Psychiatry Research 342 (2024) 116170

Contents lists available at ScienceDirect

Psychiatry Research

journal homepage: www.elsevier.com/locate/psychres

Maternal sex-hormone exposure and the risk of eating disorders in daughters

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ARTICLE INFO

Keywords:
Bulimia nervosa
Anorexia nervosa
Eating disorders
Feeding and eating disorders
Prenatal hormones
Maternal hormones

ABSTRACT

Potential effects of prenatal sex hormones on later eating disorders in offspring have been investigated with two indirect methods (finger length ratio, opposite- *versus* same-sex twins). We utilized a direct, prospective method, examining the association between prenatal sex-hormones in maternal sera and the risk of bulimia nervosa (BN) and anorexia nervosa (AN) among daughters. Females with BN (55), AN (150), sister controls without eating disorders (one per case), and population controls (one per case) were derived from Finnish registers. Maternal gestational testosterone and estradiol levels were assayed from archived specimens stored in a national serum biobank. When females with BN were compared to their sister controls, those with higher gestational testosterone levels were at an increased risk of BN. No significant associations with BN were found when the comparison was made to population controls, and when estradiol levels and testosterone/estrogen ratio were assessed. We neither found associations between gestational sex-hormone levels and the risk of AN. Among females with familial liability for BN, higher gestational testosterone exposure may have a role in later development of BN, whereas lower testosterone exposure may have a protective effect. We found no evidence for the involvement of gestational sex-hormones in the etiology of AN.

1. Introduction

Bulimia nervosa (BN) and anorexia nervosa (AN) are potentially severe eating disorders with highly disproportionate sex-distribution (Silén and Keski-Rahkonen, 2022). Along with socio-cultural influences and psychological vulnerability (Fuglset, 2019, Stackpole et al., 2023, Striegel-Moore and Bulik, 2007), the origins of the female preponderance are thought to be based on biological factors. One such biological mechanism is suggested to be the exposure to prenatal sex-hormone milieu (Raevuori et al., 2014, Klump et al., 2006, Marzola et al., 2021, Klump et al., 2017), as the primary androgen, testosterone, is crucial for the development of sexually-dimorphic characteristics. Prenatal testosterone, and higher testosterone/estrogen ratio specifically (Lutchmaya et al., 2004), drive the organizational (i.e., permanent) changes to brain structure and function. In animals, prenatal testosterone exposure and subsequent brain masculinization produce sexually dimorphic behaviors, such as increased food intake, and higher body weight with higher muscle mass in females exposed to high testosterone during gestation compared to non-exposed females (Donohoe and Stevens, 1983, Madrid et al., 1993, Wade, 1972). The absence of testosterone and lower testosterone/estrogen ratio during the prenatal period in turn prevent masculinization and result in female-typical postnatal characteristics, including decreased food intake, lower body weight, and higher body adiposity (Wade, 1972).

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https://doi.org/10.1016/j.psychres.2024.116170
Received 2 October 2023; Received in revised form 1 July 2024; Accepted 30 August 2024
Available online 5 September 2024

Results from animal models therefore suggest, that dimensions of disorder eating, i.e., persistent binge eating and persistent restrictive eating as analogs for overeating and undereating tendency, may be differently affected by the levels of prenatal sex-hormones. However, it is evident that animal models do not reach the complexity of human behavior, and their findings seldom translate straightforward to clinical phenomena.

