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Primary Amenorrhea in Anorexia Nervosa: Impact on Characteristic Masculine and Feminine Traits

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Abstract

Animal studies indicate that gonadal hormones at puberty have an effect on the development of masculine and feminine traits. However, it is unknown whether similar processes occur in humans. We examined whether women with anorexia nervosa (AN), who often experience primary amenorrhea, exhibit attenuated feminization in their psychological characteristics in adulthood due to the decrease/absence of gonadal hormones at puberty. Women with AN were compared on a number of psychological characteristics using general linear models on the basis of the presence/absence of primary amenorrhea. Although women with primary amenorrhea exhibited lower anxiety scores than those without primary amenorrhea, in general, results did not provide evidence of attenuated feminization in women with AN with primary amenorrhea. Future research should utilize novel techniques and direct hormone measurement to explore the effects of pubertal gonadal hormones on masculine and feminine traits. Copyright © 2013 John Wiley & Sons, Ltd and Eating Disorders Association.

Keywords

organizational effects; sex differences; amenorrhea; pubertal timing; anorexia nervosa

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The aetiology of anorexia nervosa (AN) and other sex-differentiated disorders (e.g. depression) remains largely unknown (Hudson, Hiripi, Pope, & Kessler, 2007; Kessler et al., 1994). A relatively unexplored area in this regard is gonadal hormone effects, which have been found to exert significant influences on sex-differentiated behaviour (for recent reviews, see Berenbaum & Beltz, 2011; Hines, 2011; Sisk & Zehr, 2005). For example, Phoenix, Roy, Gerall, and Young (1959) posited that gonadal hormones affect the brain and behaviour in two fundamentally different ways—in an organizational and an activational manner. During specific points in development (e.g. prenatal/perinatal periods), exposure to gonadal hormones will organize neural circuits such that testosterone masculinizes neural circuits in males, whereas the absence of testosterone results in the feminization of neural circuits.
in females. Organizational effects persist beyond the period of hormone exposure and do not require the continued presence of the hormone. Organizational effects also program sex-typical behavioural and physiological responses to gonadal hormones later in life. These responses are referred to as activational effects, which are transient in nature, and require the continued presence of hormone to be maintained.

Organizational effects have been confirmed by a multitude of experimental animal studies where neonatal or prepubertal castration of rodent results in men who are ‘feminized’ (in mating behaviours, aggressiveness and play fighting) (Beach & Holz, 1946; Peters, Bronson, & Whitsett, 1972; Schulz & Sisk, 2004; Taylor, Frechmann, & Royalty, 1986), whereas perinatal testosterone injections in female animals result in masculine juvenile play, sexual and aggressive behaviours (Cohen-Bendahan, van de Beek, & Berenbaum, 2005; Phoenix et al., 1959; Schulz & Sisk, 2004). In humans, the organizational effects of prenatal hormones have been examined in populations with congenital conditions that affect reproductive hormones. For example, women with congenital adrenal hyperplasia (prenatal androgen excess) display more interest in male typical behaviours and personality characteristics (e.g. aggression) and perform better on spatial tasks compared with women without congenital adrenal hyperplasia (Berenbaum, Bryk, & Beltz, 2012; Hines, 2011; Mueller, 2013).

