk for disordered eating during adolescence.

No previous studies have investigated whether prenatal testosterone's masculinizing effects on disordered eating become prominent during puberty. However, animal studies provide support for this possibility. Female rodents exposed to elevated testosterone during early development display masculinized eating behaviors in adulthood, including increased food intake (Bell & Zucker, 1971; Donohoe, 1983; Gentry & Wade, 1976; Madrid, Lopez-Bote, & Martin, 1993) and decreased saccharin consumption (Wade & Zucker, 1969a; Wade & Zucker, 1969b; Zucker, 1969). Thus, prenatal testosterone exerts organizational effects on the male-like expression of feeding. Importantly, sex differences in these eating behaviors emerge during puberty, indirectly suggesting that prenatal testosterone's influence on sex-typical feeding behaviors may first become expressed during puberty. Moreover, studies examining "typical" (i.e., gonadally intact and no hormonal manipulations) male and female rodents have demonstrated that sexually differentiated patterns of food intake and saccharin preference are not present until after pubertal onset (Cohen, Lieblich, & Ganchrow, 1982; Wade, 1972; Wade & Zucker, 1969a). That is, typical male and female rats show similar levels of food intake and saccharin consumption (Cohen et al., 1982; Wade, 1972; Wade & Zucker, 1969a) prior to puberty. In contrast, a large increase in saccharin preference and decrease in food intake occurs in females after pubertal onset, whereas saccharin consumption and food intake exhibit minimal changes in males during puberty (Cohen et al., 1982; Wade, 1972; Wade & Zucker, 1969a).

Several theories have been proposed to account for prenatal testosterone's masculinization of eating behaviors. The most popular have involved the effects of prenatal testosterone on sensitivity to ovarian hormone activation during puberty in females. Indeed, a

critical aspect of early organizational effects of testosterone is to program activational (i.e., effects of gonadal hormones that influence neural systems and behavior transiently) and sex-specific responses to gonadal hormones later in life (Arnold & Breedlove, 1985). Puberty is recognized as the traditional activational period since gonadal hormones rise with pubertal onset. In typical females, lower exposure to testosterone early in life enables the brain to respond to the activational effects of ovarian hormones on sex-typical behavior (e.g., reproduction) during and after puberty (Arnold & Breedlove, 1985). However, if females are exposed to higher levels of testosterone early in life, the brain's sensitivity to ovarian hormone activation during puberty is lessened, resulting in more masculinized behavior. For example, female rodents exposed to elevated testosterone in utero are less responsive to the effects of ovarian hormones on sexual receptivity (i.e., less lordosis in the presence of a male mounting) after puberty and in adulthood, relative to non-androgenized females (Rines & vom Saal, 1984). Likewise, female rats administered testosterone early in life (i.e., during postnatal days 1-5) show male-typical food consumption (e.g., elevated food intake) via attenuated responsiveness to ovarian hormones (Bell & Zucker, 1971; Donohoe, 1983; Gentry & Wade, 1976; Madrid, Lopez-Bote, & Martin, 1993). Saccharin preference in neonatally androgenized female rats is also masculinized (i.e., decreased) during and after puberty, regardless of the level of exogenous ovarian hormone administration (Wade & Zucker, 1969a; Wade & Zucker, 1969b; Zucker, 1969). These animal findings highlight the interplay between organizational and activational effects of gonadal hormones on feeding behaviors and demonstrate that prenatal testosterone organizes the brain to be less responsive to the activational effects of ovarian hormones during puberty and adulthood.

Prenatal testosterone's masculinizing effects on disordered eating in OS female twins may also become expressed during puberty via decreased sensitivity to the activational risk

effects of ovarian hormones. Similar to patterns of food intake in male and female rodents, sex differences in disordered eating emerge during puberty (i.e., level of disordered eating in females > males) (Culbert et al., in preparation), and importantly, ovarian hormones show activational effects on disordered eating symptoms (e.g., binge eating, body dissatisfaction, weight preoccupation) in adult women (Edler et al., 2007; Klump et al., 2008; Racine, Culbert, Keel, Sisk, Burt, & Klump, in press). Thus, prenatal testosterone may masculinize disordered eating, but the masculinized effect in OS female twins may only become evident during puberty, when prenatal testosterone may decrease responsiveness to the activational risk effects of ovarian hormones. If this were the case, differences in levels of disordered eating between OS and SS female twins would be expected to emerge only after pubertal onset. The current study was the first to investigate this hypothesis.

Specifically, this study examined levels of disordered eating in a cross-sectional sample of OS and SS male and female twins before and after pubertal onset. Levels of disordered eating were not expected to significantly differ between OS and SS male and female twins in pre-early puberty. In contrast, OS female twins were expected to show significantly lower levels of disordered eating than SS female twins, yet be similar on levels of disordered eating to male twins, during mid-late puberty.

This study also aimed to rule-out possible confounding factors for pubertal moderation of prenatal testosterone's masculinizing effects on disordered eating. First, to ensure that socialization effects from being reared with a brother in OS female twins do not account for results, levels of disordered eating were compared between OS female twins and non-twin females reared with one or more brothers. OS female twins and non-twins were expected to show similar levels of disordered eating during pre-early puberty, whereas masculinized levels of

disordered eating were expected to be prominent in OS female twins in the mid-late puberty group. Second, this study investigated whether findings could be accounted for by sex-differentiated factors that change during puberty and may vary between OS and SS twins. Adiposity and mood symptoms were selected as covariates in this regard since they have been shown to be influenced by organizational effects of testosterone (e.g., Alexanderson et al., 2011; Eisner, Dumesic, Kemnitz, Colman, & Abbott, 2003; Zuloaga, Jordan, & Breedlove, 2011), to increase risk for disordered eating (Jacobi et al., 2004), and to exhibit a sex-differentiated effect (higher in females > males; Nolen-Hoeksema, 2001; Blum, Englaro, Hanitsch, Juul, Hertel, Muller, et al., 1997) that could presumably increase risk for disordered eating in girls relative to boys. Autonomy difficulties were also covaried since separation-individuation has been one of the most influential theories regarding increased risk for disordered eating during puberty (Eggert & Klump, unpublished dissertation; Marsden, Meyer, Fuller, & Waller, 2002; Rhodes & Kroger, 1992). This theory posits that girls may develop disordered eating as a way to avoid maturation and the necessary separation from major attachment figures (Eggert & Klump, unpublished dissertation; Marsden et al., 2002; Rhodes & Kroger, 1992). Separation-individuation seemed particularly important to examine in the current study given speculations that twins may have more difficulties with autonomy than singletons, since twins must separate from parental figures and co-twins (Fichter, 1990; Holland, Sicotte, & Treasure, 1988; Klump & Leon, unpublished data). In the case of the current study, SS twins may experience even greater autonomy difficulties than OS twins since SS female twins would separate from a SS twin and a same-sex parent rather than only a same-sex parent in the case of OS twins. This study therefore aimed to ensure that higher levels of disordered eating in mid-late pubertal SS female twins, relative to mid-late pubertal OS female twins, could not be accounted for by being reared with a

brother or differential levels of depression, anxiety, adiposity, and autonomy difficulties between SS and OS twins.

METHODS

Participants

Participants were 394 twins (i.e., 178 SS female twins; 88 SS male twins; 64 OS male twins; 64 OS female twins) and 63 non-twin females, ages 10-15, from the Michigan State University Twin Registry (MSUTR; Klump & Burt, 2006). As noted previously, the MSUTR is a population-based registry of twins that were recruited across lower Michigan (Klump & Burt, 2006). Non-twin females were reared with at least one biological brother within 4 years of their own age. All adolescent twin and non-twin participants were recruited through birth records via the MDCH and MBIIP (for a more detailed description, see Klump & Burt, 2006).

Parental reports of child ethnicity and family socioeconomic status revealed that the majority of participants were Caucasian (84.7% of total sample) and largely in the middle-to-upper level of socioeconomic status (89.3% of the total sample). Twin type groups did not significantly vary in terms of ethnic backgrounds [overall sample, χ^2 (3) = 4.13, p = .25]. Mean levels of socioeconomic status exhibited trend-level differences across twin types [F (3, 390) = 2.67, p = .06], but these trend-level differences were considered trivial since mean levels corresponded to mid-to-upper socioeconomic status for all twin groups. No differences were observed between OS female twins and non-twin females in terms of ethnicity [overall sub-sample, χ^2 (1) = 1.38, p = .24] or socioeconomic status [F = .26 (1, 125), p = .61].

The majority (96.3%) of participants completed assessments in the MSUTR laboratory. Home assessments were conducted for participants who were unable to travel to the laboratory.

Levels of disordered eating symptoms did not significantly vary as a function of laboratory versus home assessments (*p*'s = .65-.86).

Measures

Disordered Eating Symptoms

Similar to study 1, disordered eating was assessed with the Minnesota Eating Behaviors Survey (MEBS; von Ranson et al., 2005) and the Eating Disorders Examination Questionnaire (EDE-Q; Fairburn & Beglin, 1994). The MEBS and EDE-Q were the only available measures of disordered eating for participants in this study.

The MEBS assesses a range of disordered eating attitudes and behaviors, including body dissatisfaction, weight preoccupation, binge eating, and compensatory behaviors. A total score is also calculated by summing all items on the MEBS. Higher scores on each of the MEBS scales indicate higher levels of disordered eating symptoms. The MEBS is designed to be used in children as young as 9 years old (von Ranson et al., 2005). Scores on the MEBS have shown robust sex differences in adolescents (Culbert et al., submitted) and adults (Culbert et al., 2008; von Ranson et al., 2005) as well as associations with organizational (Culbert et al., 2006; Klump et al., 2006) and activational (Klump et al., 2006) effects of gonadal hormones. The MEBS has also demonstrated good psychometric properties (Marderosian et al., in preparation; von Ranson et al., 2005) and exhibited a replicable factor structure (von Ranson et al., 2005) in male and female samples. In addition, the MEBS total score has also been shown to successfully discriminate between individuals with an eating disorder versus controls (von Ranson et al., 2005).

The MEBS total score, body dissatisfaction, and weight preoccupation scales were examined in analyses since these scales demonstrated good internal consistency across sex and

pubertal groups (α's = .71-.89). The binge eating and compensatory behavior subscales of the MEBS were not examined in analyses given the low alphas (α's < .65) in some sample groups (i.e., pre-early pubertal males), even when the binge eating/compensatory behavior scales were combined (α = .66).

The EDE-Q assesses disordered eating symptoms over the past 28 days, including shape concerns, weight concerns, eating concerns, and dietary restraint. A total score is comprised of items across all subscales. Higher scores on the EDE-Q scales suggest higher levels of disordered eating symptoms. The EDE-Q has demonstrated good psychometric properties in previous studies of males and females (Lavendar et al., 2010; Peterson et al., 2007; Zehr et al., 2007), including high correlations with scores attained via the Eating Disorder Examination interview (Binford et al., 2005; Carter et al., 2001; Mond et al., 2004b) and good long-term test-retest reliability (Mond et al., 2004a). The EDE-Q total score, shape concerns, and weight concerns scales also showed good internal consistency in the current study across sex and pubertal groups (α's = .73-.94), and thus, these scales were examined in analyses. The EDE-Q dietary restraint and eating concerns subscales were not examined in analyses due to low alphas in males (α's = .50-.62).

Consistent with study 1, correlations across MEBS and EDE-Q scales were moderate to high in this sample of adolescent males and females: MEBS and EDE-Q total scores (males, r = .81; females, r = .80; all *p*'s <.001), MEBS weight preoccupation and EDE-Q weight concerns (males, r = .70; females, r = .71; all *p*'s <.001), and MEBS body dissatisfaction and EDE-Q shape concerns (males, r = .62; females, r = .74; all *p*'s <.001). Thus, like study 1, this study investigated the replicability and unique masculinization effects across each disordered eating scale.

Pubertal Development

Pubertal development was assessed with the self-report Pubertal Development Scale (PDS; Petersen, Crockett, Richards, & Boxer, 1988). The PDS measures development on several secondary sex characteristics. Height spurts, underarm and pubic hair growth, and skin changes are assessed in males and females. Sex-specific characteristics are also assessed, including breast development and initiation of menses in females or voice changes in males (Petersen et al., 1988). Females dichotomously rated the onset of menses as present or absent. All other items used a four-point continuous rating scale: 1) development has not yet begun, 2) development has barely started, 3) development is definitely underway, and 4) development seems completed.

The PDS has been established as acceptable to both parents and children for measuring sensitive information about physical characteristics (Petersen et al., 1988). Previous research also supports the reliability, validity, and pubertal categorical classifications of the PDS (Petersen et al., 1988). For example, categorical classifications correlate substantially ($r \sim .70$) with clinician ratings of pubertal development (Petersen et al., 1988). Internal consistency was also good for males ($\alpha = .86$) and females ($\alpha = .81$) in the current study.

Consistent with previous research (Culbert et al., 2009; Culbert et al., in preparation; Klump, McGue, & Iacono, 2003), this study used average PDS scores to categorize participants' pubertal development as pre-early puberty (PDS score < 2.5) or mid-late puberty (PDS score ≥ 2.5). A total of 221 twins and 20 non-twin females were identified as pre-early pubertal, whereas the mid-late pubertal group consisted of a total of 173 twins and 43 non-twin females (see Table 1b).

Anxiety Symptoms

Anxiety was measured with the total score from the Multidimensional Anxiety Scale for Children (MASC; March, Parker, Sullivan, Stallings, & Conners, 1997), which is a composite score of items assessing a range of anxiety symptoms (i.e., physical symptoms, separation panic, social anxiety, and harm avoidance). Higher scores indicate higher levels of anxiety symptoms. The MASC total score was selected over subscale scores since the total score is a comprehensive measure of several anxiety symptoms. The MASC total score also showed excellent internal consistency (α's = .79-.90, across sex and pubertal groups) and exhibited the strongest correlations with the disordered eating scales (*r*'s = .20-.36, $p < .001$) relative to the MASC subscales. Importantly, the MASC has also demonstrated excellent psychometric properties in non-clinical samples of male and female children and adolescents (March et al., 1997).

Depressive Symptoms

The total score from the Children's Depression Inventory (CDI; Kovacs, 1985) was used to assess depressive symptoms, including depressive thoughts (e.g., "All bad things are my fault"), feelings (e.g., "I am sad all the time"), and behaviors (e.g., "I cannot make up my mind about things"). Elevated scores are indicative of more depressive symptoms. Compared to the subscale scores, the CDI total score exhibited the highest correlations with the disordered eating scales (r's = .42-.53, $p < .001$). The CDI total score also showed excellent internal consistency across sex and pubertal groups (α's = .82-.88) and has demonstrated excellent psychometric properties in other non-clinical samples of male and female children and adolescents (Kovacs, 1985).

Autonomy Difficulties

The separation-anxiety subscale from the Separation-Individuation Test of Adolescence (SITA) was used to assess difficulties developing autonomy. Specifically, the SITA separation-anxiety subscale examines autonomy difficulties with parents, teachers, or peers as evidenced by fears of losing emotional or physical contact (Levine, Green, & Millon, 1986). Participants evaluated a range of separation-anxiety based statements (e.g., "Being alone is a very scary idea for me" or "I worry about death a lot") using a 5-point Likert scale ranging from "strongly agree or is always true for me" to "strongly disagree or is never true for me." Higher scores indicate greater difficulties with separation-anxiety. The SITA separation-anxiety subscale was selected for use in this study since it showed the strongest associations with disordered eating in previous studies (Eggert, unpublished dissertation) and the current study (r's = .21-.37, p <.001). Past studies have supported its reliability and validity of the SITA in adolescent non-clinical and clinical samples of males and females (Eggert, unpublished dissertation; Levine et al., 1986; Levine & Saintonge, 1993; McClanahan & Holmbeck, 1992), with alphas ranging from .64-.88 among the subscales (Eggert, unpublished dissertation; Levine et al., 1986). Internal consistency for the separation-anxiety subscale in the current study was on par with those of previous reports (α = .66-.69, across sex and pubertal groups).