Previous studies in humans have used two proxy measures for prenatal testosterone and estrogen exposure on eating disorders and disordered eating behavior in humans; the ratio of the length of the 2nd and 4th fingers (2D:4D ratio), and the comparison of the members of opposite-sex twin pairs to those of same-sex twin pairs (Marzola et al., 2021). The 2D:4D ratio is negatively related to the prenatal testosterone/estrogen ratio (Manning et al., 2014, Zheng and Cohn, 2011), and a lower 2D:4D ratio, i.e. higher prenatal testosterone/estrogen ratio, has been associated with lower levels of disordered eating behavior in non-clinical populations in both sexes (Klump et al., 2006, Smith et al., 2010). In a clinical population, however, the 2D:4D ratio has been suggested to be lower in women with AN, indicating a higher prenatal testosterone/estrogen ratio, as compared to women with BN, with healthy controls in between (Quinton et al., 2011). In line, daughters of women with BN had higher prenatal testosterone exposure (based on the 2D:4D ratio) compared to daughters of mothers without an eating disorder in a population-based sample (Kothari et al., 2014). Another indirect method, comparison of the members of twin pairs, relies on the observation that due to their intrauterine position females from opposite-sex twin pairs are particularly exposed to the masculinizing fetal hormonal environment (Cohen-Bendahan et al., 2004), whereas among males from opposite-sex twin pairs, the fetal environment may have a feminizing effect (Resnick et al., 1993). Indeed, same-sex female twins have been reported to exhibit the highest levels of disordered eating behavior followed by opposite-sex female twins, opposite-sex male twins, and same-sex male twins (Culbert et al., 2008); and beginning in puberty, females from opposite-sex twin pairs expressed less, i.e. masculinized, disordered eating attitudes than females from same-sex twin pairs (Culbert et al., 2013). Analyses in U.S., Norwegian, Swedish, and Finnish twin samples found however no overall support for the hypothesis that having a female co-twin increases the risk for AN, BN, and binge-eating eating disorder (BED) in either males (from opposite-sex pairs) or females (from monozygotic or dizygotic same-sex pairs) (Lydecker et al., 2012, Raevuori et al., 2008).

Taken together, the evidence from prior studies concerning gestational sex-hormone exposure and the later risk of eating disorders in offspring is highly inconsistent and based on indirect evidence (Klump et al., 2017). Therefore, we aimed to explore direct evidence, utilizing prospectively collected maternal sera, from which sex hormone concentrations were assayed, to explore the association between gestational sex-hormone levels and the subsequent risk of bulimia nervosa (BN) and anorexia nervosa (AN) in female offspring. We included individuals with registered diagnoses of BN or AN, but no other lifetime eating disorder diagnoses, and compared their maternal sex hormone levels to those of both sister and population controls. The inclusion of sister controls allowed for controlling for the genetic risk of eating disorders.

2. Methods

2.1. Case and control identification

The study was based on probands with BN and AN identified from Finnish registries, their sister controls (one per proband), and population controls (one per proband). The study capitalized on the use of the following Finnish registries: the Finnish Hospital Discharge Register, the Finnish Central Population Register, and the Finnish Maternity Cohort. All individuals in these registries can be identified by a unique personal identification number assigned at birth or migration, which allows for linkages between each of the registries.

The sampling frame was defined such that source cohort members were within the typical age of onset range for BN and AN (Silén et al., 2020). Diagnoses were retrieved from the Finnish Hospital Discharge Register. The Hospital Discharge Register (computerized data) identifies all recorded diagnoses for all psychiatric hospital admissions. All Finnish citizens and permanent residents are entitled to Finland's national health insurance maintained by the state and financed through tax revenues. This registry covers all mental and general hospitals, as well as all inpatient wards of local health centers, military wards, prison hospitals, and private hospitals. The registry contains the hospital identification code, dates and length of stay, and primary diagnoses at discharge, together with three possible subsidiary diagnoses. Diagnostic information is based on clinical (hospital) diagnoses made by the attending physician. The sampling of probands covered all offspring born in Finland from 1991-2000.