Despite the robustness of the results from animal studies and the intriguing findings from human models, more work is needed in translating these findings to human populations. However, exploring the impact of gonadal hormones on behaviour in humans is difficult because we are unable to manipulate the reproductive system in humans as can be carried out in animals. A possible strategy for examining these effects in humans is to explore proxies, such as a delayed pubertal onset (Berenbaum & Beltz, 2011). This design is particularly important, as puberty is believed to be an additional period of development when gonadal hormones organize the nervous system and influence brain and behaviour development in humans (Hines, 2011; Neufang et al., 2009; Schulz, Molenda-Figueira, & Sisk, 2009; Sisk & Zehr, 2005). In animals, hamsters that have their testes removed after the perinatal period of sexual differentiation and before puberty behave in a less masculinized manner (e.g. reduced sexual and aggressive behaviours), and prolonged testosterone replacement after puberty fails to normalize these behaviours (Schulz & Sisk, 2004). Although studied less extensively, similar patterns are emerging in females such that a prepubertal ovariectomy in female rats results in male-like food guarding strategies that do not change with exogenous ovarian hormone exposure in adulthood (Field, Whishaw, Forgie, & Pellis, 2004). Further, a recent report in a sample of adolescents with AN observed that reductions in grey matter in the brain during acute AN are reversible upon weight restoration and the increase of certain gonadal hormones (i.e. follicle-stimulating hormone) (Mainz, Schulte-Ruther, Fink, Herpertz-Dahlmann, & Konrad, 2012). Together, these results suggest that puberty is a time-sensitive window for hormone organization and brain development.

Women with AN frequently experience primary amenorrhea (Pinheiro et al., 2007), defined as menarche after age 16 years (Master-Hunter & Heiman, 2006). Primary amenorrhea is a naturally occurring phenomenon that can be explored to examine the behavioural effects of a partially or permanently delayed pubertal onset, which may stall the developmental process entirely. However, primary amenorrhea is uncommon, making this difficult to explore in population-based samples. Thus, because women with AN frequently experience primary amenorrhea, this population may represent a proxy population, where an adequate sample of women with primary amenorrhea can be obtained, to explore the organizational effects of gonadal steroids on sex-differentiated behaviour. Using this proxy model, if organizational effects at puberty do affect sex differences in behaviour, we expect women with AN who experienced primary amenorrhea to exhibit increased male typical behaviours and characteristics in adulthood. In other words, these women may be permanently ‘defeminized’ because of the absence of organizational effects of gonadal hormones during adolescent brain development.

We explore this possibility by comparing adult women with AN, with and without primary amenorrhea, on a number of psychological disorders and personality traits that are characteristic of women (i.e. depression and anxiety) or men (i.e. substance-use disorders) (Kessler et al., 1994). If adolescence is a sensitive period for pubertal hormones to organize sex-typical behaviours, we expect elevated male typical disorders and traits in women with AN who experienced primary amenorrhea. Although the main focus of our report is on the effects of primary amenorrhea on sex-typical behaviour (using AN as a proxy population), an added benefit is that we are also able to examine the effects of primary amenorrhea on AN features that may have significant clinical implications.

**Material and methods**

**Participants**

Participants were women from the National Institute of Health-funded Genetics of Anorexia Nervosa Study. A detailed description of this investigation can be found elsewhere (Kaye et al., 2008). This study was approved by the local institutional review and ethics boards. All participants provided informed consent prior to participation.

Probands were at least 16 years old, ill with or recovered from a lifetime diagnosis of Diagnostic and Statistical Manual of Mental Disorders, fourth edition (DSM-IV) AN and with or without amenorrhea, at least 3 years prior to study entry and by age 45 years. Low weight was defined as a body mass index (BMI) at or below 18 kg/m² for women, which corresponds to the fifth percentile BMI values of the National Health and Nutrition Examination Survey (Hebebrand, Himmelmann, Hesseker, Schafer, & Remschmidt, 1996) epidemiological sample of women aged 27–29 years, the average age of probands in our previous studies (Kaye et al., 2000). Probands were required to have at least one first-, second- or third-degree relative with AN (excluding parents and monozygotic twins) willing to participate in the study. Exclusion criteria for probands were regular binge eating (binge-eating episodes occurring at least twice a week for at least 3 months), a maximum lifetime BMI exceeding 30 kg/m², a history of severe central nervous system trauma that renders diagnosis uncertain, psychotic disorder or developmental disability, or...
a medical, neurological or substance-use disorder that may confound the diagnosis of AN or interfere with responding cogently to assessments.