Adiposity

Adiposity was measured using body mass index (BMI). BMI was calculated [Weight (in kilograms)/Height (in meters) squared] from measurements obtained with a wall mounted ruler

and digital scale. BMI correlated highly with estimates of body fat percentage[4] (r's = .76-.93, p <.001 across twin and non-twins), suggesting that BMI is a good indicator of adiposity.

Statistical Analyses

A small number of participants were missing more than 10% of the items on the MEBS body dissatisfaction (n = 3) and weight preoccupation (n = 2) subscales, the MASC total score (n = 3), the CDI total score (n = 2), and the SITA separation anxiety subscale (n = 4). Thus, scores on these measures were coded as missing. Scores were prorated for participants missing 10% or fewer of the scale items.

The CDI total score and BMI were log transformed ($\log_{10} X + 1$) prior to analyses due to positive skew. In addition, age and pubertal status were significantly correlated (r = .67, p < .001). Age also showed small associations with the disordered eating scores, mood symptoms, autonomy, and BMI variables (male and female r's = -.14 to .26). Thus, age was regressed from all MEBS scales as well as the mood, autonomy, and BMI variables, and standardized residual scores were used in analyses. The use of standardized residual scores would ensure that the effects of age were accounted for in all statistical models.

Twin Type Comparisons

Mixed linear models (MLM) were used to examine whether the masculinizing effects on disordered eating become pronounced only after pubertal onset. As noted previously, MLM is an ideal statistical method given the dyadic nature of twin data. MLM accounts for the non-independence of twin dyads by nesting the lower-level unit (i.e., individual twin variable) within

[4] Body fat percentage was measured using bioelectrical impedance analysis (BIA), which is a painless procedure that passes electrical signals through fat, lean mass, and water to evaluate adiposity. BIA was only conducted on participants who completed laboratory assessments. Thus, body fat percentage data from the BIA procedure was not included as a covariate since participants missing these data (n = 23; 5% of the total sample) were not missing at random.

an upper-level unit (i.e., family variable shared by co-twins). Mean differences in disordered eating were examined as a function of twin type (i.e., all male, OS female, and SS female twins) and pubertal status (i.e., pre- early pubertal and mid-late pubertal groups). Consistent with previous research (e.g., see study 1, chapter 2; Baker et al. 2009; Raevouri et al., 2008), SS and OS male twins did not significantly differ on levels of disordered eating in any pubertal group (*p*'s = .30-.97). Thus, SS and OS male twins were combined in analyses (denoted "all males").

Multiple MLM models were conducted. MLM models initially examined the main effect of twin type, the main effect pubertal status, and the interaction between twin type and pubertal status on levels of disordered eating. A significant interaction would suggest that the influence of twin type on disordered eating varies between pre-early puberty and mid-late puberty. In the presence of significant twin type by pubertal status interaction effects, two follow-up "main effects" analyses were conducted to identify the specific twin types (i.e., OS twins, SS twins, males) that differed on disordered eating in each pubertal group (i.e., pre-early puberty versus mid-late puberty). The first models were termed "simple main effect" models. Simple main effect models examined the main effect of twin type on disordered eating and were only adjusted for age and zygosity. The second, "covariate main effect" models, examined the main effect of twin type on disordered eating, covarying age, zygosity depressive symptoms, anxiety symptoms, adiposity, and autonomy difficulties.

All main effect models were conducted using both OS female twins and SS female twins as reference groups to obtain pair-wise twin type comparisons. Obtaining all pair-wise comparisons amongst twin types was important for the examination of sex difference effects as well as prenatal testosterone effects on levels of disordered eating. Moreover, it was important to confirm the presence of sex difference effects since masculinization of OS female twins would

only be expected in the presence of sex difference effects. Categorical covariates were effect coded (zygosity[5]: monozygotic = 1, dizygotic = -1) and continuous covariates (i.e., mood, autonomy, and adiposity) were centered prior to analyses.

Female OS Twin and Non-twin Comparisons

Analysis of variance (ANOVA) and analysis of covariance (ANCOVA) models examined mean differences in levels of disordered eating as a function of participant type (i.e., OS female twins and non-twin females) and pubertal group (i.e., pre-early puberty and mid-late puberty). Consistent with twin comparison models, several models were conducted. First, ANOVA models examined the main effect of participant type, main effect of pubertal group, and participant type by pubertal group interactions on levels of disordered eating. Second, follow-up "simple main effect" ANOVA models (i.e., adjusted only for age) and "covariate main effect" ANCOVA models (i.e., adjusted for age, mood symptoms, autonomy, and adiposity) examined the main effect of participant type on levels of disordered eating separately for each pubertal group. Continuous covariates (i.e., mood, autonomy, and adiposity) were centered prior to analyses. Significantly lower (i.e., more masculinized) levels of disordered eating in OS female twins, relative to non-twin females, would indicate that being reared with a male sibling does not account for masculinization effects in OS female twins. Further, if masculinization effects are only prominent in the pubertal group, findings would suggest that prenatal testosterone's masculinizing effects on disordered eating likely emerges after pubertal onset.

[5]Although no significant differences in mean levels of disordered eating were detected between SS monozygotic and dizygotic twins (male twins: *p*'s = .44 -.80; female twins: *p*'s = .22-.99), irrespective of pubertal status, zygosity was accounted for in all analyses. Covarying zygosity ensured that twin type effects on disordered eating could not be accounted for by increased concordance for high levels of disordered eating in SS female monozygotic twins or low levels of disordered eating in SS male monozygotic twins.

RESULTS

Descriptive Statistics

Twin and non-twin participates exhibited a range of disordered eating attitudes and behaviors (see Table 1b), suggesting there was ample variability to examine differences in levels of disordered eating within each pubertal group. A number of participants also scored above the mean clinical score for the MEBS total score (15.55, von Ranson et al., 2005; see Table 1b), but not surprisingly, the number of participants scoring above the clinical mean was higher for participants in the pubertal, rather than pre-pubertal, group.

Twin Type Comparisons

Findings from the MLM models confirmed masculinization of disordered eating in OS female twins only after pubertal onset. Significant twin type by pubertal status interaction effects were observed for all disordered eating scores except the MEBS body dissatisfaction score, which showed trend-level interaction effects (see Table 2b). Simple main effect models indicated no significant differences on levels of disordered eating amongst twin types in the pre-early pubertal group (see Table 3b). However, significant main effects for twin type were present in the mid-late pubertal group (see Table 3b). Pair-wise comparisons indicted significantly lower levels of disordered eating in male twins compared to SS female twins, whereas OS female twins fell intermediate to male twins and SS female twins on mean levels of disordered eating in the mid-late pubertal group (see Table 3b and Figures 1b-2b). The masculinization of OS female twins in the mid-late pubertal group appeared to be small-to-medium in magnitude (see Figures 1b-2b).

Results from the covariate main effect models largely paralleled those of the simple main effect models. Minimal differences in disordered eating were observed across twin types in the

pre-early pubertal group when models were adjusted for adiposity (i.e., BMI), depressive symptoms, anxiety symptoms, and autonomy difficulties (see Table 3b and Figures 1b-2b). Conversely, twin type exhibited trend-level to significant main effects across disordered eating symptoms in the mid-late pubertal group. Pair-wise comparisons from the covariate main effect models indicated that mid-late pubertal SS female twins continued to exhibit significantly higher levels of disordered eating than mid-late pubertal males (see Table 3b and Figures 1b-2b). Levels of disordered eating in OS female twins also continued to fall intermediate to males and SS female twins in mid-late puberty, even after controlling for the effects of mood symptoms, autonomy difficulties, and adiposity (see Table 3b and Figures 1b-2b).

Notably, the masculinization of disordered eating in OS female twins was smaller in magnitude in the covariate adjusted models than the simple main effect models. Decreases in the estimated magnitude of masculinization of mid-late pubertal OS female twins appeared to be due to slight decreases in disordered eating in SS female twins and slight increases in disordered eating in OS female and male twins, after controlling for covariates (see Figures 1b-2b). These changes in levels of disordered eating across twin types indicated a sex-differentiated effect of the covariates on disordered eating.

Taken together, findings suggest that OS female twins show more masculinized levels of disordered eating after pubertal onset, and importantly, mood symptoms, autonomy difficulties, and adiposity do not completely account for these effects.

Female OS Twin and Non-twin Comparisons

ANOVA and ANCOVA results are presented in Tables 4b and 5b, respectively. Notably, interaction effects between participant type (i.e., OS female twins versus non-twins) and pubertal group were non-significant (see Table 4b). However, the lack of significant participant type by

pubertal group interactions effects were likely due to a lack of statistical power, particularly given the small sample sizes in the pre-early pubertal group (female non-twins, n = 20; OS female twins, n = 31). Notably, inspection of the means in Table 1b indicated that differences in mean levels of disordered eating between OS female twins and non-twin females appeared to only be present in the mid-late pubertal group. Indeed, simple and covariate main effect models (in combination with Cohen's d effect sizes) suggested substantial mean differences between OS female twins and non-twins on levels of disordered eating, but as expected, these differences varied by pubertal status. OS female twins and non-twin females generally showed similar levels of disordered eating in the pre-early pubertal group (see Table 5b). In contrast, mid-late pubertal OS female twins showed substantially lower (i.e., more masculinized) levels of disordered eating than non-twin females, even after accounting for adiposity, mood symptoms, and autonomy (see Table 5b). Similar to the twin type comparisons, masculinization of disordered eating in OS female twins appeared to be small-to-medium in magnitude in the mid-late pubertal group (see Table 5b).

Differences in disordered eating between OS female twins and non-twin females did not appear to be influenced by the magnitude of age differences between non-twin females and their brothers. ANCOVA models were re-run after selecting only non-twin females whose closest-in-age brother was no more than 2 years older or younger (pre-early pubertal, n = 16; mid-late pubertal, n = 28). Despite a reduction in sample size, results remained consistent with the full sample. Minimal differences in levels of disordered eating were observed in the pre-early pubertal group of female OS twins and non-twins (Cohen's d = .10-.16), yet OS female twins showed lower levels of disordered eating than the non-twin females in the mid-late pubertal group (Cohen's d = .42-.51) even after accounting for adiposity, mood symptoms, and

autonomy. Post-hoc analyses were also re-run with mid-late pubertal non-twin females whose closest-in-age brother was no more than 1 year older or younger (n = 10). Again, results were strikingly similar to the full sample, in that mid-late pubertal OS female twins showed masculinized levels of disordered eating relative to non-twin females reared with a brother $\pm$1 year age difference, even after adjusting for covariates (Cohen's d = .33-.77). Associations between disordered eating and the magnitude of the age differences between non-twin females and their closest-in-age brother were also small and non-significant (r's = .04-.16, all *p*'s > .05).

Together, these results confirm and extend those of the twin type comparisons. Although interaction models were non-significant, pair-wise comparisons via simple and covariate main effect models indicated that the masculinization of disordered eating in OS female twins becomes more prominent after pubertal onset. Thus, being reared with a male sibling does not appear to account for the emergence of masculinized levels of disordered eating in opposite-sex female twins during puberty.

DISCUSSION

Findings from this study suggest that prenatal testosterone's masculinizing effects on disordered eating emerge during puberty. Within this pilot sample, masculinized levels of disordered eating in OS female twins primarily became evident after pubertal onset (irrespective of age). Levels of disordered eating demonstrated linear effects based on expected levels of prenatal testosterone exposure, but only in the mid-late pubertal group. Mid-late pubertal male twins exhibited the lowest (i.e., most masculinized) levels of disordered eating, followed by OS female twins, and then SS female twins. Together, these findings suggest that increased levels of prenatal testosterone exposure may masculinize disordered eating and underlie the emergence of sex differences in risk for disordered eating during puberty.

Several possible explanations for the pubertal moderation of prenatal testosterone's masculinizing effects on disordered eating were investigated. Despite some reduction in the magnitude of effects, findings indicated that the masculinization of disordered eating in mid-late pubertal OS female twins does not appear to be completely accounted for by mood symptoms, autonomy difficulties, adiposity, or socialization effects of being reared with a brother. OS female twins in mid-late puberty exhibited lower levels of disordered eating than non-twin females who were reared with a brother and fell intermediate to male and SS female twins on levels of disordered eating, even after controlling for mood, autonomy difficulties, and adiposity. Other, unexamined factors therefore likely play a role in the effects of puberty on the emergence of masculinization of disordered eating in OS female twins.

Speculatively, the combined effects of prenatal and pubertal hormone exposure may play a role in the masculinization of disordered eating and underlie the pubertal emergence of these effects in OS female twins. The two-stage model of hormone-dependent development of sex-typical characteristics posits that elevated exposure to prenatal testosterone early in life (prenatally/perinatally) organizes the central nervous system to be male-like (Arnold & Breedlove, 1985; Schulz, Molenda-Figueira, & Sisk, 2009). Indeed, female rodents exposed to elevated levels of testosterone early in life show male-like patterns of food intake (i.e., increased) and saccharin consumption (i.e., decreased) in adulthood (Bell & Zucker, 1971; Madrid, Lopez-Bote, & Martin, 1993; Wade, 1972; Wade & Zucker, 1969a; Wade & Zucker, 1969b; Zucker, 1969), highlighting a prenatal organizational effect of testosterone on feeding behaviors. Puberty marks a second period of organization, in that pubertal gonadal hormones further organize neural circuits for sex-typical behavior during adolescent brain development (Schulz et al., 2009). For example, postural strategies for food defense are masculinized in females that are not exposed to

ovarian hormones during puberty (i.e., ovariectomized prior to puberty), whereas adult ovariectomy does not modify this behavior (Field, Whishaw, Forgie, & Pellis, 2004). These findings highlight puberty as a critical window for organization of female-typical food defense strategies.

The theory posits that the two-stage prenatal and pubertal organization of neural systems results in a "template" upon which circulating hormones act during adolescence and adulthood to activate sex-typical behavior (Schulz et al., 2009). Thus, circulating gonadal hormones are thought to activate organized neural circuits to facilitate the expression of female-like or male-like behavior. For example, females rodents androgenized (i.e., exogenously administered testosterone) early in life show decreased sensitivity to the effects of ovarian hormones on eating behaviors in adulthood, resulting in masculinized food intake (i.e., increased food intake; Bell & Zucker, 1971; Donohoe, 1983; Gentry & Wade, 1976; Madrid, Lopez-Bote, & Martin, 1993) and saccharin preference (i.e., decreased saccharin consumption; Wade & Zucker, 1969a; Wade & Zucker, 1969b; Zucker, 1969). Taken together, it is likely that organizational and activational effects of gonadal hormones drive sexually differentiated eating behavior in animals, and thus, may be biological mechanisms underlying sexually differentiated risk for disordered eating symptoms in humans. Specifically, elevated exposure to testosterone during prenatal development in OS twins may organize the central nervous system to be more "male-like". Altered sensitivity to gonadal hormones in OS twins during puberty (as a result of elevated exposure to testosterone prenatally) may further promote the organization of a more "male-like" neural system. The activational effects of circulating gonadal hormones on a masculinized neural system may then result in decreased expression of female-like eating pathology (e.g., binge eating, body dissatisfaction, weight preoccupation), and instead, result in more male-like patterns

of disordered eating (e.g., low levels of binge eating, body dissatisfaction, weight preoccupation) during puberty and adulthood.