To be included in the study, the proband had to 1) be female; born 1991–2000, 2) have either the ICD-10 diagnosis of bulimia nervosa (F50.2) or anorexia nervosa (F50.0), but not both, and no other lifetime eating disorder diagnoses (other diagnoses in the ICD-10 category F50 or in the ICD-9 categories 307.1, 307.5), retrieved from the Finnish Hospital Discharge Register; 3) have an inpatient-treatment for BN or AN, and 4) have a biological full-sister (sister control) born between 1984-2012 (median 1994, 5th to 95th percentile 1986–2001) identified from the Finnish Central Population Register. For those with BN, comorbid lifetime psychiatric diagnoses (excluding lifetime AN and other eating disorders) were permitted due to the low number of probands with BN without any psychiatric comorbidity. No comorbid diagnoses of lifetime psychiatric disorders were allowed for those with AN. Analogously with the probands, sister controls of probands with BN could have had lifetime diagnoses for psychiatric disorders other than eating disorders (ICD-9 290–319, excluding 307.1, 307.5, F10.0–F99, excluding F50), whereas sister controls of those with AN were not allowed to have any ICD-9/ICD-10 diagnoses of psychiatric disorders (ICD-9 290–319 / F10.0 - F99) based on the information in the Hospital Discharge Register. Population controls were selected based on the date of birth (± 146 days), sex, and place of birth from the Central Population Register. The computerized nationwide Central Population Register includes comprehensive data on place of birth, date of emigration, date of death, place of residence, and biological parents, including their birth dates. Exclusion criteria for population controls included any ICD-9/ICD-10 diagnoses for eating disorders (F50) in the Hospital Discharge Register. Adherence to these criteria produced a sample of 75 probands with BN and 198 with AN, their sister controls (one per each proband), and population controls (one per each proband). Thereafter, 20 triplets of BN and 48 triplets of AN were excluded due to low-quality or a too small amount of serum sample in any member of the triplet (the proband, sister control, population control). Only full triplets with analyzable samples were included, which produced a final sample of 55 probands with BN and 150 probands with AN, their sister controls (55 for probands with BN and 150 for probands with AN), and population controls (55 for probands with BN and 150 for probands with AN).

2.2. Sex-hormone assay

Maternal samples of probands, sister controls, and population controls were derived from the Finnish Maternity Cohort, which consists of virtually all pregnancies in Finland with archived prenatal serum specimens drawn beginning in 1983 (the total number of samples is over 1 million). Sera were drawn during the first or early second trimester (5th to 95th percentile: months 2–4 of pregnancy) from over 98% of pregnant women in Finland, following informed consent, for screening for HIV, syphilis, and hepatitis. One maternal serum sample was obtained for each pregnancy. Serum samples were stored as one aliquot at −25°C in a protected biorepository at Biobank Borealis in Oulu, Finland. The FMC can be linked with other Finnish nationwide registers using a unique personal identification code, which has been assigned to all residents of Finland since 1971.

Measurements of the sex-hormone levels were conducted by laboratory personnel blind to case-control-sister control status. Quantitative analyses of testosterone and estradiol were performed using chemiluminescent microparticle immunoassays with Architect ci8200 analyzer (Abbott Diagnostics, Abbott Park, IL) according to the manufacturer's instructions. Estradiol as the most potent and the primary form of estrogen during reproductive years was chosen as a proxy for estrogens. The samples were analyzed for serum total testosterone and estradiol concentrations (nmol/l) (ARCHITECT 2nd Generation). Internal control samples of pooled serum derived from pregnant women in the first trimesters were included in each set of daily assays. The coefficient of variation (cv%, SD/mean) for testosterone was 4.8% and for estradiol <5%. The lowest limits of detection were 0.05 nmol/l for testosterone and 0.04 nmol/l for estradiol.

2.3. Statistical analysis

The distribution of hormone levels was assessed with histograms and with Shapiro-Wilk tests. The analyses of the relationship between gestational estradiol and testosterone levels and testosterone/estradiol (T/E) ratio and offspring eating disorders were based on a matched case–control design. We conducted a matched analysis using conditional logistic regression. Two comparisons were made: probands (cases) versus sister controls and probands (cases) versus population controls. We first examined gestational estradiol and testosterone levels and T/E ratio as continuous variables in relation to the risk of AN and BN in offspring. Due to skewed distribution, the variables were log-transformed before analysis. Then, in order to facilitate the interpretation of the data, prenatal estradiol and testosterone levels and T/E ratio were additionally categorized into tertiles with cut-points defined based on the values of these biomarkers in population controls. The analyses were adjusted for maternal age at the expected date of delivery, gestational age (pregnancy weeks) at the time of the collection of the serum sample, and calcium concentration of the sample (a marker of its quality). Adjustment for comorbidities was not possible due to low number of affected individuals. This analytic scheme has been used in previous studies with similar settings (Brown et al., 2014, Chudal et al., 2020). Differences in means between probands vs. sister or population controls were assessed using linear fixed effects models. Statistical analyses were performed with the Statistical Package for the Social Sciences (SPSS), version 28.0, and Stata, version 18.