Inclusion criteria for affected family members were identical except that relatives were permitted to engage in regular binge eating, did not have to meet AN criteria 3 years prior to the study and could have an additional lifetime diagnosis of bulimia nervosa. However, affected family members were required to have maintained a low weight for at least 3 months. Additional relatives with eating disorders could be included in the study once a proband and a relative meeting the inclusion criteria were enrolled.

Measures

Anorexia nervosa pathology

Lifetime AN diagnoses, inclusion/exclusion criteria and current illness status (presence of at least one AN symptom in the last year was categorized as currently ill) were assessed using a modified version of Module H of the Structured Clinical Interview for Axis I Disorders (First, Spitzer, Gibbon, & Williams, 1997). The Structured Interview for Anorexia Nervosa and Bulimic Syndromes (Fichter, Herpertz, Quadflieg, & Herpertz-Dahlmann, 1998) was used to confirm lifetime AN diagnosis and obtain age at menarche, age at lowest weight, current BMI, lifetime lowest illness-related BMI and lifetime highest BMI. From these assessments, the following AN subtypes were created: (i) AN restricting type (AN with no regular binge eating or purging, defined as fewer than five times ever); (ii) AN purging type (AN with purging but no regular binge eating (i.e. fewer than five times ever)); (iii) AN binge/purge type (AN with regular binge eating and/or purging); and (iv) lifetime history of both AN and bulimia nervosa.

We constructed two menstrual status variables for analysis. First, we coded primary amenorrhea as present if menarche occurred at or after age 16 years and primary amenorrhea as absent if menarche occurred before age 16 years. This definition of primary amenorrhea is based on the current typical chronological age for age at menarche (Master-Hunter & Heiman, 2006; McDowell, Brody, & Hughes, 2007). Second, in an effort to rule out the possibility that significant effects were due to early-onset AN, we explored group differences on the basis of the ordering of age of AN onset versus age of menarche (‘AN-menses onset’). The groups included the following: (i) no menarche onset by age 16 years, with AN onset prior to the onset of menarche [i.e. primary amenorrhea with AN onset occurring before menarche (PRIME-AN1MN2)]; (ii) no menarche onset by age 16 years, with AN onset after the onset of menarche [i.e. primary amenorrhea with AN onset occurring after menarche (PRIME-AN2MN1)]; (iii) no menarche onset by age 16 years, with AN onset prior to the onset of menarche [i.e. primary amenorrhea with AN onset occurring after menarche (AN1MN2)]; (iv) menarche onset by age 16 years, with AN onset prior to the onset of menarche [i.e. primary amenorrhea with AN onset occurring before menarche (AN2MN1)]; and (v) menarche onset by age 16 years, with AN onset after the onset of menarche [i.e. no primary amenorrhea with AN onset occurring after menarche (AN3MN1)].

Personality and symptom measures

The Temperament and Character Inventory (TCI; Cloninger, Przybeck, Svrakic, & Wetzel, 1994) was used to assess the personality dimensions of novelty seeking and harm avoidance. Impulsivity was evaluated using the cognitive, non-planning and motor subscales of the Barratt Impulsiveness Scale-11 (BIS; Patton, Stanford, & Barratt, 1995), and the State-Trait Anxiety Inventory Form Y (STAI-Y; Spielberger, Gorsuch, & Luchene, 1970) was used to assess state anxiety. Obsessions and compulsions were examined using the Yale-Brown Obsessive Compulsive Scale (Goodman et al., 1989), which is a semi-structured interview designed to rate the presence and severity of obsessions and compulsions typically found among individuals with obsessive-compulsive disorder.