Findings from this study warrant future investigations that can directly examine these hypotheses. For example, animal models of eating disorder characteristics (e.g., binge-proneness; activity-based anorexia) could be used to directly investigate the role of prenatal and pubertal gonadal hormone exposure on sexually-differentiated expression of eating disorder phenotypes. To determine if prenatal testosterone organizes sex differences in binge-eating or risk for activity-based anorexia, studies could compare the developmental emergence of binge-proneness or susceptibility to activity-based anorexia in gonadally intact male and female rats as well as neonatally androgenized females. Follow-up studies could subsequently be conducted to identify if organizational and activational effects of puberty further modify individual and sex differences in behavioral phenotypes. Specifically, comparisons could be made between intact rats (i.e., males, females, neonatally androgenized females) versus gonadoectomized rats (i.e., males, females, neonatally androgenized females) with and without exogenous hormone exposure during puberty and adulthood. Comparisons across groups would allow for a direct examination of whether ovarian hormones organize and activate binge proneness or susceptibility to activity-based anorexia during puberty. Even more, findings could also directly demonstrate whether exposure to early testosterone prevents or alters pubertal organization and/or activational effects of ovarian hormones on female-typical risk of these eating disorder phenotypes.

Although findings from this study are novel, several limitations must be noted. First, this study was cross-sectional. Longitudinal studies will be necessary to ensure that the differences observed between pubertal groups are in fact reflective of developmental trends. Second, sample sizes were relatively small, particularly for OS twins and non-twin females, where standard

errors and deviations of the means were rather broad. Future research should examine larger samples of twins and non-twins to replicate our findings. Increased sample sizes would also allow for a more comprehensive examination of puberty's effects across all stages of puberty (pre-puberty vs. early puberty vs. mid-puberty vs. late puberty) to more clearly establish the timing of the emergence of masculinization effects on disordered eating and whether masculinization effects become linearly pronounced across stages of development.

Third, disordered eating symptoms were measured in a community-based sample rather than a clinical sample of individuals with eating disorders, and thus, it is unclear whether these findings generalize to clinical eating disorders. Nonetheless, conducting this study in a clinical sample would be near impossible given the low prevalence of eating disorders before puberty. Given that disordered eating symptoms show prospective associations with eating disorder risk (Jacobi et al.) and a variety of disordered eating symptoms were also observed in all of our sample groups, our findings are likely informative for etiologic models of eating disorders.

Finally, we were unable to directly assess levels of prenatal testosterone exposure, and instead, used twin type as a proxy of differential exposure. However, it is difficult to overcome this limitation since direct measures of prenatal testosterone would be difficult to obtain in human studies. Future studies should thus examine other models of prenatal testosterone exposure in humans (e.g., girls with congenital adrenal hyperplasia) and animals (e.g., intrauterine position effects in animal models of disordered eating) to confirm the emergence of masculinization of disordered eating during pre-to-early adolescence, and in particular, during puberty.

To date, psychosocial explanations have largely been used to explain epidemiological features of eating disorders, including sex differences in prevalence and increases in risk after

puberty. However, findings from this study are significant in suggesting that prenatal testosterone exposure likely plays a role in sex and developmental differences in risk for disordered eating. Prenatal testosterone's masculinizing effects on disordered eating were not accounted for by mood symptoms, autonomy difficulties, adiposity, or socialization effects from being reared with a brother. Investigations of other key developmental factors associated with puberty (e.g., gonadal hormones) are now warranted.

Chapter 4: Overall Summary and Conclusions

This project aimed to integrate basic science and clinical research to improve the understanding of hormonal contributions to risk for eating disorders. The series of studies conducted herein were the first to investigate possible developmental differences in the expression of prenatal testosterone's masculinizing or protective effects on risk for disordered eating.

Findings from studies 1 and 2 indicate that prenatal testosterone exerts protective effects on disordered eating, but these effects are not static across development. First, the masculinizing or protective effects of prenatal testosterone on disordered eating can only occur in the presence of sex differences, and thus, they are not detectable prior to the emergence of sex differences in disordered eating (i.e., prior to mid-late puberty). Second, prenatal testosterone's protective effects on disordered eating appear to be most strongly expressed during "average" risk periods (i.e., after pubertal onset and during young adulthood) and appear to be completely attenuated during the "peak" period of risk for the onset of eating disorders (i.e., mid-to-late adolescence). In other words, OS female twins only appear feminized or "female-like" on levels of disordered eating during the highest period of risk for eating disorder onset. Prenatal testosterone's protective effects on disordered eating in OS female twins may thus be "trumped" by other risk factors during mid-to-late adolescence. The attenuation of prenatal testosterone's protective effects on disordered eating during the peak period of risk for eating disorders is perhaps not surprising given that the protective effects generally appear to be relatively small-to-medium in magnitude (i.e., effect sizes ~ .20-.50), at least in females. Nonetheless, these findings are novel in that they suggest possible developmental windows of expression for the protective effects of prenatal testosterone on disordered eating. Future studies can aim to identify factors (e.g.,

dieting) that may underlie developmental changes in the protective effects of prenatal testosterone exposure on risk for disordered eating.

Findings from study 2 extend those of study 1 by aiming to identify possible mechanisms underlying prenatal testosterone's protective effects on disordered eating. Study 2 demonstrated that the masculinizing effects of prenatal testosterone on disordered eating emerge during puberty and do not appear to be due to being reared with a brother or several other developmental- and sex- moderated factors (e.g., mood, autonomy, adiposity). Thus, these findings serve as the first critical step in understanding the interplay between prenatal testosterone exposure and risk for disordered eating during puberty. Findings also set the foundation for future translational research that can directly examine the interplay between prenatal testosterone exposure and pubertal hormonal effects (e.g., sensitivity to ovarian hormones) using animal and human models.

Overall, findings contribute to a growing literature on sex and developmental differences in risk for disordered eating. Prenatal testosterone's protective effects on disordered eating are prominent after puberty during all developmental periods except mid-to-late adolescence. During mid-to-late adolescence, the protective effects of prenatal testosterone exposure on disordered eating appear to be trumped or washed-out by other female-specific risk factors. Results from this project therefore necessitate a re-thinking of current conceptualizations of etiologic risk for disordered eating, as both sociocultural and biological models will likely be needed to understand sex differentiated risk for eating disorders across development.

APPENDICES

APPENDIX A

Tables: 1a-7a and 1b-5

Table 1a. Descriptive Statistics for Twins and Non-Twins.

Sample Descriptives	**Twin/Participant Type**			
	Overall Full Sample, Ages 15-30[a]			
	All Males	**OS Females**	**SS Females**	**Non-Twin Females**
Sample Size (n):	279-304	120-129	299-322	71-74
Mean Age (SD)	20.44 (3.01)	20.57 (3.41)	20.54 (2.64)	19.74 (2.18)
MEBS Scores:				
Total Score				
Mean (SD)	**3.86 (4.10)**	**7.51 (6.07)**	**8.33 (6.15)**	**9.14 (5.29)**
Range (max score = 30)	0-21	0-28	0-29	1-21
% > mean clinical cut-off	1.99%	10.85%	14.69%	14.86%
Body Dissatisfaction				
Mean (SD)	**0.95 (1.59)**	**2.21 (2.23)**	**2.67 (2.20)**	**2.52 (1.99)**
Range (max score = 6)	0-6	0-6	0-6	0-6
Weight Preoccupation				
Mean (SD)	**1.38 (1.68)**	**2.78 (2.45)**	**3.00 (2.42)**	**3.54 (2.15)**
Range (max score = 8)	0-7	0-8	0-8	0-8
Binge Eating/Compensatory Behaviors				
Mean (SD)	**1.10 (1.35)**	**1.94 (2.21)**	**2.04 (2.26)**	**2.40 (1.96)**
Range (max score = 13)	0-8	0-11	0-12	0-10
Raw EDE-Q Scores:				
Total Score				
Mean (SD)	**0.66 (0.81)**	**1.39 (1.18)**	**1.50 (1.21)**	**1.56 (1.21)**
Range (max score = 6)	0-4	0-5	0-6	0-6
Shape Concerns				
Mean (SD)	**0.94 (1.14)**	**1.91 (1.56)**	**2.08 (1.60)**	**2.26 (1.48)**
Range (max score = 6)	0-6	0-6	0-6	0-6

Table 1a. Descriptive Statistics for Twins and Non-Twins (cont'd).

Sample Descriptives	Twin/Participant Type			
	Overall Full Sample, Ages 15-30[a]			
	All Males	**OS Females**	**SS Females**	**Non-Twin Females**
Raw EDE-Q Scores:				
Weight Concerns				
Mean (SD)	**0.71 (1.02)**	**1.63 (1.43)**	**1.75 (1.47)**	**1.85 (1.51)**
Range (max score = 6)	0-6	0-6	0-6	0-6
Dietary Restraint				
Mean (SD)	**0.64 (0.99)**	**1.20 (1.33)**	**1.23 (1.30)**	**1.27 (1.30)**
Range (max score = 6)	0-5	0-6	0-6	0-5

Note: OS = opposite-sex twins; SS = same-sex twins; SD = standard deviation; MEBS = Minnesota Eating Behaviors Survey; EDE-Q = Eating Disorder Examination Questionnaire. Raw mean scores = not adjusted for any covariate (e.g., age, zygosity). [a] = Non-Twin female descriptive statistics reflect data for ages 15-23.

Table 2a. Descriptive Statistics by Participant Type and Age Group.

Sample Descriptives	**Twin/Participant Type**											
	Ages 15-20				***Ages 21-23***				***Ages 24-30***			
	Males	**OS-F**	**SS-F**	**NT**	**Males**	**OS-F**	**SS-F**	**NT**	**Males**	**OS-F**	**SS-F**	**NT**
Sample Size (n):	162-185	64-73	195-214	50-53	86-87	39	65-66	20-21	30	17	38-42	--
Mean Age (SD)	18.68 (1.86)	18.37 (2.14)	19.01 (1.28)	18.84 (1.88)	22.01 (0.76)	22.04 (0.73)	22.32 (0.91)	22.02 (0.80)	26.70 (1.87)	26.62 (2.21)	25.57 (1.27)	--
Raw MEBS Scores:												
Total Score												
Mean (SD)	**3.60 (3.84)**	**8.08 (6.63)**	**7.88 (6.02)**	**8.56 (4.97)**	**4.45 (4.69)**	**6.60 (5.27)**	**9.19 (6.27)**	**10.62 (5.89)**	**3.73 (3.74)**	**7.18 (4.75)**	**9.24 (6.47)**	--
Range (max score = 30)	0-21	0-28	0-29	1-21	0-19	0-19	0-22	2-21	0-14	1-14	0-25	--
% > mean clinical cut-off	2.72%	13.70%	11.80%	11.32%	3.45%	7.70%	22.72%	19.05%	0.00%	0.00%	16.67%	--
Body Dissatisfaction												
Mean (SD)	**0.83 (1.52)**	**2.25 (2.31)**	**2.41 (2.14)**	**2.33 (2.01)**	**1.11 (1.68)**	**1.97 (2.11)**	**3.17 (2.28)**	**3.00 (1.92)**	**1.17 (1.78)**	**2.59 (2.24)**	**3.21 (2.20)**	--
Range (max score = 6)	0-6	0-6	0-6	0-6	0-6	0-6	0-6	0-6	0-6	0-6	0-6	--
Weight Preoccupation												
Mean (SD)	**1.25 (1.59)**	**2.96 (2.57)**	**2.95 (2.44)**	**3.54 (2.29)**	**1.63 (1.84)**	**2.49 (2.28)**	**3.00 (2.37)**	**3.54 (1.83)**	**1.43 (1.63)**	**2.65 (2.34)**	**3.26 (2.43)**	--
Range (max score = 8)	0-7	0-8	0-8	0-8	0-7	0-8	0-7	0-8	0-6	0-7	0-8	--
BE/CB												
Mean (SD)	**1.05 (1.32)**	**2.15 (2.53)**	**1.94 (2.20)**	**2.36 (2.05)**	**1.29 (1.51)**	**1.72 (1.88)**	**2.35 (2.26)**	**2.50 (1.73)**	**0.83 (0.87)**	**1.53 (1.28)**	**2.07 (2.53)**	--
Range (max score = 13)	0-8	0-11	0-12	0-10	0-7	0-8	0-9	0-7	0-3	0-3	0-10	--

Table 2a. Descriptive Statistics by Participant Type and Age Group (cont'd).

Sample Descriptives	Twin/Participant Type											
	Ages 15-20				*Ages 21-23*				*Ages 24-30*			
	Males	**OS-F**	**SS-F**	**NT**	**Males**	**OS-F**	**SS-F**	**NT**	**Males**	**OS-F**	**SS-F**	**NT**
Raw EDE-Q Scores:												
Total Score	**0.55 (0.68)**	**1.51 (1.28)**	**1.42 (1.23)**	**1.53 (1.23)**	**0.84 (1.00)**	**1.19 (1.11)**	**1.59 (1.11)**	**1.63 (1.18)**	**0.69 (0.78)**	**1.39 (0.90)**	**1.74 (1.25)**	--
Range (max score = 6)	0-3	0-5	0-6	0-6	0-4	0-5	0-5	0-4	0-4	0-4	0-5	--
Shape Concerns												
Mean (SD)	**0.82 (1.02)**	**2.06 (1.68)**	**1.97 (1.58)**	**2.20 (1.53)**	**1.17 (1.32)**	**1.59 (1.44)**	**2.27 (1.52)**	**2.41 (1.38)**	**0.97 (1.15)**	**2.09 (1.34)**	**2.38 (1.76)**	--
Range (max score = 6)	0-5	0-6	0-6	0-6	0-6	0-6	0-6	0-5	0-5	0-5	0-6	--
Weight Concerns												
Mean (SD)	**0.60 (0.89)**	**1.80 (1.63)**	**1.63 (1.45)**	**1.85 (1.54)**	**0.91 (1.26)**	**1.41 (1.19)**	**1.93 (1.46)**	**1.86 (1.47)**	**0.76 (0.80)**	**1.48 (1.13)**	**2.04 (1.53)**	--
Range (max score = 6)	0-5	0-6	0-6	0-6	0-6	0-5	0-6	0-5	0-3	0-4	0-6	--
Dietary Restraint												
Mean (SD)	**0.51 (0.83)**	**1.28 (1.37)**	**1.20 (1.37)**	**1.29 (1.36)**	**0.86 (1.18)**	**1.06 (1.25)**	**1.16 (1.15)**	**1.22 (1.16)**	**0.75 (1.07)**	**1.26 (1.37)**	**1.47 (1.16)**	--
Range (max score = 6)	0-4	0-6	0-6	0-5	0-5	0-5	0-5	0-4	0-5	0-4	0-5	--

Note: Males = all same-sex and opposite-sex male twins; OS-F = opposite-sex female twins; SS-F = same-sex female twins; NT = non-twin females; SD = standard deviation; MEBS = Minnesota Eating Behaviors Survey; EDE-Q = Eating Disorder Examination Questionnaire; BE/CB = Binge Eating/Compensatory Behaviors. Raw mean scores = not adjusted for any covariate (e.g., age, zygosity).

Table 3a. MLM Twin Type by Age Interaction Results.

Model	Statistics	
	F (df, df)	*p*-value
Minnesota Eating Behaviors Survey		
Total Score		
Twin Type	35.09 (2, 481.57)	**<.001**
Age Group	0.21 (2, 426.01)	.81
Twin Type x Age Group	1.79 (4, 482.54)	.13
Body Dissatisfaction		
Twin Type	39.05 (2, 467.96)	**<.001**
Age Group	1.75 (2, 420.27)	.18
Twin Type x Age Group	1.21 (4, 469.76)	.31
Weight Preoccupation		
Twin Type	26.08 (2, 484.00)	**<.001**
Age Group	0.02 (2, 420.28)	.98
Twin Type x Age Group	1.12 (4, 483.61)	.35
Binge Eating/Compensatory Behaviors		
Twin Type	10.76 (2, 512.56)	**<.001**
Age Group	0.86 (2, 424.76)	.42
Twin Type x Age Group	0.78 (4, 506.13)	.54
Eating Disorder Examination Questionnaire		
Total Score		
Twin Type	32.70 (2, 444.28)	**<.001**
Age Group	0.26 (2, 392.06)	.78
Twin Type x Age Group	2.20 (4, 446.25)	.07
Shape Concerns		
Twin Type	34.66 (2, 437.16)	**<.001**
Age Group	0.43 (2, 387.47)	.65

Table 3a. MLM Twin Type by Age Interaction Results (cont'd).