2.4. Ethical considerations

The necessary permissions have been received from the register-keeping organizations, and the study sites (Finnish Institute for Health and Welfare and University of Turku, where the register linkages were performed). Ethical permits for the study were obtained from the hospital district of Southwest Finland Ethics Committee (Record number 7/2007), and the Finnish Institute of Health and Welfare (8/2016). Data handling was performed according to the Finnish data protection legislation and the rules of the Finnish Institute of Health and Welfare. Because the data concerning the offspring (probands and controls) were completely register-based, personal informed consent was not required. The authors did not have access to personal identification data, only pseudonymized data were analyzed.

3. Results

3.1. Characteristics of the study population

Characteristics of the study sample are presented in Table 1. The total sample size, including probands, their sister controls, and population controls, was 615. Participants' birth years ranged between 1984 – 2012, (median 1993, 5th to 95th percentile 1989–1999). The sampling time point (gestational weeks) of the maternal serum samples was significantly earlier in sister controls compared to that of population controls ($p = 0.034$). There were no significant differences in other background variables between cases vs. population controls or cases vs. sister controls (Table 1). Of probands with BN, 29 were first-born siblings, whereas respectively, 15 and 27 of their sister controls and population controls were first-born. Likewise of probands with AN, 61 were first-born siblings, and for their sister controls and population controls, the respective was true for 49 and 47 participants The differences in birth order were not significant (BN triplets: X^2 [10] = 13.293, $p = 0.208$; AN triplets: X^2 [16] = 19.901, $p = 0.225$, See Supplementary Table 1).

3.2. Psychiatric comorbidity and morbidity

Of probands with BN, 19 (34.5 %) of 55 had no record of a comorbid lifetime psychiatric disorders. The rest 36 (65.5 %) individuals had at least one comorbid psychiatric disorder. Of the 55 BN-sister controls, eight (14.5 %) had at least one psychiatric diagnosis, and of the 55 BN-population controls, also eight (14.5 %) had at least one psychiatric diagnosis. The sampling of probands with AN and their sister controls did not allow the presence of records of other psychiatric diagnoses. Of the population controls of AN, 20 individuals (13.3 %) had at least one non-eating disorder psychiatric diagnosis. See supplementary Table 2 for a detailed description of psychiatric diagnoses.

Table 1
Characteristics of the study sample.

		Bulimia nervosa (F50.2)			Anorexia nervosa (F50.0)		
		Probands	Population controls	Sister controls	Probands	Population controls	Sister controls
	N	**55**	**55**	**55**	**150**	**150**	**150**
Birth year	**Mean**	1992	1992	1993	1994	1994	1993
	(SD)	(1.7)	(1.7)	(4.9)	(2.5)	(2.5)	(4.4)
	Range	1991-1997	1991-1997	1984-2006	1991–2000	1991-2000	1984–2012
Sampling year of the maternal serum sample	**Mean**	1992	1992	1992	1993	1993	1993
	(SD)	(1.8)	(1.8)	(4.8)	(2.6)	(2.6)	(4.5)
	Range	1990–1997	1990–1997	1984–2006	1990–2000	1990–2000	1984–2012
Maternal age (years) at the expected date of delivery	**Mean**	28.6	29.1	29.2	29.2	29.9	29.6
	(SD)	(5.0)	(4.3)	(5.5)	(4.3)	(5.2)	(4.8)
	Range	19.0–42.2	18.6–40.5	20.3–42.3	19.0-41.1	17.8-44.0	20.0-41.9
Sampling time (gestational week) of the maternal serum sample	**Mean**	11.0	10.9	11.0	11.1	11.8	10.9
	(SD)	(3.3)	(3.2)	(3.1)	(2.7)	(3.4)	(2.8)
	Range	4.0–21.7	5.6–20.7	4.7–21.6	6.4–18.9	4.3–24.1	5.1–16.6

Abbreviations: N: number, SD: standard deviation.