Psychiatric and substance-use disorders

The DSM-IV lifetime history of major depression was assessed with the Diagnostic Interview for Genetics Studies (Nurnberger et al., 1994). The Structured Clinical Interview for Axis I Disorders was used to assess DSM-IV defined anxiety (agoraphobia, generalized anxiety disorder, obsessive-compulsive disorder, panic disorder, post-traumatic stress disorder, social phobia and specific phobia) and substance disorders. An ‘any anxiety disorder’ variable was created indicating whether the individual met, in their lifetime, the criteria for any of the listed anxiety disorders. Psychoactive substance-use disorder was considered present if a participant met the criteria for abuse or dependence of sedatives, hallucinogens, stimulants, cocaine, marijuana or opioids. Alcohol-use disorder was similarly defined.

Statistical procedures

General linear models using PROC GENMOD in SAS version 9.2 (Cary, NC, USA) (SAS Institute Inc, 2004) were used to examine whether women with primary amenorrhea exhibit masculinized psychological characteristics, compared with women with no primary amenorrhea. Generalized estimating equation modelling was applied to correct for the non-independence of the family data. The specific methods used to implement the generalized estimating equation are detailed elsewhere (Klump et al., 2000).

Our first set of models examined whether primary amenorrhea influenced scores on the dependent measures. Our second set of models were conducted on any personality and additional comorbidity measures that were significant in the first set of models and assessed group differences on the basis of the ordering of age of AN onset versus age at menarche. Cohen’s d effect sizes were calculated for all results in the first set of models and for all significant results in the second set of models. Current illness status, duration of illness, age at interview and AN subtype were included as covariates. Continuous variables were standardized prior to analysis.

Results

Demographics

A total of 841 participants (388 probands and 453 relatives) were eligible for the current report. Sixty-five per cent of participants reported an age of AN onset between the ages of 11 and 17 years, with an average total duration of illness of 10 years and an average duration of illness of 3 years during the ages of 11–17 years. Thus, a majority of our participants had AN during adolescence, which spanned well beyond the
adolescent years. Approximately 10% \((n = 79)\) of the sample was classified as having primary amenorrhea. Mean age at the time of interview for those with primary amenorrhea was 28.89 years \((SD = 10.41)\) and for those without primary amenorrhea was 29.72 years \((SD = 11.30)\). Mean age at onset of menses was 17.46 years \((SD = 2.29)\) for the primary amenorrhea group and 12.74 years \((SD = 1.22)\) for the no primary amenorrhea group, the latter of which is similar to the population average \((McDowell et al., 2007)\). A majority of the women with primary amenorrhea \((71\%; n = 56)\) reported AN onset prior to menarche and were placed in the PRIME-AN1MN2 group. The other 23 \((29\%)\) were placed in the PRIME-AN2MN1 group. Of the women without primary amenorrhea, 74 \((10\%)\) reported AN onset prior to menarche and were placed in the AN1MN2 group, whereas the majority of the participants \((90\%; n = 622)\) were placed in the AN2MN1 group.

Individuals with primary amenorrhea differed significantly from those with no primary amenorrhea on several eating disorder features (Table 1). Those with primary amenorrhea were significantly younger at the time of lowest weight, had a lower lifetime lowest BMI and had a longer duration of illness. There were no significant differences for current BMI or current illness status: 66% of women with primary amenorrhea and 68% of women without primary amenorrhea endorsed experiencing at least one eating disorder symptom in the year prior to the assessment suggesting that the primary amenorrhea group likely did not have more severe eating disorder features at the time of interview.

**Comparisons between anorexia nervosa with and without primary amenorrhea**

Significant differences between the primary amenorrhea group and the no primary amenorrhea group were observed for TCI novelty seeking, BIS motor and STAI state anxiety (Table 1). Contrary to hypotheses, the primary amenorrhea group scored significantly lower on these measures, and effect sizes, in general, indicated a small-to-medium effect. There were no significant differences between the amenorrhea groups for any psychiatric disorder (Table 2).