Model	Statistics	
	F (df, df)	*p*-value
Eating Disorder Examination Questionnaire		
Shape Concerns		
Twin Type x Age Group	2.28 (4, 438.93)	.06
Weight Concerns		
Twin Type	32.73 (2, 439.70)	**<.001**
Age Group	0.20 (2, 385.39)	.82
Twin Type x Age Group	2.14 (4, 441.09)	.08
Dietary Restraint		
Twin Type	14.11 (2, 480.72)	**<.001**
Age Group	1.05 (2, 407.41)	.35
Twin Type x Age Group	1.56 (4, 478.58)	.18

Note: Models were adjusted for zygosity.

Table 4a. MLM Simple Main Effect Models across Twin Type.

Model	Mean (Standard Deviation)			Twin Type Main Effect	Twin Type Pair-wise Comparisons		
					OS-F vs. All Males	OS-F vs. SS-F	SS-F vs. All Males
	All Males	OS-F	SS-F	F (df, df)	t (df)	t (df)	t (df)
Age Group: 15-20 Years							
Minnesota Eating Behaviors Survey							
Total Score	3.54 (5.75)	7.86 (5.44)	7.81 (6.71)	**37.01*** (2, 387.05)**	**-6.66*** (302.93)**	-0.63 (409.22)	**-6.67*** (240.66)**
Body Dissatisfaction	0.83 (2.18)	2.19 (1.95)	2.43 (2.55)	**33.06*** (2, 408.20)**	**-5.98*** (287.59)**	0.83 (390.50)	**-6.57*** (242.99)**
Weight Preoccupation	1.21 (2.36)	2.86 (2.22)	2.89 (2.69)	**32.30*** (2, 374.67)**	**-6.05*** (310.15)**	0.10 (407.61)	**-6.51*** (242.98)**
Binge Eating/Compensatory Behaviors	1.03 (1.94)	2.11 (2.01)	1.88 (2.21)	**10.61*** (2, 357.29)**	**-3.63*** (325.75)**	-0.38 (416.57)	**-3.63*** (245.01)**
Eating Disorder Examination Questionnaire							
Total Score	0.53 (1.17)	1.49 (1.06)	1.41 (1.36)	**38.90*** (2, 357.50)**	**-7.23*** (265.59)**	-0.48 (358.68)	**-6.44*** (216.61)**
Shape Concerns	0.80 (1.58)	2.06 (1.39)	1.94 (1.83)	**37.87*** (2, 364.52)**	**-7.25*** (260.87)**	-0.51 (352.54)	**-6.21*** (217.32)**
Weight Concerns	0.59 (1.44)	1.74 (1.30)	1.64 (1.67)	**35.78*** (2, 350.76)**	**-6.92*** (269.18)**	-0.50 (357.46)	**-6.27*** (218.52)**
Dietary Restraint	0.47 (1.23)	1.24 (1.21)	1.19 (1.40)	**22.29*** (351.80)**	**-5.25*** (305.19)**	-0.44 (400.24)	**-5.21*** (233.33)**

Table 4a. MLM Simple Main Effect Models across Twin Type (cont'd).

Model	Mean (Standard Deviation)			Twin Type Main Effect	Twin Type Pair-wise Comparisons		
					OS-F vs. All Males	OS-F vs. SS-F	SS-F vs. All Males
	All Males	OS-F	SS-F	F (df, df)	t (df)	t (df)	t (df)
Age Group: 21-23 Years							
Minnesota Eating Behaviors Survey							
Total Score	4.41 (5.80)	5.90 (5.44)	9.53 (6.36)	**13.00*** (158.01)**	-1.54 (127.14)	**2.95** (150.25)**	**-5.08*** (101.56)**
Body Dissatisfaction	1.11 (2.14)	1.88 (2.08)	3.22 (2.35)	**16.56*** (2, 155.68)**	**-2.09* (127.14)**	**2.90** (155.70)**	**-5.69*** (101.07)**
Weight Preoccupation	1.63 (2.28)	2.23 (2.18)	3.14 (2.58)	**7.33*** (2, 164.00)**	-1.59 (122.56)	**1.85† (154.37)**	**-3.73*** (101.24)**
Binge Eating/Compensatory Behaviors	1.28 (1.99)	1.52 (1.94)	2.45 (2.15)	**4.62** (2, 152.23)**	-0.50 (124.87)	**1.99* (157.02)**	**-3.03** (98.63)**
Eating Disorder Examination Questionnaire							
Total Score	0.83 (1.13)	1.10 (1.08)	1.65 (1.22)	**9.01*** (2, 151.38)**	-1.37 (129.78)	**2.29* (151.50)**	**-4.23*** (101.00)**
Shape Concerns	1.16 (1.52)	1.49 (1.47)	2.34 (1.66)	**10.17*** (2, 154.13)**	-1.29 (127.12)	**2.59** (153.45)**	**-4.50*** (99.66)**
Weight Concerns	0.88 (1.41)	1.24 (1.31)	2.03 (1.52)	**11.54*** (2, 152.52)**	-1.54 (128.87)	**2.66** (148.38)**	**-2.66** (148.38)**
Dietary Restraint	0.86 (1.26)	0.99 (1.19)	1.20 (1.33)	1.91 (2, 148.25)	-0.86 (131.91)	0.81 (152.44)	**-1.91* (101.94)**

Table 4a. MLM Simple Main Effect Models across Twin Type (cont'd).

Model	Mean (Standard Deviation)			Twin Type Main Effect	Twin Type Pair-wise Comparisons		
					OS-F vs. All Males	OS-F vs. SS-F	SS-F vs. All Males
	All Males	OS-F	SS-F	F (df, df)	t (df)	t (df)	t (df)
Age Group: 24-30 Years							
Minnesota Eating Behaviors Survey							
Total Score	3.56 (5.64)	7.42 (5.57)	9.02 (6.08)	**8.35*** (2, 62.41)**	**-2.50** (57.81)**	0.97 (71.49)	**-3.93*** (49.89)**
Body Dissatisfaction	1.14 (2.19)	2.62 (2.16)	3.17 (2.40)	**7.79** (2, 63.74)**	**-2.53** (56.27)**	0.84 (71.26)	**-3.73*** (48.83)**
Weight Preoccupation	1.35 (2.26)	2.78 (2.27)	3.22 (2.36)	**6.11** (2, 58.94)**	**-2.16* (60.81)**	0.67 (71.44)	**-3.40*** (51.77)**
Binge Eating/Compensatory Behaviors	0.74 (1.85)	1.63 (2.05)	1.94 (1.96)	**2.65† (2, 57.84)**	-1.48 (63.72)	0.24 (74.13)	**-2.23* (52.39)**
Eating Disorder Examination Questionnaire							
Total Score	0.64 (1.13)	1.46 (1.06)	1.74 (1.21)	**8.73*** (2, 62.25)**	**-2.73** (57.46)**	0.90 (67.10)	**-3.99*** (52.43)**
Shape Concerns	0.88 (1.57)	2.21 (1.51)	2.40 (1.68)	**8.69*** (2, 55.37)**	**-2.97** (55.89)**	0.41 (65.75)	**-3.92*** (49.42)**
Weight Concerns	0.72 (1.34)	1.54 (1.27)	2.03 (1.43)	**8.25*** (2, 61.66)**	**-2.28* (57.42)**	1.28 (68.04)	**-2.72** (53.52)**
Dietary Restraint	0.73 (1.27)	1.26 (1.16)	1.47 (1.30)	**3.84* (2, 63.09)**	-1.60 (59.41)	0.88 (66.57)	**-2.71** (53.52)**

Note: SS-F = same-sex female twins; OS-F = opposite-sex female twins; All Males = opposite-sex and same-sex male twins; df = degrees of freedom. Models were adjusted for zygosity.

†$p < .10$, *$p < .05$, **$p < .01$, ***$p < .001$

Table 5a. MLM Simple Main Effect Models across Same-Sex and Newly Recruited Opposite-Sex Twins Ages 21-23.

Model	**Mean (Standard Deviation)**			**Twin Type Main Effect**	**Twin Type Pair-wise Comparisons**		
					New OS-F vs. All Males	New OS-F vs. SS-F	SS-F vs. All Males
	All Males	OS-F	SS-F	F (df, df)	t (df)	t (df)	t (df)
Age Group: 21-23 Years							
Minnesota Eating Behaviors Survey							
Total Score	4.55 (6.11)	5.61 (5.21)	9.37 (6.29)	**10.17*** (2, 98.85)**	-0.78 (103.86)	**2.47* (105.82)**	**-4.50*** (79.14)**
Body Dissatisfaction	1.15 (2.24)	1.78 (2.02)	3.21 (2.36)	**13.12*** (2, 97.10)**	-1.25 (99.44)	**2.45* (109.81)**	**-5.12*** (78.23)**
Weight Preoccupation	1.72 (2.41)	2.08 (2.08)	3.09 (2.58)	**4.98** (2, 99.60)**	-0.68 (98.91)	**1.66† (109.67)**	**-3.16** (79.35)**
Binge Eating/Compensatory Behaviors	1.32 (2.14)	1.54 (2.00)	2.40 (2.14)	**4.31* (2, 88.83)**	-0.41 (103.25)	1.51 (101.12)	**-2.91** (75.79)**
Eating Disorder Examination Questionnaire							
Total Score	0.88 (1.20)	1.14 (1.03)	1.62 (1.21)	**6.41** (2, 93.71)**	-0.97 (105.23)	**1.59† (103.10)**	**-3.58*** (78.33)**
Shape Concerns	1.24 (1.60)	1.65 (1.43)	2.31 (1.65)	**7.31*** (2, 93.90)**	-1.10 (102.68)	**1.62† (105.69)**	**-3.82*** (77.10)**
Weight Concerns	0.96 (1.51)	1.31 (1.29)	1.99 (1.53)	**7.83*** (2, 93.70)**	1.00 (105.77)	**1.84† (102.53)**	**-3.96*** (78.88)**
Dietary Restraint	0.85 (1.30)	0.91 (1.07)	1.19 (1.29)	1.47 (2, 93.71)	-0.27 (105.62)	0.93 (102.20)	**-1.70† (79.68)**

Note: SS-F = same-sex female twins; New OS-F = newly recruited opposite-sex female twins that were not included in analyses conducted in the Culbert et al. (2008) manuscript; All Males = opposite-sex and same-sex male twins; df = degrees of freedom. Models were adjusted for zygosity.

†$p < .10$, *$p < .05$, **$p < .01$, ***$p < .001$

Table 6a. ANCOVA Participant Type by Age Interaction Results.

Model	**Statistics**	
	F (df, df)	*p*-value
Minnesota Eating Behaviors Survey		
Total Score		
Participant Type	7.20 (1, 181)	**.008**
Age Group	0.00 (1, 181)	.99
Participant Type x Age Group	3.82 (1, 181)	.06
Body Dissatisfaction		
Participant Type	3.45 (1, 180)	.07
Age Group	0.09 (1, 180)	.77
Participant Type x Age Group	2.00 (1, 180)	.16
Weight Preoccupation		
Participant Type	5.36 (1, 178)	**.02**
Age Group	0.74 (1, 178)	.39
Participant Type x Age Group	0.39 (1, 178)	.54
Binge Eating/Compensatory Behaviors		
Participant Type	6.73 (1, 180)	**.01**
Age Group	0.04 (1, 180)	.85
Participant Type x Age Group	0.77 (1, 180)	.38
Eating Disorder Examination Questionnaire		
Total Score		
Participant Type	3.85 (1, 170)	**.05**
Age Group	0.02 (1, 170)	.89
Participant Type x Age Group	2.50 (1, 170)	.11
Shape Concerns		
Participant Type	5.25 (1, 170)	**.02**
Age Group	0.18 (1, 170)	.67

Table 6a. ANCOVA Participant Type by Age Interaction Results (continued).

Model	Statistics	
	F (df, df)	*p*-value
Eating Disorder Examination Questionnaire		
Shape Concerns		
Participant Type x Age Group	2.32 (1, 170)	.13
Weight Concerns		
Participant Type	2.80 (1, 172)	.09
Age Group	0.28 (1, 172)	.460
Participant Type x Age Group	1.42 (1, 172)	.24
Dietary Restraint		
Participant Type	1.42 (1, 172)	.24
Age Group	0.68 (1, 172)	.41
Participant Type x Age Group	0.21 (1, 172)	.65

Note: Models were adjusted for ethnicity.

Table 7a. ANCOVA Simple Main Effect Models for Female Opposite-Sex Twins and Non-Twins.

Model	Mean (Standard Deviation)		Mean Difference Effect Size	Participant Type Main Effect
	OS Female Twins	Non-Twin Females	Cohen's d	F (df, df)
Age Group: 15-20 Years				
Minnesota Eating Behaviors Survey				
Total Score	7.99 (5.94)	8.69 (5.94)	.12	0.42 (1, 123)
Body Dissatisfaction	2.21 (2.16)	2.38 (2.18)	.08	0.21 (1, 122)
Weight Preoccupation	2.93 (2.46)	3.59 (2.53)	.26	2.11 (1, 120)
Binge Eating/Compensatory Behaviors	2.13 (2.33)	2.39 (2.34)	.11	0.40 (1, 123)
Eating Disorder Examination Questionnaire				
Total Score	1.48 (1.34)	1.58 (1.27)	.08	0.16 (1, 113)
Shape Concerns	2.02 (1.73)	2.25 (1.64)	.14	0.54 (1, 113)
Weight Concerns	1.76 (1.70)	1.90 (1.59)	.08	0.24 (1, 114)
Dietary Restraint	1.23 (1.44)	1.36 (1.35)	.09	0.50 (1, 114)

Table 7a. ANCOVA Simple Main Effect Models for Female Opposite-Sex Twins and Non-Twins (cont'd).

Model	Mean (Standard Deviation)		Mean Difference Effect Size	Participant Type Main Effect
	OS Female Twins	Non-Twin Females	Cohen's d	F (df, df)
Age Group: 21-23 Years				
Minnesota Eating Behaviors Survey				
Total Score	6.46 (5.52)	10.88 (5.54)	.79	**8.49** (1, 57)**
Body Dissatisfaction	1.98 (2.09)	3.00 (2.10)	.48	**3.20† (1, 57)**
Weight Preoccupation	2.45 (2.16)	3.60 (2.18)	.52	**3.72* (1, 57)**
Binge Eating/Compensatory Behaviors	1.66 (1.84)	2.61 (1.90)	.50	**5.43* (1, 56)**
Eating Disorder Examination Questionnaire				
Total Score	1.18 (1.31)	1.90 (1.35)	.47	**3.91* (1, 56)**
Shape Concerns	1.59 (1.48)	2.53 (1.52)	.62	**5.32* (1, 56)**
Weight Concerns	1.41 (1.46)	2.08 (1.48)	.45	**2.77† (1, 57)**
Dietary Restraint	1.06 (1.25)	1.14 (1.26)	.06	0.71 (1, 57)

Note: df = degrees of freedom; Models were adjusted for ethnicity.

†$p < .10$, *$p < .05$, **$p < .01$, ***$p < .001$

Table 1b. Descriptive Statistics for Twins and Non-Twins.