3.3. Prenatal estradiol and testosterone levels and T/E ratio and the risk of BN and AN in daughters

Crude values of gestational estradiol and testosterone levels, and T/E ratio, without taking into account the gestational week during sampling, are presented in Table 2.

Prenatal estradiol and testosterone levels, and T/E ratio, when treated as log-transformed continuous variables, had no statistically significant association with the risk for BN or AN (Table 3). The results for prenatal estradiol, testosterone, and T/E ratio by tertiles and offspring risk for BN and AN are presented in Table 4. When comparing BN probands to sister controls, there was an elevated risk for BN in the middle tertile (Odds ratio [*OR*] 4.42, 95% confidence interval [*CI*] 1.16–16.78, $p = 0.03$); the OR in the highest tertile was even higher (5.43, 95% *CI* 0.88–33.63), although statistically marginally significant ($p = 0.07$). In the respective comparison of testosterone levels to population controls, no differences emerged; and prenatal estradiol or T/E ratio were not associated with the risk for BN. Prenatal estradiol or testosterone levels or T/E ratio were not associated with the risk for AN.

4. Discussion

For the first time to our knowledge, this study utilized a direct method to examine a gestational sex-hormone exposure, specified from serum samples of pregnant mothers, to assess its association with later eating disorders among daughters. The results suggest that females exposed to higher prenatal testosterone levels were at increased risk for BN when compared to their biological sisters without eating disorders. However, when compared to female population controls, no difference in testosterone exposure emerged. This suggests that among females with familial liability for BN, higher gestational testosterone exposure may have a role in developing BN, and lower testosterone exposure might in turn have a protective effect. We found no evidence of the involvement of prenatal sex-hormone exposure and the risk of AN in daughters, either when compared to the biological sisters or population controls without eating disorders.

An important caveat in this research area, affecting interpretations of results across studies, concerns the conceptualization and operationalization of disordered eating behavior. As a concept, “disordered eating” may include various types of restrictive eating, binge-eating, and compensatory behaviors such as purging or fasting - either alone or in any combination. In addition, differentiating binge eating from overeating is at times vague; combined with eating large amounts of food, an effort to control eating, and hence perceived “loss of control over one’s eating” as required for binge-eating, may be challenging to define. Previous studies have used different self-report instruments emphasizing slightly different aspects of symptoms of disorder eating; the Minnesota Eating Behavior Survey (MEBS) (Klump et al., 2006, Culbert et al., 2008, 2013, 2015) includes Binge Eating subscale, while the Eating disorder inventory (EDI/ EDI-2) (Raevuori et al., 2008, Baker et al., 2009, Oinonen and Bird, 2012) and the Eating Attitudes Test (EAT-26) (Oinonen and Bird, 2012), include Bulimia subscale. The Eating Disorder Examination Questionnaire (EDE-Q) (Smith et al., 2010, Culbert et al., 2008, 2013, 2015) does not include either subscale despite including items covering binge-eating and compensatory behaviors. Most studies did not specify which subscales were elevated. Of those that reported subscale-specific results, one found an association between higher prenatal testosterone and increased bulimia symptoms, while two studies found an association between lower prenatal testosterone and less bulimic symptoms. In addition, despite assumed to be salient regarding the effect of prenatal testosterone exposure, studies did not assess lifetime binge eating or persistent over-eating behavior. As a result, most studies that report a link between prenatal testosterone exposure and offspring disordered eating, do not specify types of disordered eating behavior (“under-“ or “overeating”, or both), or take into account the longitudinal course of participants’ disordered eating behavior, but merely report an association between increased or decreased testosterone level and recent or current eating disorder symptoms (Klump et al., 2006, Smith et al., 2010, Quinton et al., 2011, Culbert et al., 2008, 2013, 2015, Baker et al., 2009, Oinonen and Bird, 2012).