We examined whether significant group differences in TCI novelty seeking, BIS motor and STAI state anxiety were independent of the ordering of AN onset versus menarche (Table 3). For novelty seeking, the PRIME-AN1MN2 and PRIME-AN2MN1 groups differed significantly from the AN1MN2 group. For state anxiety, the PRIME-AN1MN2 group differed significantly from the AN1MN2 group, and the PRIME-AN2MN1 group differed significantly from both the AN1MN2 and AN2MN1 groups. For both measures, the PRIME-AN1MN2 and PRIME-AN2MN1 groups reported lower scores than the

### Table 1 Comparisons of women with anorexia nervosa with and without primary amenorrhea

<table>
<thead>
<tr>
<th>Eating disorder features</th>
<th>Primary amenorrhea Mean (SD)</th>
<th>No primary amenorrhea Mean (SD)</th>
<th>Amenorrhea (\chi^2)-value</th>
<th>Cohen’s (d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age low weight</td>
<td>17.43 (5.00)</td>
<td>19.40 (6.00)</td>
<td>5.00*</td>
<td>0.36</td>
</tr>
<tr>
<td>Current BMI</td>
<td>19.45 (2.52)</td>
<td>19.32 (2.52)</td>
<td>0.10</td>
<td>0.05</td>
</tr>
<tr>
<td>Lifetime highest BMI</td>
<td>22.30 (2.38)</td>
<td>22.01 (2.94)</td>
<td>4.10</td>
<td>0.10</td>
</tr>
<tr>
<td>Lifetime lowest BMI</td>
<td>14.00 (2.11)</td>
<td>14.70 (1.90)</td>
<td>6.60*</td>
<td>0.35</td>
</tr>
<tr>
<td>Duration of illness (years)</td>
<td>10.54 (8.06)</td>
<td>9.30 (8.32)</td>
<td>6.62*</td>
<td>0.16</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Temperament and Character Inventory</th>
</tr>
</thead>
<tbody>
<tr>
<td>Novelty seeking</td>
</tr>
<tr>
<td>Harm avoidance</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Barratt Impulsiveness Scale-II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cognitive</td>
</tr>
<tr>
<td>Non-planning</td>
</tr>
<tr>
<td>Motor</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>State-Trait Anxiety Inventory Form Y</th>
</tr>
</thead>
<tbody>
<tr>
<td>State anxiety</td>
</tr>
<tr>
<td>Trait anxiety</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Yale-Brown Obsessive Compulsive Scale</th>
</tr>
</thead>
<tbody>
<tr>
<td>Obsessions</td>
</tr>
<tr>
<td>Compulsions</td>
</tr>
</tbody>
</table>

*Note: Chi-square values represent group comparisons from the logistic regression analyses. When appropriate, age at interview, status of illness and anorexia nervosa subtype were entered into the models as covariates. Significant results are in bold.

SD, standard deviation; BMI, body mass index.

\*\(p < .05\).

\**p < .05\.
AN2MN1 also scored significantly lower on state anxiety than AN1MN2.

Discussion

We examined the hypothesis that women with AN who experienced primary amenorrhea would display attenuated feminization in adulthood due to the organizational effects of gonadal hormones at puberty. In contrast to our hypothesis, we did not find evidence of attenuated feminization. Although we observed significant differences between women with and without primary amenorrhea on several personality characteristics, in general, results were in the opposite direction of attenuated feminization. Women with AN and primary amenorrhea scored lower on novelty seeking and impulsivity, both of which would be expected to be higher than those with normal menstrual onset if a defeminization effect was present.

More in line with our hypothesis, women with AN and primary amenorrhea exhibited lower levels of state anxiety. Although this finding aligns with those for general levels of anxiety exhibited in humans (McLean & Anderson, 2009), it is contrary to observations in women with Turner’s syndrome (genetic disorder of androgen deficiency) who typically exhibit increased levels of anxiety, depression and arousal in response to facial expressions of fear (Schmidt et al., 2006; Skuse et al., 2005). We also observed no significant differences for any anxiety disorder diagnosis. This suggests that either defeminization is specific to state anxiety or the effect is spurious. Although the state anxiety group difference was of medium effect size, overall, our results do not provide convincing evidence for a defeminization effect.