Sample Descriptives	**Twin/Participant Type**											
	Pre-Early Pubertal Group				***Mid-Late Pubertal Group***				***Overall Full Sample***			
	Males	**OS-F**	**SS-F**	**NT**	**Males**	**OS-F**	**SS-F**	**NT**	**Males**	**OS-F**	**SS-F**	**NT**
Sample Size (n):	112	31	76-78	20	40	33	100	43	152	64	175-178	63
Mean Age (SD)	12.06 (1.36)	11.70 (1.09)	11.47 (0.97)	12.11 (1.26)	13.96 (1.30)	14.13 (1.35)	13.27 (1.14)	13.77 (1.36)	12.56 (1.58)	12.95 (1.73)	12.49 (1.39)	13.24 (1.53)
Raw MEBS Scores:												
Total Score												
Mean (SD)	**4.47 (4.60)**	**4.26 (4.78)**	**5.08 (4.42)**	**4.90 (3.46)**	**4.03 (4.36)**	**5.69 (4.90)**	**7.53 (5.99)**	**8.00 (5.76)**	**4.36 (4.53)**	**5.00 (4.86)**	**6.47 (5.51)**	**7.02 (5.32)**
Range (max score = 30)	0-19	0-18	0-18	0-13	0-17	0-18	0-24	0-21	0-19	0-18	0-24	0-21
% > mean clinical cut-off	4.46	3.23	3.66	0.00	5.00	6.10	13.00	13.95	4.61	4.69	8.99	9.52
Body Dissatisfaction												
Mean (SD)	**0.79 (1.44)**	**0.90 (1.58)**	**0.95 (1.32)**	**0.95 (1.32)**	**0.80 (1.34)**	**1.33 (1.81)**	**1.91 (2.08)**	**2.18 (2.07)**	**0.79 (1.41)**	**1.13 (1.70)**	**1.50 (1.85)**	**1.79 (1.96)**
Range (max score = 6)	0-6	0-6	0-5	0-4	0-4	0-6	0-6	0-6	0-6	0-6	0-6	0-6
Weight Preoccupation												
Mean (SD)	**1.62 (1.83)**	**1.65 (1.85)**	**2.07 (1.98)**	**2.05 (1.32)**	**1.28 (1.75)**	**2.00 (1.93)**	**3.02 (2.37)**	**3.12 (2.40)**	**1.53 (1.53)**	**1.83 (1.89)**	**2.60 (2.25)**	**2.86 (2.23)**
Range (max score = 8)	0-7	0-6	0-7	0-5	0-7	0-7	0-8	0-8	0-7	0-7	0-8	0-8
Raw EDE-Q Scores:												
Total Score												
Mean (SD)	**0.65 (0.84)**	**0.66 (0.97)**	**0.75 (0.86)**	**0.71 (0.85)**	**0.57 (0.60)**	**0.95 (0.86)**	**1.32 (1.20)**	**1.37 (1.24)**	**0.63 (0.79)**	**0.81 (0.92)**	**1.10 (1.10)**	**1.16 (1.17)**
Range (max score = 6)	0-5	0-4	0-4	0-4	0-3	0-4	0-5	0-5	0-5	0-4	0-5	0-5

Table 1b. Descriptive Statistics for Twins and Non-Twins (cont'd).

Sample Descriptives	Twin/Participant Type								*Overall Full Sample*			
	Pre-Early Pubertal Group				*Mid-Late Pubertal Group*							
	Males	**OS-F**	**SS-F**	**NT**	**Males**	**OS-F**	**SS-F**	**NT**	**Males**	**OS-F**	**SS-F**	**NT**
Raw EDE-Q Scores:												
Shape Concerns												
Mean (SD)	**0.77 (1.10)**	**0.85 (1.22)**	**0.96 (1.18)**	**0.94 (1.14)**	**0.77 (0.90)**	**1.38 (1.28)**	**1.81 (1.55)**	**1.89 (1.65)**	**0.77 (1.05)**	**1.12 (1.27)**	**1.45 (1.46)**	**1.59 (1.56)**
Range (max score = 6)	0-6	0-4	0-5	0-5	0-4	0-5	0-6	0-5	0-6	0-5	0-6	0-6
Weight Concerns												
Mean (SD)	**0.72 (1.01)**	**0.81 (1.16)**	**0.89 (1.02)**	**0.85 (1.04)**	**0.63 (0.85)**	**1.03 (1.18)**	**1.55 (1.56)**	**1.59 (1.56)**	**0.70 (0.97)**	**0.93 (1.17)**	**1.26 (1.39)**	**1.36 (1.45)**
Range (max score = 6)	0-6	0-4	0-4	0-5	0-4	0-5	0-6	0-6	0-6	0-5	0-6	0-6

Note: Males = all same-sex and opposite-sex male twins; OS-F = opposite-sex female twins; SS-F = same-sex female twins; NT = non-twin females; SD = standard deviation; MEBS = Minnesota Eating Behaviors Survey; EDE-Q = Eating Disorder Examination Questionnaire. Raw mean scores = not adjusted for any covariate (e.g., age, zygosity).

Table 2b. MLM Twin Type by Pubertal Status Interaction Results.

Model	Statistics	
	F (df, df)	*p*-value
Minnesota Eating Behaviors Survey		
Total Score		
Twin Type	5.64 (2, 252.580	**.004**
Pubertal Group	3.03 (1, 338.38)	.08
Twin Type x Pubertal Group	3.32 (2, 334.14)	**.04**
Body Dissatisfaction		
Twin Type	6.28 (2, 261.45)	**.002**
Pubertal Group	2.79 (1, 322.34)	.10
Twin Type x Pubertal Group	2.53 (2, 332.74)	.08
Weight Preoccupation		
Twin Type	9.05 (2, 251.73)	**<.001**
Pubertal Group	1.37 (1, 338.00)	.24
Twin Type x Pubertal Group	4.22 (2, 332.50)	**.02**
Eating Disorder Examination Questionnaire		
Total Score		
Twin Type	6.55 (2, 244.94)	**.002**
Pubertal Group	3.86 (1, 338.29)	**.05**
Twin Type x Pubertal Group	5.35 (2, 325.44)	**.005**
Shape Concerns		
Twin Type	8.94 (2, 244.31)	**<.001**
Pubertal Group	3.75 (1, 329.64)	.06
Twin Type x Pubertal Group	4.99 (2, 322.53)	**.007**
Weight Concerns		
Twin Type	6.38 (2, 244.19)	**.002**
Pubertal Group	3.28 (1, 327.41)	.07
Twin Type x Pubertal Group	4.01 (2, 320.88)	**.02**

Note: Models were adjusted for age and zygosity.

Table 3b. MLM Simple and Covariate Main Effect Models across Twin Type.

Model	Mean (Standard Deviation)			Twin Type Main Effect	Twin Type Pair-wise Comparisons		
					OS-F vs. All Males	OS-F vs. SS-F	SS-F vs. All Males
	All Males	OS-F	SS-F	F (df, df)	t (df)	t (df)	t (df)
Simple Effects Models							
Pre-Early Pubertal Group							
Minnesota Eating Behaviors Survey							
Total Score	4.61 (5.02)	4.41 (4.44)	4.98 (5.10)	0.21 (2, 142.68)	0.20 (124.30)	0.58 (144.78)	-0.55 (125.43)
Body Dissatisfaction	0.79 (1.47)	0.77 (1.47)	1.04 (1.56)	0.81 (2, 137.89)	0.01 (129.79)	0.85 (145.68)	-1.47 (117.34)
Weight Preoccupation	1.64 (2.05)	1.75 (1.88)	2.02 (2.15)	0.85 (2, 144.97)	-0.36 (117.45)	0.66 (150.83)	-1.30 (124.26)
Eating Disorder Examination Questionnaire							
Total Score	0.70 (0.99)	0.62 (0.83)	0.73 (1.03)	0.23 (2, 143.66)	0.38 (108.79)	0.68 (150.91)	-0.49 (123.53)
Shape Concerns	0.79 (1.29)	0.83 (1.11)	0.94 (1.33)	0.47 (2, 148.55)	-0.05 (113.99)	0.68 (146.38)	-0.95 (121.23)
Weight Concerns	0.79 (1.17)	0.70 (0.99)	0.87 (1.25)	0.40 (2, 143.53)	0.34 (109.43)	0.85 (151.59)	-0.75 (123.97)

Table 3b. MLM Simple and Covariate Main Effect Models across Twin Type (cont'd).

Model	Mean (Standard Deviation)			Twin Type Main Effect	Twin Type Pair-wise Comparisons		
					OS-F vs. All Males	OS-F vs. SS-F	SS-F vs. All Males
	All Males	OS-F	SS-F	F (df, df)	t (df)	t (df)	t (df)
Simple Effects Models							
Mid-Late Pubertal Group							
Minnesota Eating Behaviors Survey							
Total Score	4.04 (5.55)	6.07 (5.80)	7.43 (6.33)	**5.04** (2, 118.11)**	**-1.73† (109.53)**	1.16 (122.90)	**-3.15** (112.42)**
Body Dissatisfaction	0.80 (1.90)	1.37 (2.00)	1.90 (2.11)	**4.66** (2, 117.15)**	-1.30 (119.42)	1.40 (122.97)	**-3.05** (116.14)**
Weight Preoccupation	1.25 (2.24)	2.24 (2.22)	2.98 (2.50)	**8.09*** (2, 118.69)**	**-2.09* (115.37)**	**1.64† (121.99)**	**-4.01*** (116.85)**
Eating Disorder Examination Questionnaire							
Total Score	0.57 (1.09)	0.99 (1.02)	1.31 (1.14)	**6.70** (2, 115.26)**	**-1.78† (132.38)**	1.58 (119.15)	**-3.66*** (123.91)**
Shape Concerns	0.75 (1.44)	1.41 (1.38)	1.80 (1.54)	**7.83*** (2, 114.08)**	**-2.09* (128.07)**	1.54 (120.50)	**-3.96*** (122.22)**
Weight Concerns	0.64 (1.44)	1.06 (1.38)	1.53 (1.45)	**5.70** (2, 118.09)**	-1.35 (140.41)	**1.70† (117.82)**	**-3.32*** (127.15)**

Table 3b. MLM Simple and Covariate Main Effect Models across Twin Type (cont'd).

Model	Mean (Standard Deviation)			Twin Type Main Effect	Twin Type Pair-wise Comparisons		
					OS-F vs. All Males	OS-F vs. SS-F	SS-F vs. All Males
	All Males	OS-F	SS-F	F (df, df)	t (df)	t (df)	t (df)
Covariate Effects Models							
Pre-Early Pubertal Group							
Minnesota Eating Behaviors Survey							
Total Score	4.43 (3.85)	4.83 (3.93)	5.30 (3.95)	1.06 (2, 162.89)	-0.13 (134.43)	0.84 (138.71)	-1.44 (119.09)
Body Dissatisfaction	0.77 (1.29)	0.94 (1.15)	1.05 (1.35)	1.60 (2, 153.82)	-0.70 (126.96)	0.50 (135.21)	**-1.76† (93.39)**
Weight Preoccupation	1.61 (1.74)	1.76 (1.78)	2.10 (1.80)	1.66 (2, 165.47)	-0.51 (132.39)	0.75 (140.75)	**-1.82† (124.06)**
Eating Disorder Examination Questionnaire							
Total Score	0.62 (0.70)	0.65 (0.66)	0.72 (0.71)	0.37 (2, 155.23)	-0.22 (122.60)	0.41 (137.76)	-0.86 (111.64)
Shape Concerns	0.74 (0.95)	0.86 (0.91)	0.93 (0.97)	1.14 (2, 154.34)	-0.81 (124.21)	0.28 (136.47)	-1.42 (114.12)
Weight Concerns	0.69 (0.87)	0.75 (0.79)	0.88 (0.89)	0.70 (2, 152.11)	-0.39 (119.51)	0.49 (138.08)	-1.17 (111.66)

Table 3b. MLM Simple and Covariate Main Effect Models across Twin Type (cont'd).

Model	Mean (Standard Deviation)			Twin Type Main Effect	Twin Type Pair-wise Comparisons		
					OS-F vs. All Males	OS-F vs. SS-F	SS-F vs. All Males
	All Males	OS-F	SS-F	F (df, df)	t (df)	t (df)	t (df)
Covariate Effects Models							
Mid-Late Pubertal Group							
Minnesota Eating Behaviors Survey							
Total Score	5.07 (4.36)	6.17 (5.02)	7.07 (5.10)	**2.46† (2, 108.43)**	-1.15 (94.29)	0.78 (117.98)	**-2.19* (103.44)**
Body Dissatisfaction	1.06 (1.73)	1.38 (1.87)	1.84 (1.93)	**2.82† (2, 101.93)**	-0.85 (104.93)	1.10 (114.31)	**-2.36* (103.98)**
Weight Preoccupation	1.58 (1.97)	2.20 (2.08)	2.85 (2.27)	**4.91** (113.14)**	**-1.79† (100.46)**	1.12 (116.81)	**-3.08** (111.02)**
Eating Disorder Examination Questionnaire							
Total Score	0.72 (0.96)	1.01 (0.94)	1.24 (1.03)	**3.77* (2, 105.83)**	-1.37 (113.94)	1.14 (112.57)	**-2.75** (113.10)**
Shape Concerns	0.97 (1.25)	1.43 (1.25)	1.70 (1.36)	**4.80** (2, 108.81)**	**-1.66† (117.83)**	1.09 (113.37)	**-3.10** (112.73)**
Weight Concerns	0.84 (1.26)	1.07 (1.25)	1.45 (1.32)	**2.96† (2, 114.43)**	-0.92 (125.66)	1.25 (111.60)	**-2.40* (116.75)**

Note: SS-F = same-sex female twins; OS-F = opposite-sex female twins; All Males = opposite-sex and same-sex male twins; df = degrees of freedom; Simple Effect Models were adjusted for age and zygosity only. Covariate Effect Models were adjusted for age, zygosity, depressive symptoms, anxiety symptoms, body mass index, and autonomy difficulties. Depressive symptoms, body mass index, and autonomy difficulties were significant covariates in all pre-early pubertal group models (p's < .01). Anxiety was a non-

significant covariate in all pre-early pubertal models (p's > .05). In the mid-late pubertal group models, depressive symptoms, anxiety symptoms, and body mass index were significant covariates (p's < .01). Autonomy was a non-significant covariate in the mid-late pubertal group models for the Minnesota Eating Behaviors Survey, but exhibited trend-level or significant covariate effects for the Eating Disorders Examination Questionnaire scales: total score (p = .08), shape concerns (p = .06), weight concerns scale (p = .02).

†p < .10, *p < .05, **p < .01, ***p < .001

Table 4b. ANOVA Participant Type by Pubertal Status Interaction Results.

Model	Statistics	
	F (df, df)	*p*-value
Minnesota Eating Behaviors Survey		
Total Score		
Participant Type	2.55 (1, 123)	.11
Pubertal Group	5.37 (1, 123)	**.02**
Participant Type x Pubertal Group	0.88 (1, 123)	.35
Body Dissatisfaction		
Participant Type	1.83 (1, 123)	.18
Pubertal Group	4.35 (1, 123)	**.04**
Participant Type x Pubertal Group	1.69 (1, 123)	.20
Weight Preoccupation		
Participant Type	5.44 (1, 123)	**.02**
Pubertal Group	1.87 (1, 123)	.17
Participant Type x Pubertal Group	0.41 (1, 123)	.52
Eating Disorder Examination Questionnaire		
Total Score		
Participant Type	1.48 (1, 122)	.22
Pubertal Group	4.64 (1, 122)	**.03**
Participant Type x Pubertal Group	1.04 (1, 122)	.31
Shape Concerns		
Participant Type	1.34 (1, 122)	.25
Pubertal Group	4.75 (1, 122)	**.03**
Participant Type x Pubertal Group	0.87 (1, 122)	.35
Weight Concerns		
Participant Type	1.53 (1, 122)	.22
Pubertal Group	3.70 (1, 122)	.06
Participant Type x Pubertal Group	1.17 (1, 122)	.28

Table 5b. Simple and Covariate Main Effect Models for Female Opposite-Sex Twins and Non-Twins.