Focusing on diagnostic eating disorders instead of disordered eating behavior, as we did, has similar shortcomings. In DSM-5 (American Psychiatric Association 2013) and DSM-IV (American Psychiatric Association 1994) classifications, a key diagnostic criterion of AN is a significantly low weight, manifesting with or without binge-eating/purging behavior. By contrast, in ICD-10 (World Health Organization 1992), individuals with frequent binge-eating/purging behavior are diagnosed with BN, despite potential significant underweight. Therefore ICD-10 AN (F50.0), such as in the current study, covers primarily and only restricting type AN, whereas DSM-5/DSM-IV AN includes both restricting and binge-eating/purging types, and ICD-10 BN could in the DSM classification be diagnosed as binge-eating/purging type AN.

Keeping the above described limitations in mind, both studies using 2D:4D ratio as a proxy measure and twin studies comparing females from opposite-sex to same-sex pairs have yielded varying results. Focusing on diagnostic eating disorders, two out of three studies using 2D:4D ratios reported an association between increased prenatal testosterone and DSM-IV AN (including females with restricting and binge-eating/purging type, 16,33); and another also reported an association between lower prenatal testosterone and DSM-IV BN (Quinton et al., 2011). Conversely, a third, population-based cohort study, reported that daughters of women with self-reported lifetime BN had higher prenatal testosterone exposure compared to daughters of mothers without an eating disorder (Kothari et al., 2014). Of twin studies, a study combining three twin samples from United States, Norway, and Sweden (Lydecker et al., 2012) did not show an overall association between the twin type and lifetime DSM-IV AN, BN, or BED, but in the Swedish sample, having a female co-twin (indicating lower prenatal testosterone exposure), was associated with broadly defined BN when males and females were assessed together. A study in Finnish twins did likewise not show an overall association with twin zygosity and DSM-IV or broad AN or BN, but a marginally decreased risk of developing broad AN (*OR* 0.65, 95% *CI* 0.39–1.08, p=0.10) was demonstrated among women from opposite-sex twin pairs, indicating protective effect of higher prenatal testosterone for these phenotypes. Differences in study samples and in DSM and ICD classification systems complicate meaningful comparison of these findings to the results of our current study. If anything, some findings from the studies using 2D:4D ratio may be speculated to be parallel with our current results. These include higher prenatal testosterone in females with DSM-IV AN (Quinton et al., 2011,

Table 2
Prenatal estradiol and testosterone levels and testosterone / estradiol ratio.

	Bulimia Nervosa (F50.2)			Anorexia Nervosa (F50.0)		
Estradiol level						
	N	**Mean**	**SD**	***N***	**Mean**	**SD**
Probands	55	7.63	5.21	150	8.27	6.26
Population controls	55	8.93	7.33	150	9.64	7.58
Sister controls	55	7.78	6.95	150	7.83	5.72
Testosterone level						
Probands	55	2.69	1.38	150	2.66	1.34
Population controls	55	2.86	1.07	150	2.59	1.36
Sister controls	55	2.57	1.43	150	2.56	1.14
Testosterone / estradiol ratio						
Probands	55	0.65	0.78	150	0.54	0.51
Population controls	55	0.65	0.66	150	0.46	0.52
Sister controls	55	0.62	0.73	150	0.53	0.51

Table 3
The association of log-transformed prenatal estradiol levels, testosterone levels and testosterone/estradiol ratio with AN and BN.

	Probands with BN *versus* population controls		Probands with BN *versus* sister controls		Probands with AN *versus* population controls		Probands with AN *versus* sister controls	
	***OR* (95 % *CI*)**	***p*-value**	***OR* (95 % *CI*)**	***p*-value**	***OR* (95 % *CI*)**	***p*-value**	***OR* (95 % *CI*)**	***p*-value**
Estradiol level	0.64 (0.27–1.49)	0.30	0.93 (0.39–2.20)	0.87	0.93 (0.60–1.44)	0.75	0.90 (0.53–1.54)	0.71
Testosterone level	0.54 (0.20–1.47)	0.23	3.08 (0.50–19.08)	0.23	1.12 (0.68–1.83)	0.66	1.01 (0.41–2.48)	0.99
Testosterone/ estradiol ratio	0.98 (0.45–2.15)	0.96	1.46 (0.58–3.68)	0.42	1.16 (0.76–1.76)	0.50	1.16 (0.61–2.18)	0.65

Abbreviations: CI: confidence interval; OR: odds ratio

Table 4
The associations of tertiles of prenatal estradiol levels, testosterone levels, and testosterone/estradiol ratio with AN and BN.