Model	Simple Effect Models				Covariate Effect Models			
	Mean (SD)		Mean Difference Effect Size	Participant Type Main Effect	Mean (SD)		Mean Difference Effect Size	Participant Type Main Effect
	OS-F	NT	Cohen's d	F (df, df)	OS-F	NT	Cohen's d	F (df, df)
Pre-Early Pubertal Group								
Minnesota Eating Behaviors Survey								
Total Score	4.23 (4.39)	4.95 (4.40)	.16	0.24 (1, 49)	4.47 (2.97)	4.38 (2.91)	.03	0.01 (1, 42)
Body Dissatisfaction	0.87 (1.49)	0.99 (1.50)	.08	0.00 (1, 49)	0.85 (0.96)	0.82 (0.94)	.03	0.01 (1, 42)
Weight Preoccupation	1.62 (1.82)	2.25 (1.82)	.34	1.43 (1, 49)	1.67 (1.43)	2.17 (1.40)	.35	1.42 (1, 42)
Eating Disorder Examination Questionnaire								
Total Score	0.64 (0.93)	0.74 (0.94)	.03	0.02 (1, 48)	0.64 (0.74)	0.64 (0.72)	.01	0.00 (1, 42)
Shape Concerns	0.81 (1.18)	0.99 (1.19)	.04	0.03 (1, 48)	0.84 (1.08)	0.88 (1.06)	.00	0.00 (1, 42)
Weight Concerns	0.77 (1.10)	0.90 (1.11)	.03	0.01 (1, 48)	0.81 (0.90)	0.75 (0.89)	.07	0.06 (1, 42)

Table 5b. Simple and Covariate Main Effect Models for Female Opposite-Sex Twins and Non-Twins.

Model	Simple Effect Models				Covariate Effect Models			
	Mean (SD)		Mean Difference Effect Size	Participant Type Main Effect	Mean (SD)		Mean Difference Effect Size	Participant Type Main Effect
	OS-F	NT	Cohen's d	F (df, df)	OS-F	NT	Cohen's d	F (df, df)
Mid-Late Pubertal Group								
Minnesota Eating Behaviors Survey								
Total Score	5.70 (5.46)	8.01 (5.46)	.42	**3.49† (1, 74)**	6.00 (4.74)	7.77 (4.73)	.37	2.51 (1, 69)
Body Dissatisfaction	1.34 (1.99)	2.18 (1.97)	.64	**3.71† (1, 74)**	1.49 (1.71)	2.07 (1.70)	.34	2.09 (1, 69)
Weight Preoccupation	1.97 (2.22)	3.14 (2.23)	.52	**4.87* (1, 74)**	2.07 (2.00)	3.06 (2.00)	.49	**4.44* (1, 69)**
Eating Disorder Examination Questionnaire								
Total Score	0.94 (1.10)	1.38 (1.10)	.39	**2.85† (1, 74)**	1.03 (0.92)	1.31 (0.92)	.31	1.70 (1, 70)
Shape Concerns	1.35 (1.49)	1.92 (1.50)	.35	2.43 (1, 74)	1.49 (1.26)	1.81 (1.25)	.28	1.39 (1, 70)
Weight Concerns	1.02 (1.43)	1.61 (1.42)	.39	**2.93† (1, 74)**	1.15 (1.16)	1.50 (1.16)	.28	1.65 (1, 70)

Note: OS-F = opposite-sex female twins; NT = female non-twins; df = degrees of freedom; simple effects models = adjusted for age only; covariate effects models = adjusted for age, depressive symptoms, anxiety symptoms, body mass index, and autonomy difficulties. Significant covariate effects were observed for depressive symptoms, body mass index, and autonomy difficulties in pre-

early pubertal group models (p's < .01) and for depressive symptoms, anxiety symptoms, and body mass index in the mid-late pubertal group models (p's < .01).

†$p < .10$, *$p < .05$, **$p < .01$, ***$p < .001$

APPENDIX B

Figures: 1a-3a and 1b-2b

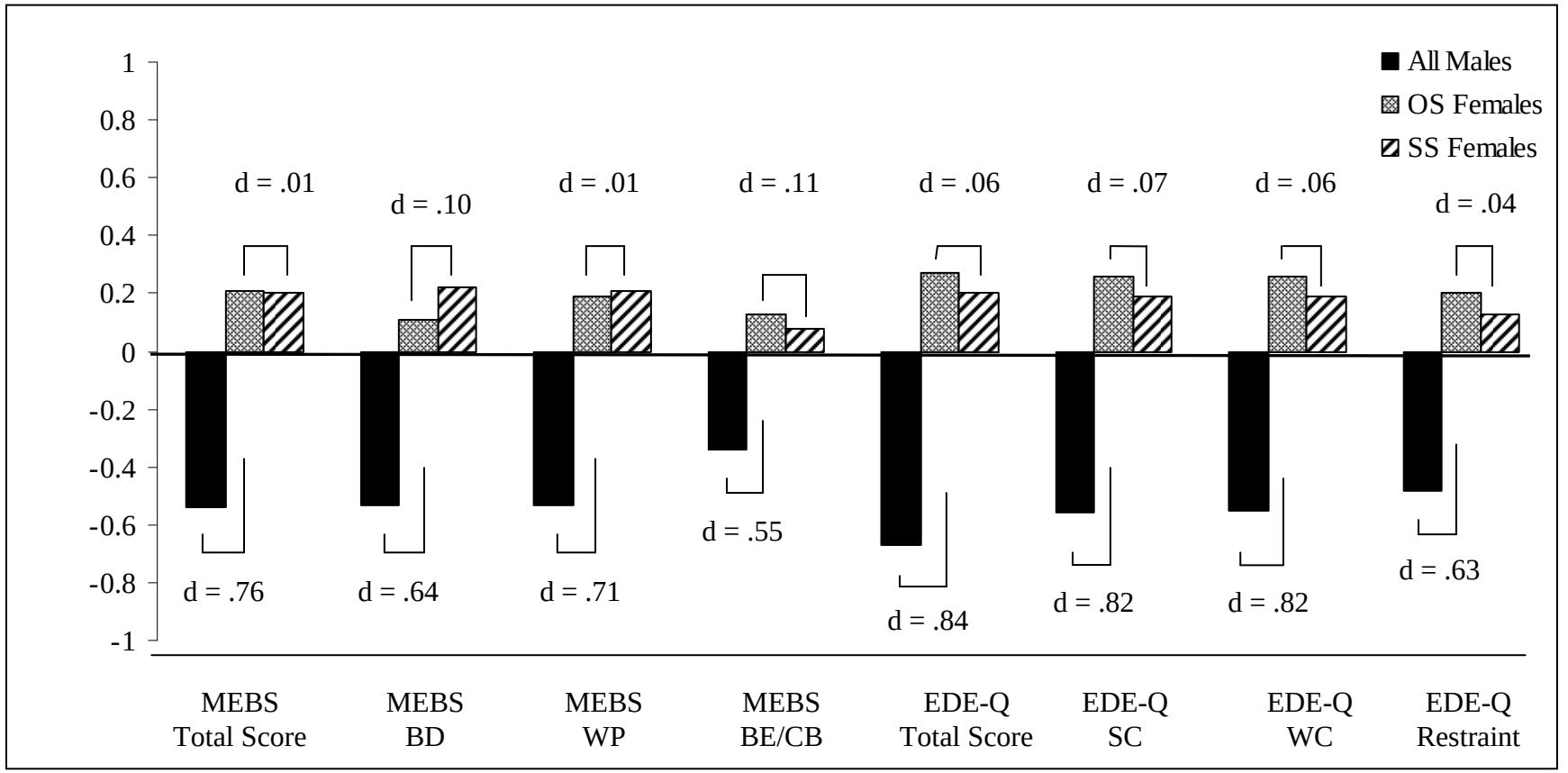

Figure 1a. Standardized Mean Disordered Eating Score by Twin Type, Ages 15-20. OS = Opposite-Sex; SS = Same-Sex; MEBS = Minnesota Eating Behaviors Survey; EDE-Q = Eating Disorder Examination Questionnaire; BD = Body Dissatisfaction; WP = Weight Preoccupation; BE/CB = Binge Eating/Compensatory Behaviors; SC = Shape Concerns; WC = Weight Concerns; d = Cohen's d effect size.

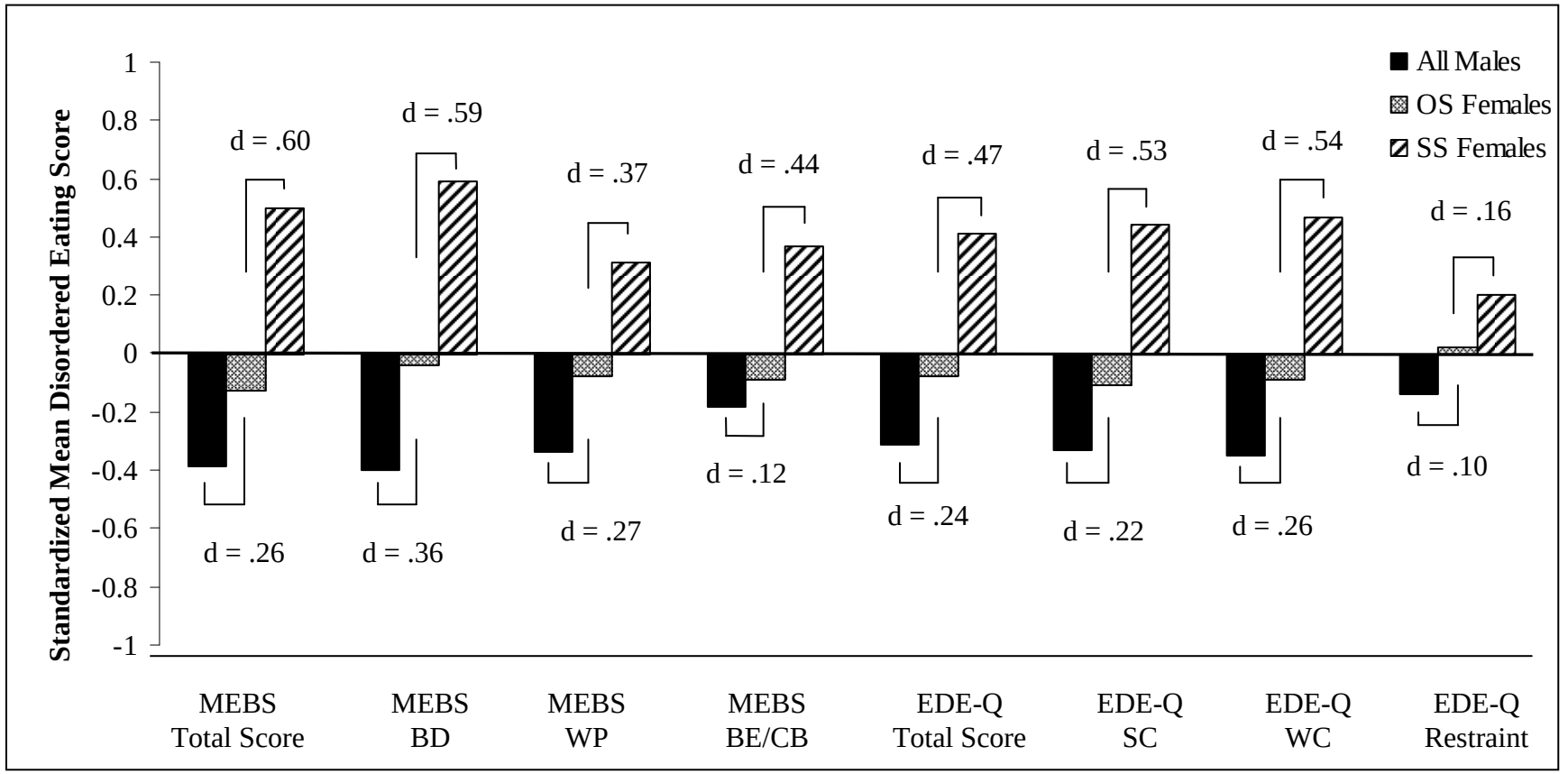

Figure 2a. Standardized Mean Disordered Eating Score by Twin Type, Ages 21-23. OS = Opposite-Sex; SS = Same-Sex; MEBS = Minnesota Eating Behaviors Survey; EDE-Q = Eating Disorder Examination Questionnaire; BD = Body Dissatisfaction; WP = Weight Preoccupation; BE/CB = Binge Eating/Compensatory Behaviors; SC = Shape Concerns; WC = Weight Concerns; d = Cohen's d effect size.

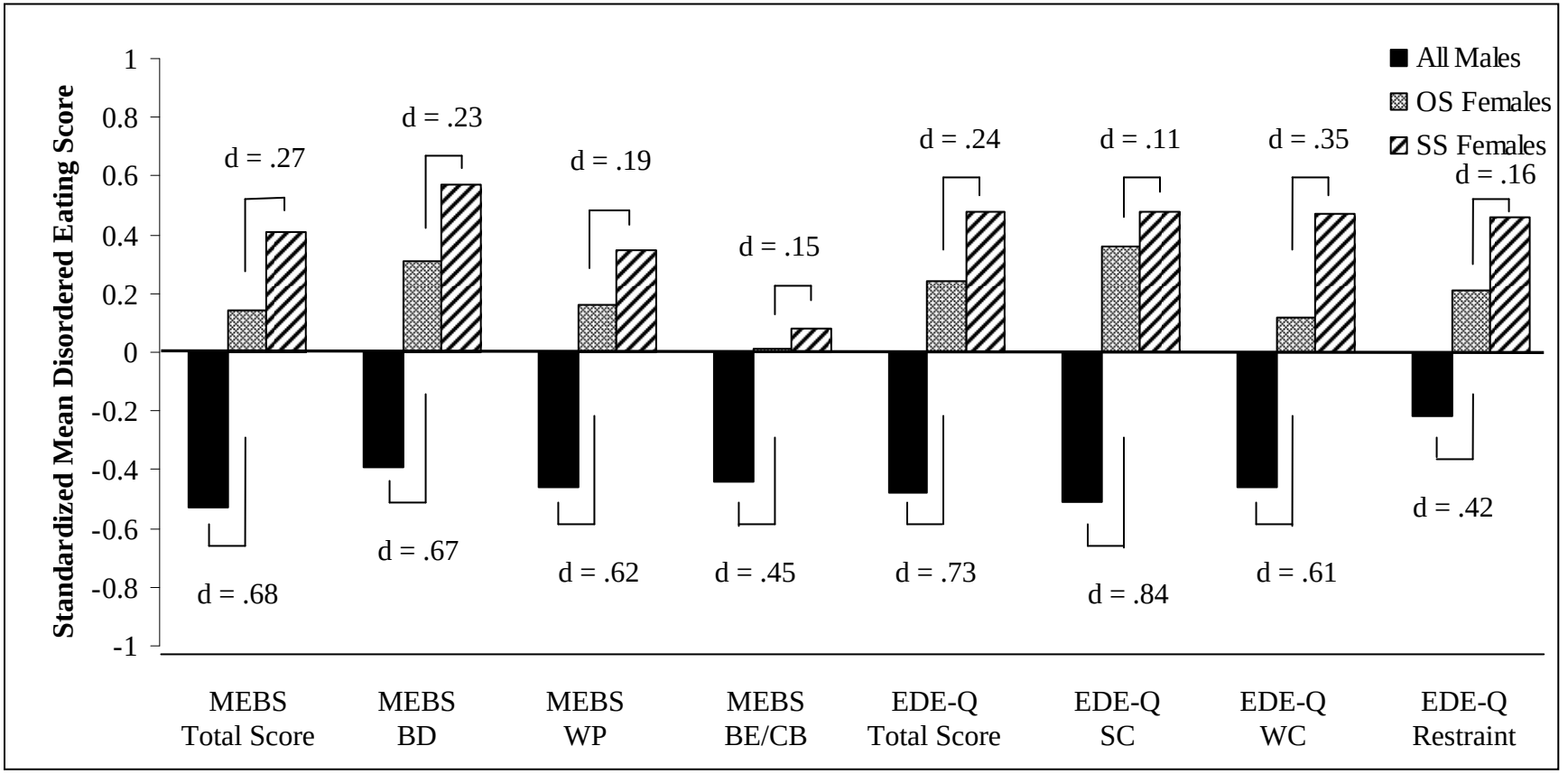

Figure 3a. Standardized Mean Disordered Eating Score by Twin Type, Ages 24-30. OS = Opposite-Sex; SS = Same-Sex; MEBS = Minnesota Eating Behaviors Survey; EDE-Q = Eating Disorder Examination Questionnaire; BD = Body Dissatisfaction; WP = Weight Preoccupation; BE/CB = Binge Eating/Compensatory Behaviors; SC = Shape Concerns; WC = Weight Concerns; d = Cohen's d effect size.