	Probands with BN *versus* population controls		Probands with BN *versus* sister controls		Probands with AN *versus* population controls		Probands with AN *versus* sister controls	
Estradiol level								
	***OR* (95 % *CI*)**	***p*-value**	***OR* (95 % *CI*)**	***p*-value**	**OR (95 % *CI*)**	***p*-value**	***OR* (95 % *CI*)**	***p*-value**
First tertile	1.00		1.00		1.00		1.00	
Second tertile	0.90 (0.24–3.37)	0.88	1.22 (0.33–4.50)	0.76	1.18 (0.62–2.24)	0.61	0.89 (0.46–1.73)	0.74
Third tertile	0.99 (0.18–5.51)	0.99	2.40 (0.27–21.10)	0.43	1.02 (0.43–2.40)	0.97	0.96 (0.36–2.51)	0.93
Testosterone level								
First tertile	1.00		1.00		1.00		1.00	
Second tertile	0.80 (0.31–2.06)	0.65	4.42 (1.16–16.78)	0.03	1.04 (0.59–1.83)	0.88	0.76 (0.37–1.56)	0.45
Third tertile	0.57 (0.19–1.73)	0.32	5.43 (0.88–33.63)	0.07	1.31 (0.75–2.27)	0.34	0.99 (0.41–2.42)	0.98
Testosterone / estradiol ratio								
First tertile	1.00		1.00		1.00		1.00	
Second tertile	0.98 (0.30–3.23)	0.97	0.88 (0.24–3.18)	0.84	0.93 (0.46–1.90)	0.85	1.30 (0.57–2.94)	0.53
Third tertile	0.51 (0.13–2.00)	0.33	1.33 (0.25–7.18)	0.74	1.29 (0.62–2.68)	0.50	2.24 (0.74–6.80)	0.16

Romero-Martínez and Moya-Albiol, 2014), if higher testosterone values were derived specifically from women with binge/purge-type AN (representing the same phenotype as ICD-10 BN in our study). This could also reflect connection between higher prenatal testosterone and later binge-purge behavior. Higher prenatal testosterone in daughters of women with BN (Kothari et al., 2014) in turn refers to the potential role of increased prenatal testosterone associated with BN and manifesting within families, that was, albeit differently, demonstrated also in our study.

Prenatal testosterone may increase the risk for BN via its influence on increasing the appetite and tendency for overeating. A connection between higher prenatal testosterone and increased appetite and increased eating in general, has been demonstrated in animal models (Donohoe and Stevens, 1983, Madrid et al., 1993, Asarian and Geary, 2013). Studies investigating longitudinal trajectories of eating behaviors have found that individuals who later develop clinical binge eating, such as in BN or BED, have had a tendency for overeating and higher BMI already in early childhood (Fernández-Aranda et al., 2007, Herle et al., 2020, Micali, 2005). Tendency to overeat, along with a tendency to weight gain and sociocultural pressures for slimness, may lead to attempts to maintain strict dietary control. Binge eating episodes result if an individual is temporarily disinhibited in maintaining the control; combined with recurrent inappropriate compensatory behavior, such as self-induced vomiting or laxative abuse, this leads to a vicious cycle distinctive of BN. Moreover, both fetal androgen receptor sensitivity and prenatal exposure for testosterone are influenced by genetic factors (Kothari et al., 2014, Chamberlain et al., 1994), and the exposure to prenatal testosterone has been suggested to be a mechanism for the intergenerational transmission risk for BN (Kothari et al., 2014). It has been suggested that females with an elevated familial risk for BN may have heightened sensitivity for fetal androgen, and combined with higher exposure to prenatal testosterone, these together would contribute to the risk for developing BN (Kothari et al., 2014). Accordingly, those with heightened sensitivity for fetal androgen exposure may be protected by lower prenatal testosterone level, whereas in those without heightened androgen sensitivity, prenatal testosterone level may not have an influence for the risk of BN. In line in our study, females with familiar risk for BN and higher relative testosterone exposure compared to their biological sisters, were at increased risk for BN, and among population control females without familial risk for BN, prenatal testosterone appeared not to have a similar effect.

4.1. Strengths and limitations

Strengths of the study included direct assessment of the prenatal sex-hormone levels from prospectively collected maternal serum samples, whereas all previous studies have relied on proxy measures. Maternal serum levels of estradiol and testosterone have been shown to represent a robust index of prenatal exposure to these hormones and to reflect their concentration in both maternal and fetal compartments (Meulenberg and Hofman, 1991). The inclusion of sister controls allowed controlling for the genetic vulnerability for eating disorders,

and for familial environmental factors, such as socioeconomical background (Frisell, 2021). All participants were drawn systematically from national registries to minimize selection bias. Our participants were hospital-treated for narrow ICD-10 diagnosis of BN or AN, instead of DSM-5/DSM-IV diagnosis. These ICD-10 diagnoses of severe BN or AN, with the exclusion of other lifetime eating disorder diagnoses, characterize binge-eating and restricting eating disorder phenotypes relatively well compared to studies using DSM eating disorder diagnoses or instruments of disordered eating behavior.

Yet limitations of our study include lack of longitudinal information – beyond eating disorder diagnoses –concerning participants' (disordered) eating behavior, and in particular, of their binge-eating /overeating tendency versus other types of disordered eating behavior. Diagnostic information were based solely on register data; it is possible that some participants had undiagnosed psychiatric disorder that may confound the results. It is likewise possible that sister control or population control participants may have had undiagnosed eating disorders. Despite nation-wide sampling, a major limitation of the study is relatively low number of participants with eating disorders, particularly those with BN; results may have been more robust in a larger sample and we cannot exclude type II error. Some of the individuals with BN and their sister controls had psychiatric comorbidities/morbidities that may confound the results. On the other hand, psychiatric comorbidity is innate in severe BN, hence likely reflecting clinical reality and the accumulation of familial risk for psychiatric disorders. Finally, as the most potent and primary form of estrogen during pregnancy, estradiol alone was used as a proxy for the overall maternal estrogen level (including estrone, estriol, and estradiol).

4.2. Conclusion

In conclusion, we demonstrated that compared to sister controls without an eating disorder, a higher level of prospectively documented maternal testosterone exposure in pregnancy was associated with later BN among females, or conversely, females exposed to lower testosterone levels than their sister-controls, were at decreased risk of BN. This suggests that the organizational effect of prenatal testosterone on brain structure and function, and subsequent enduring drive to increase food intake, may have a role in the later development of BN in girls and women with familial liability for this eating disorder. Future studies should pay attention to thorough characterization of participants' longitudinal course of disordered eating behavior, regardless of their focus on diagnostic eating disorders or symptoms of disordered eating.

CRediT authorship contribution statement

Emma Saure: Writing – original draft, Writing – review & editing. **Pyry N. Sipilä:** Formal analysis, Writing – review & editing. **Heljä-Maria Surcel:** Conceptualization, Resources, Writing – review & editing. **Antti Latvala:** Writing – review & editing. **Anni Heiskala:** Data curation, Writing – review & editing. **Jouko Miettunen:** Writing – review & editing. **Marja Laasonen:** Writing – review & editing. **Tuulia Lepistö-Paisley:** Writing – review & editing. **Anu Raevuori:** Conceptualization, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors have no conflicts of interest to declare that are relevant to the content of this article.

Funding

The study was supported by grant 259764 (to A.R.) from the Research Council of Finland, and by grant 20227571 and 20237703 from the Yrjö Jahnsson Foundation (to E.S.) and by grant from the Jalmari and Rauha Ahokas Foundation (to E.S.).

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.psychres.2024.116170.

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