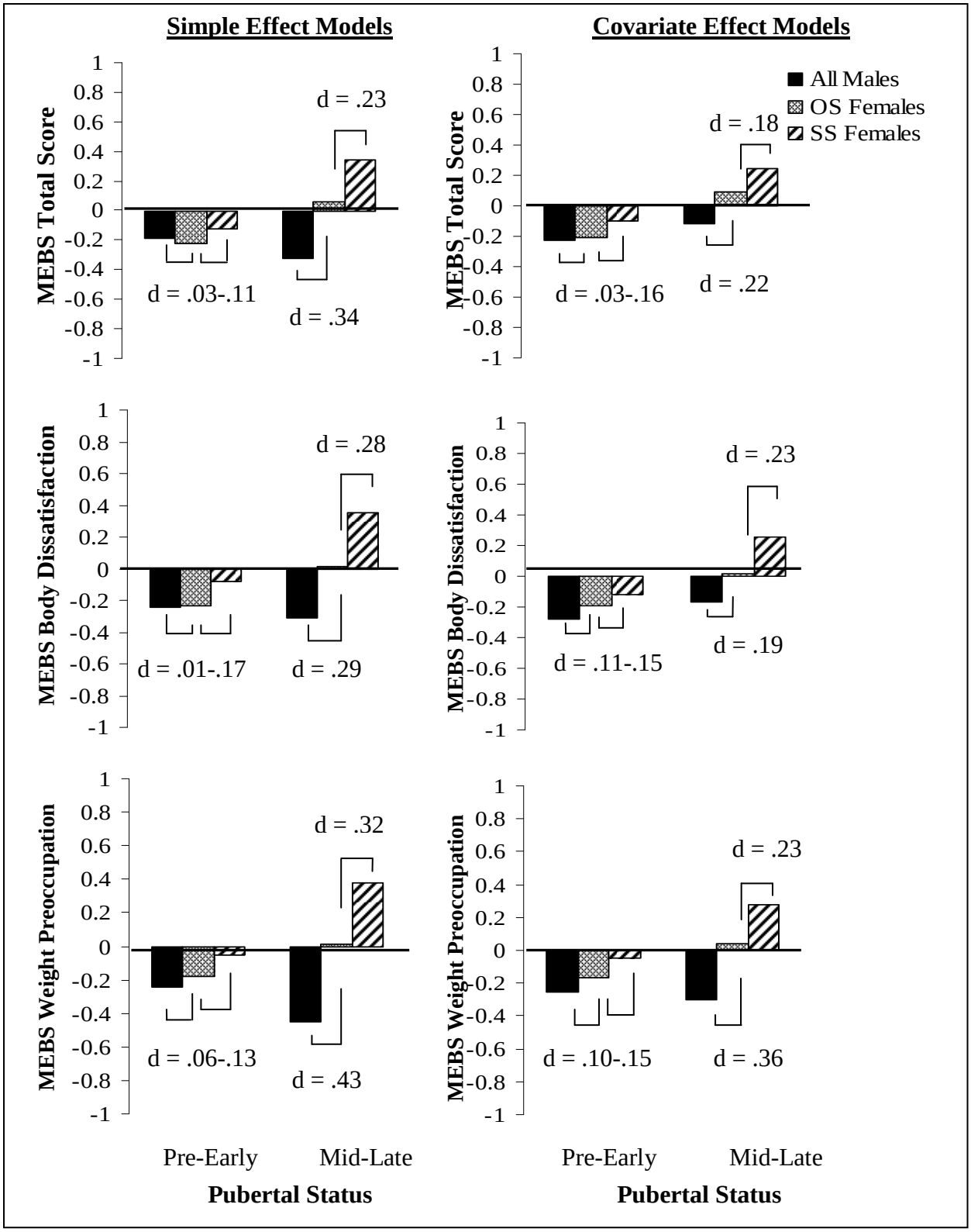

Figure 1b. Standardized Mean MEBS Disordered Eating Score by Twin Type and Pubertal Status for Simple and Covariate Main Effect Models. OS = Opposite-Sex; SS = Same-Sex; MEBS = Minnesota Eating Behaviors Survey; d = Cohen's d effect size.

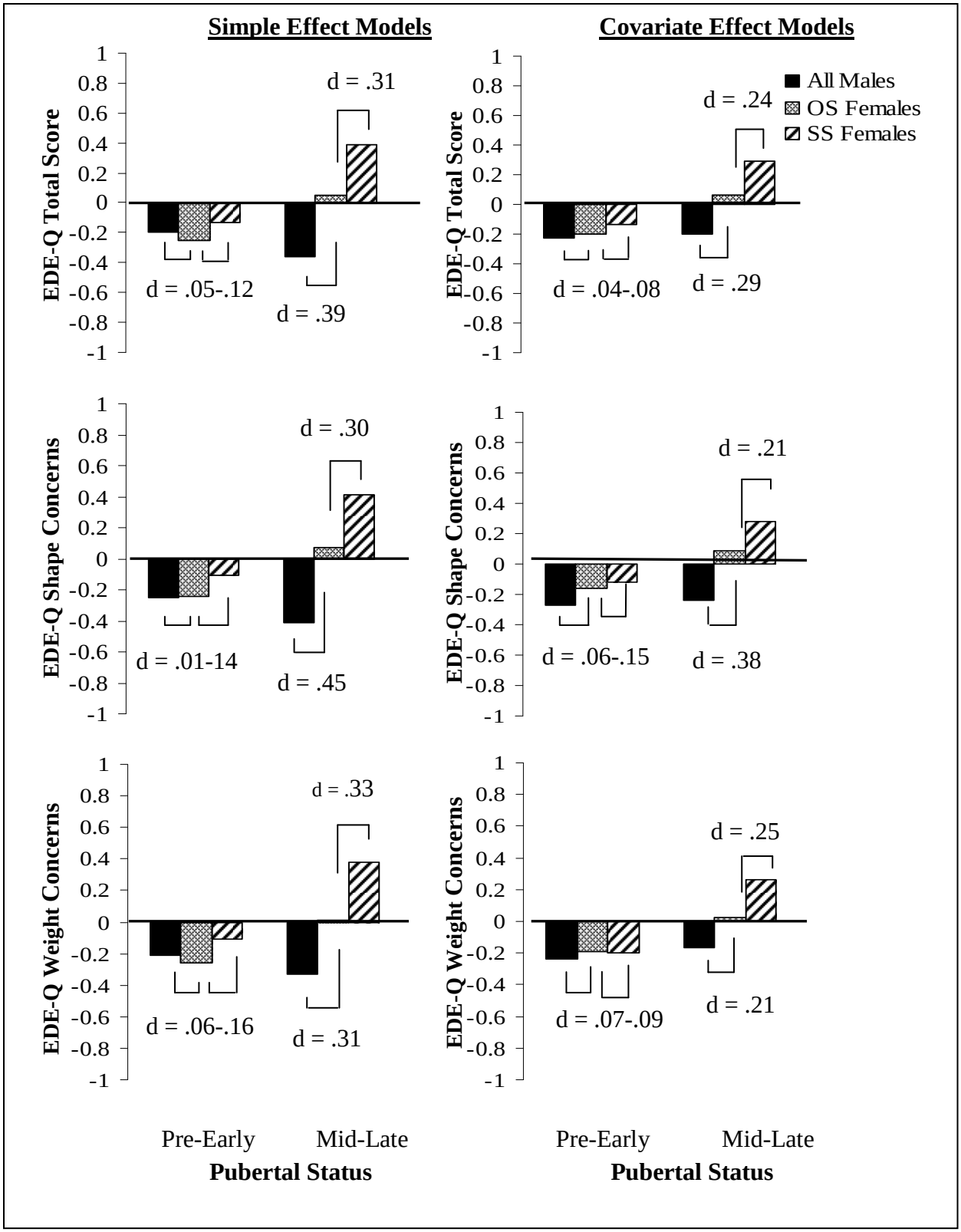

Figure 2b. Standardized Mean EDE-Q Disordered Eating Score by Twin Type and Pubertal Status for Simple and Covariate Main Effect Models. OS = Opposite-Sex; SS = Same-Sex; EDE-Q = Eating Disorder Examination Questionnaire; d = Cohen's d effect size.

REFERENCES

REFERENCES

American Psychiatric Association (2000). *Diagnostic and Statistical Manual of Mental Disorders, fourth Edition – Text Revision (DSM-IV-TR).* Washington, DC.

Alexanderson, C., Henningsson, S., Lichtenstein, P., Holmang, A., & Eriksson, E. (in press). Influence of having a male twin on body mass index and risk for dyslipidemia in middle-aged and old women. *International Journal of Obesity*.

Arnold, A.P. & Breedlove, S.M. (1985). Organizational and activational effects of sex steroids on brain and behavior: a reanalysis. *Hormones and Behavior, 19,* 469-498.

Attie, I. & Brooks-Gunn, J. (1989). Development of eating problems in adolescent girls: a longitudinal study. *Developmental Psychology, 25,* 70-79.

Baker, J.H., Lichtenstein, P., & Kendler, K.S. (2009). Intrauterine testosterone exposure and risk for disordered eating. *The British Journal of Psychiatry, 194,* 375-376.

Bell, D.D. & Zucker, I. (1971). Sex differences in body weight and eating: organization and activation by gonadal hormones in the rat. *Physiology and Behavior, 7,* 27-34.

Berenbaum, S.A., Bryk, K.K., Nowak, N., Quigley, C.A., & Moffat, S. (2009). Fingers as a marker of prenatal androgen exposure. *Endocrinology, 150,* 5119-5124.

Binford, R.B., Le Grange, D., & Jellar, C.C. (2005). Eating Disorders Examination versus the Eating Disorders Examination-Questionnaire in adolescents with full and partial-syndrome bulimia nervosa and anorexia nervosa. *International Journal of Eating Disorders, 37,* 44-49.

Blanchard, A. & Lyons, M. (2010). An investigation into the relationship between digit length ratio (2D:4D) and psychopathy. *The British Journal of Forensic Practice, 12,* 23-31.

Blum, W.F., Englaro, P., Hanitsch, S., Juul, A., Hertel, N.T., Muller, J., et al. (1997). Plasma leptin levels in healthy children and adolescents: dependence on body mass index, body fat mass, gender, pubertal stage, and testosterone. *The Journal of Clinical Endocrinology and Metabolism, 82,* 2904-2910.

Breedlove, S.M. (1994). Sexual differentiation of the human nervous system. *Annual Review of Psychology, 45,* 389-418.

Brown, W.M., Hines, M., Fane, B.A., & Breedlove, S.M. (2002). Masculinized finger length patterns in human males and females with congenital adrenal hyperplasia. *Hormones and Behavior, 42,* 380-386.

Bulik, C.M. (2002). Eating disorders in adolescents and young adults. *Child and Adolescent*

Psychiatric Clinics of North America, 11, 201-218.

Carter, J.C., Aime, A.A., & Mills, J.S. (2001). Assessment of bulimia nervosa: a comparison of interview and self-report questionnaire methods. *International Journal of Eating Disorders, 30*, 187-192.

Clemens, L.G., Gladue, B.A., & Coniglio, L.P. (1978). Prenatal endogenous androgenic influences on masculine sexual behavior and genital morphology in male and female rats. *Hormones and Behavior, 10*, 40-53.

Cohen-Bendahan, C.C., Buitelaar, J.K., van Goozen, S.H., & Cohen-Kettenis, P.T. (2004). Prenatal exposure to testosterone and functional cerebral lateralization: a study in same-sex and opposite-sex twin girls. *Psychoneuroendocrinology, 29*, 911-916.

Cohen-Bendahan, C.C, Buitelaar, J.K., van Goozen, S.H., Orlebeke, J.F., Cohen-Kettenis, P.T. Is there an effect of prenatal testosterone on aggression and other behavioral traits? A study comparing same-sex and opposite-sex twin girls. (2005). *Hormones and Behavior, 47*, 230-237.

Collaer, M. & Hines, M. (1995). Human behavioral sex differences: a role for gonadal hormones during early development? *Psychological Bulletin, 118*, 55-107.

Collinson, S.L., Lim, M., Chaw, J.H., Verma, S., Sim, K., Rapisarda, A., & Chong, S.A. (2010). Increased ratio of 2nd to 4th digit (2D:4D) in schizophrenia. *Psychiatric Research, 176*, 8-12.

Culbert, K.M., Breedlove, S.M., Burt, S.A., & Klump, K.L. (2008). Prenatal hormone exposure and risk for eating disorders: a comparison of opposite-sex and same-sex twins. *Archives of General Psychiatry, 65*, 329-336.

Culbert, K.M., Burt, S.A., McGue, M., Iacono, W.G., & Klump, K.L. (2009). Puberty and the genetic diathesis of disordered eating attitudes and behaviors. *Journal of Abnormal Psychology, 118*, 788-796.

Culbert, K.M., Burt, S.A., Sisk, C.L., Nigg, J.T., & Klump, K.L. (in preparation). The emergence of sex differences in disordered eating attitudes and behaviors during puberty.

Culbert, K.M., Racine, S., & Klump, K.L. (2011). The influence of gender and puberty on the heritability of disordered eating symptoms. *Current Topics in Behavioral Neuroscience, 6*, 177-185.

De Bruin, E.I., Verheij, F., Wiegman, T., & Ferdinand, R.F. (2006). Differences in finger length ratio between males with autism, pervasive developmental disorder-not otherwise specified, ADHD, and anxiety disorders. *Developmental Medicine and Child Neurology, 48*, 962-965.

Dempsey, P., Townsend, G., & Richards, L. (1999). Increased tooth crown size in females with twin brothers: evidence for hormonal diffusion between human twins in utero. *American Journal of Human Biology, 11,* 577-586.

Donohoe, T. P., & Stevens, R. (1983). Effects of ovariectomy, estrogen treatment and CI-628 on food inake and body weight in female rats treated neonatally with gonadal hormones. *Physiology and Behavior, 31,* 325-329.

Edler, C., Lipson, S.F., & Keel, P.K. (2007). Ovarian hormones and binge eating in bulimia nervosa. *Psychological Medicine, 37,* 131-141.

Eggert, J. (unpublished dissertation). Separation-individuation and disordered eating in adolescence.

Eisner, J.R., Dumesic, D.A., Kemnitz, J.W., Colman, R.J., & Abbott, D.H. (2003). Increased adiposity in female rehesus monkeys exposed to androgen excess during early gestation. *Obesity Research, 11,* 279-286.

Fairburn, C.G. & Beglin, S.J. (1994). Assessment of eating disorders: interview or self-report questionnaire? *International Journal of Eating Disorders, 16,* 363-370.

Field, A.E., Camargo, C.A., Barr Taylor, C., Berkey, C.S., Roberts, S.B., & Colditz, G.A. (2001). Peer, parent, and media influences on the development of weight concerns and frequent dieting among preadolescent and adolescent girls and boys. *Pediatrics, 107,* 54-60.

Field, E.F., Whishaw, I.Q., Forgie, M.L., & Pellis, S.M. (2004). Neonatal and pubertal, but not adult, ovarian steroids are necessary for the development of female-typical patterns of dodging to protect a food item. *Behavioral Neuroscience, 118,* 1293-1304.

Fichter, M.M. & Noegel, R. (1990). Concordance for bulimia nervosa in twins. *International Journal of Eating Disorders, 9,* 255-263.

Gandelman, R., vom Saal, F.S., & Reinisch, J.M. (1977). Contiguity to male fetuses affects morphology and behavior of female mice. *Nature, 266,* 722-724.

Garner, D.M. (1991). Eating Disorder Inventory 2, Professional Manual. Odessa, FL: Psychological Assessment Resources.

Gentry, R.T., & Wade, G.N. (1976). Sex differences in sensitivity of food intake, body weight, and running-wheel activity to ovarian steroids in rats. *Journal of Comparative and Physiological Psychology, 90,* 747-754.

Glinianaia, S.V., Magnus, P., Harris, J.R., & Tambs, K. (1998). Is there a consequence for fetal growth of having an unlike-sexed cohabitant in utero? *International Journal of Epidemiology, 27,* 657-659.

Heatherton, T.F., Mahamedi, F., Striepe, M., Field, A.E., & Keel, P. (1997). A 10-year longitudinal study of body weight, dieting, and eating disorder symptoms. *Journal of Abnormal Psychology, 106,* 117-125.

Heil, M., Kavsek, M., Rolke, B., Beste, C., & Jansen, P. (2011). Mental rotation in female fraternal twins: evidence for intra-uterine hormone transfer? *Biological Psychology, 86,* 90-93.

Hermes, S.F. & Keel, P.K. (2003). The influence of puberty and ethnicity on awareness and internalization of the thin ideal. *International Journal of Eating Disorders, 33,* 465-467.

Holland, A.J., Sicotte, N., & Treasure, J. (1988). Anorexia nervosa: evidence for a genetic basis. *Journal of Psychosomatic Research, 32,* 561-571.

Hollingshead, A. (1975). Four factor index of social status. New Haven, CT: Yale University.

Hudson, J.I., Hiripi, E., Pope, H.G., & Kessler, R.C. (2007). The prevalence and correlates of eating disorders in the National Comorbidity Survey Replication. *Biological Psychiatry, 61,* 348-358.

Jacobi, C., Hayward, C., de Zwaan, M., Kraemer, H.C., & Stewart, A. (2004). Coming to terms with risk factors for eating disorders: application of risk terminology and suggestions for a general taxonomy. *Psychological Bulletin, 130,* 19-65.

Keel, P.K., Baxter, M.G., Heatherton, T.F., & Joiner, T.E. (2007). A 20-year longitudinal study of body weight, dieting, and eating disorder symptoms. *Journal of Abnormal Psychology, 116,* 422-432.

Klump, K.L. & Burt, S.A. (2006). The Michigan State University Twin Registry (MSUTR): genetic, environmental and neurobiological influences on behavior across development. *Twin Research and Human Genetics, 9,* 971-977.

Klump, K.L., Culbert, K.M., Slane, J.D., Burt, S.A., Sisk, C.L., & Nigg, J.T. (submitted). The effects of puberty on genetic risk for disordered eating: evidence for a sex difference.

Klump, K.L., Gobrogge, K.L., Perkins, P., Thorne, D., Sisk, C.L., & Breedlove, S.M. (2006). Preliminary evidence that gonadal hormones organize and activate disordered eating. *Psychological Medicine, 12,* 1-8.

Klump, K.L., Keel, P.K., Culbert, K.M., & Edler, C. (2008). Ovarian hormones and binge eating: exploring phenotypic associations in community samples. *Psychological Medicine, 38,* 1749-1757.

Klump, K.L., Keel, P.K., Sisk, C.L., & Burt, S.A. (2010). Preliminary evidence that estradiol moderates genetic effects on disordered eating attitudes and behaviors during puberty.

Psychological Medicine, 40, 1745-1754.

Klump, K.L. & Leon, G.R. (unpublished data). The validity of the equal environments and representativeness assumptions for twins at risk for the development of eating disorders.

Klump, K.L., McGue, M., & Iacono, W.G. (2000). Age differences in genetic and environmental influences on eating attitudes and behaviors in female adolescent twins. *Journal of Abnormal Psychology, 109,* 239-251.

Lavender, J.M., De Young, K.P., & Anderson, D.A. (2010). Eating Disorder Examination Questionnaire (EDE-Q): norms for undergraduate men. *Eating Behaviors, 11*, 119-121.

Levine, J., Green, C., & Millon, T. (1986). The Separation-Individuation Test of Adolescence. *Journal of Personality Assessment, 50,* 123-137.

Levine, J. & Saintonge, S. (1993). Psychometric properties of the Separation-Individuation Test of Adolescence within a clinical population. *Journal of Clinical Psychology, 49*, 492-507.

Lewinsohn, P.M., Striegel-Moore, R.H., & Seeley, J.R. (2000). Epidemiology and natural course of eating disorders in young women from adolescence to young adulthood. *Journal of American Academy of Child and Adolescent Psychiatry, 39*, 1284-1292.

Linville, D., Stice, E., Gau, J., & O'Neil, M. (in press). Predictive effects of mother and peer influences on increases in adolescent eating disorder risk factors and symptoms: a 3-year longitudinal study. *International Journal of Eating Disorders.*

Lummaa, V., Pettay, J.E., & Russell, A.F. (2007). Male twins reduce fitness of female co-twins in humans. *Proceedings of the National Academy of Sciences of the United States of America, 104*, 10915-10920.

Lutchmaya, S., Baron-Cohen, S., Raggatt, P., Knickmeyer, R., & Manning, J. (2004). 2^{nd} and 4^{th} digit ratios, fetal testosterone and estradiol. *Early Human Development, 77*, 23-28.

Madrid, J.A., Lopez-Bote, C., & Martin, E. (1993). Effects of neonatal androgenization on the circadian rhythm of feeding behavior in rats. *Physiology and Behavior, 53*, 329-335.

Malas, M., Dogan, S., Evcil, E.H., & Desdicioglu, K. (2006). Fetal development of the hands, digits, and digit ratios (2D:4D). *Early Human Development, 82*, 469-475.

Maloney, M.J., McGuire, J., Daniels, S.R., & Specker, B. (1989). Dieting behavior and eating attitudes in children. *Pediatrics, 84*, 482-489.

Manning, J.T., Scutt, D., Wilson, J., & Lewis-Jones, D.I. (1998). The ratio of 2^{nd} to 4^{th} digit length: a predictor of sperm numbers and concentrations of testosterone, luteinizing hormone and oestrogen. *Human Reproduction, 13,* 3000-3004.

March, J.S., Parker, J., Sullivan, K., Stallings, P., Conners, C.K. (1997). The Multidimensional Scale for Children (MASC): factor structure, reliability, and validity. *Journal of the American Academy of Child and Adolescent Psychiatry, 36,* 554-565.

Marsden, P., Meyer, C., Fuller, M., & Waller, G. (2002). The relationship between eating psychopathology and separation-individuation in young nonclinical women. *Journal of Nervous and Mental Disease, 190,* 710-713.

Marderosian, A., Wu, Y., Culbert, K.M., Burt, S. A., Nigg, J. T., & Klump, K. L. (in preparation). Psychometric properties of the Minnesota Eating Behaviors Survey in pre-adolescent and adolescent girls and boys.

Martel, M.M., Gobrogge, K.L., Breedlove, S.M., Nigg, J.T. (2008). Masculinized finger-length ratios of boys, but not girls, are associated with attention-deficit/hyperactivity disorder. *Behavioral Neuroscience, 122,* 273-281.

McClanahan, G. & Holmbeck, G. (1992). Separation-Individuation, family functioning, and psychological adjustment in college students: a construct validity study of the separation-individuation test of adolescence. *Journal of Personality Assessment, 59,* 468-485.

McFadden, D. (1993). A masculinizing effect on the auditory systems of human females having male co-twins. *Proceedings of the National Academy of Sciences of the United States of America, 90,* 11900-11904.

Miller, E.M. (1994). Prenatal sex hormone transfer: a reason to study opposite-sex twins. *Personality and Individual Differences, 17,* 511-529.

Miller, E.M. (1995). Reported myopia in opposite sex twins: a hormonal hypothesis. *Optometry and Vision Science, 72,* 34-36.

Miller, E.M. & Martin, N. (1995). Analysis of the effect of hormones on opposite-sex twin attitudes. *Acta Geneticae Medicae et Gemellologiae, 44,* 41-52.

Mond, J.M., Hay, P.J., Rodgers, B., Owen, C., & Beaumont, P.J.V. (2004a). Temporal stability of the eating disorder examination questionnaire. *International Journal of Eating Disorders, 36,* 195-203.

Mond, J.M., Hay, P.J., Rodgers, B., Owen, C., & Beaumont, P.J.V. (2004b). Validity of the eating disorder examination questionnaire (EDE-Q) in screening for eating disorders in community samples. *Behavioral Research and Therapy, 42,* 551-567.

Nolen-Hoeksema, S. (2001). Gender differences in depression. *Current Directions in Psychological Science, 10,* 173-176.

Ohzeki, T., Otahara, H., Hanaki, K., Motozumi, H., Shiraki, K. (1993). Eating attitudes test in

boys and girls aged 6-18 years: decrease in concerns with eating in boys and the increase in girls with their ages. *Psychopathology, 26,* 117-121.

Okten, A., Kalyoncu, M., & Yari, N. (2002). The ratio of second- and fourth-digit lengths and congenital adrenal hyperplasia due to 21-hydroxylase deficiency. *Early Human Development, 70,* 47-54.

Patton, G.C., Selzer, R., Coffey, C., Carlin, J.B., & Wolfe, R. (1999). Onset of adolescent eating disorders: population based cohort study over 3 years. *British Medical Journal, 318,* 765-768.

Peper, J.S., Brouwer, R.M., van Baal, G.C., Schnack, H.G., van Leeuwen, M., Boomsma, D.I., Kahn, R.S., & Hulshoff Pol, H.E. (2009). Does having a twin brother make for a bigger brain? *European Journal of Endocrinology, 160,* 739-746.

Petersen, A.C., Crockett, L., Richards, M., & Boxer, A. (1988). A self-report measure of pubertal status: reliability, validity, and initial norms. *Journal of Youth and Adolescence, 17,* 117-133.

Peterson, C.B., Crosby, R.D., Wonderlich, S.A., Joiner, T., Crow, S.J., Mitchell, J.E., et al. (2007). Psychometric properties of the eating disorder examination-questionnaire: factor structure and internal consistency. *International Journal of Eating Disorders, 40,* 386-389.

Quinton, S.J., Smith, A.R., & Joiner, T. (in press). The 2^{nd} to 4^{th} digit ratio (2D:4D) and eating disorder diagnosis in women. *Personality and Individual Differences.*

Racine, S.E., Culbert, K.M., Keel, P.K., Sisk, C.L., Burt, S.A., & Klump, K.L. (in press). Differential associations between ovarian hormones and disordered eating symptoms across the menstrual cycle in women. *International Journal of Eating Disorders.*

Raevuori, A., Kaprio, J., Hoek, H.W., Sihvola, E., Rissanen, A., Keski-Rahkonen, A. (2008). Anorexia and bulimia nervosa in same-sex and opposite-sex twins: lack of association with twin type in a nationwide study of Finnish twins. *American Journal of Psychiatry, 165,* 1604-1610.

Resnick, S., Gottesman, I., & McGue, M. (1993). Sensation seeking in opposite-sex twins: an effect of prenatal hormones? *Behavioral Genetics, 23,* 323-329.

Rhodes, B. & Kroger, J. (1992). Parental bonding and separation-individuation difficulties among late adolescent eating disordered women. *Psychiatry and Human Development, 22,* 249-263.

Rines, J.P. & vom Saal, F.S. (1984). Fetal effects on sexual behavior and aggression in young and old female mice treated with estrogen and testosterone. *Hormones and Behavior, 18,* 117-129.

Ryan, B.C. & Vandenbergh, J.G. (2002). Intrauterine position effects. *Neuroscience and Biobehavioral Reviews, 26,* 665-678.

Schulz, K.M., Molenda-Figueira, H.A., & Sisk, C.L. (2009). Back to the future: the organizational-activational hipótesis adapted to puberty and adolescence. *Hormones and Behaviors, 55,* 597-604.

Shinwell, E.S., Reichman, B., Lerner-Geva, L., Boyko, V., Blickstein, I. & the Israel Neonatal Network (2007). "Masculinizing" effect on respiratory morbidity in girls from unlike-sex preterm twins: a possible transchorionic paracrine effect. *Pediatrics, 120,* 447-453.

Slutske, W.S., Bascom, E.N., Meier, M.H., Medland, S.E., & Martin, N.G. (in press). Sensation seeking in females from opposite-versus same-sex twin pairs: hormone transfer or sibling imitation? Behavior Genetics.

Smith, A.R., Hawkeswood, S.E., & Joiner, T.E. (2010). The measure of a man: associations between digit ratio and disordered eating in males. *International Journal of Eating Disorders, 43,* 543-548.

Steinhausen, H.C., Gavez, S., & Metzke, C.W. (2005). Psychosocial correlates, outcome, and stability of abnormal adolescent eating behavior in community samples of young people. *International Journal of Eating Disorders, 37,* 119-126.

Stice, E., Agras, W.S., & Hammer, L.D. (1999). Risk factors for the emergence of childhood eating disturbances: a five-year prospective study. *International Journal of Eating Disorders, 25,* 375-387.

Stice, E., Killen, J.D., Hayward, C., & Taylor, C.B. (1998). Age of onset for binge eating and purging during late adolescence: a found year survival analysis. *Journal of Abnormal Psychology, 107,* 671-675.

Stice, E., Marti, C.N., Shaw, H., & Jaconis, M. (2009). An 8-year longitudinal study of the natural history of threshold, subtreshold, and partial eating disorders from a community sample of adolescents. *Journal of Abnormal Psychology, 118,* 587-597.

Stice, E., Ng, J., & Shaw, H. (2010). Risk factors and prodromal eating pathology. *Journal of Child Psychology and Psychiatry, 51,* 518-525.

Striegel-Moore, R.H. & Bulik, C.M. (2007). Risk factors for eating disorders. *American Psychologist, 62,* 181-198.

von Ranson, K.M., Klump, K.L., Iacono, W.G., & McGue, M. (2005). The Minnesota Eating Behavior Survey: a brief measure of disordered eating attitudes and behaviors. *Eating Behaviors, 4,* 373-392.

Vuoksimaa, E., Eriksson, C.J.P., Pulkkinen, L., Rose, R.J., Kaprio, J. (2010). Decreased prevalence of left-handedness among females with male co-twins: evidence suggesting prenatal testosterone transfer in humans? *Psychoneuroendocrinology, 35*, 1462-1472.

Vuoksimaa, E., Kaprio, J., Kremen, W.S., Hokkanen, L., Viken, R.J., Tuulio-Henriksson, & Rose, R.J. (2010). Having a male co-twin masculinizes mental rotation performance in females. *Psychological Science, 21*, 1069-1071.

Wade, G.N. (1972). Gonadal hormones and behavioral regulation of body weight. *Physiology and Behavior, 8*, 523-534.

Wade, G. N., & Zucker, I. (1969a). Hormonal and developmental influences on rat saccharin preferences. *Journal of Comparative & Physiological Psychology, 69*, 201-300.

Wade, G. N., & Zucker, I. (1969b). Taste preferences of female rats: modification by neonatal hormones, food deprivation and prior experience. *Physiology and Behavior, 4*, 935-943.

Zehr, J.L., Culbert, K.M., Sisk, C.L., & Klump, K.L. (2007). An association of early puberty with disordered eating and anxiety in a population of undergraduate women and men. *Hormones and Behavior, 52*, 427-435.

Zucker, I. (1969). Hormonal determinants of sex differences in saccharin preference, food intake and body weight. *Physiology and Behavior, 4*, 595-602.