Table 2: Comparisons of women with anorexia nervosa with and without primary amenorrhea on the prevalence of lifetime psychiatric disorders

<table>
<thead>
<tr>
<th>Diagnosis*</th>
<th>Primary amenorrhea (n = 79) % (n)</th>
<th>No primary amenorrhea (n = 696) % (n)</th>
<th>χ²-value</th>
<th>Cohen’s d</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDD</td>
<td>No diagnosis: 30.26 (23)</td>
<td>Diagnosis: 69.74 (53)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anxiety disorder</td>
<td>34.77 (27)</td>
<td>65.82 (52)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AUD</td>
<td>83.33 (65)</td>
<td>17.95 (14)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PSUD</td>
<td>92.21 (71)</td>
<td>9.10 (7)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: Chi-square values represent group comparisons from the logistic regression analyses. MDD, major depressive disorder; AUD, alcohol-use disorder; PSUD, psychoactive substance-use disorder. Percentages were calculated on the basis of the total number of participants with available information for the specific diagnosis.

Table 3: Analyses comparing groups based on the onset of anorexia nervosa versus the onset of menses

<table>
<thead>
<tr>
<th>PRIME-AN1MN2; (n = 56)</th>
<th>PRIME-AN2MN1; (n = 23)</th>
<th>AN1MN2; (n = 74)</th>
<th>AN2MN1; (n = 622)</th>
<th>AN-menses onset</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (SD)</td>
<td>Mean (SD)</td>
<td>Mean (SD)</td>
<td>Mean (SD)</td>
<td>χ²-value</td>
</tr>
<tr>
<td>Temperament and Character Inventory</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Novelty seeking</td>
<td>15.29 (6.71)</td>
<td>13.09 (6.37)</td>
<td>18.18 (6.25)</td>
<td>17.36 (6.78)</td>
</tr>
<tr>
<td>Barratt Impulsiveness Scale-II</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Motor</td>
<td>19.66 (4.02)</td>
<td>18.48 (2.87)</td>
<td>20.46 (3.88)</td>
<td>20.34 (4.14)</td>
</tr>
<tr>
<td>State-Trait Anxiety Inventory Form Y</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>State anxiety</td>
<td>43.29 (12.98)</td>
<td>37.65 (13.79)</td>
<td>49.40 (13.84)</td>
<td>44.65 (13.59)</td>
</tr>
</tbody>
</table>

Note: Chi-square values represent group comparisons from the logistic regression analyses. Age at interview, status of illness and anorexia nervosa (AN) subtype were entered into all models as covariates. Significant results are in bold. PRIME-AN1MN2, a primary amenorrhea group in which the AN began before menses; PRIME-AN2MN1, a primary amenorrhea group in which the AN began after menses; AN1MN2, a no primary amenorrhea group in which the AN began before menses; AN2MN1, a no primary amenorrhea group in which the AN began after menses; SD, standard deviation. *p < .05.
There are several possible explanations for our largely null results. First, these effects are not present. Although animal studies have demonstrated significant evidence for organizational effects of gonadal hormones, most prominently in males, it is unclear how well results from animal studies translate to a human population and to females. Second, gonadal hormones at puberty have an organizational effect that our study was not able to capture. Although the significant differences we found remained regardless of the order of occurrence of AN and menarche, the extent and nature of the effects of AN on brain and behaviour remain incompletely understood. For example, AN typically has an onset during adolescence, and having AN during this sensitive period may affect brain development, structure and function (Buehren et al., 2011; Chui et al., 2008; Katzman, Zipursky, Lambe, & Mikulis, 1997; Katzman et al., 1996; Mainz et al., 2012), suggesting that women with AN may have additional factors confounding the organizational effects of hormones. Further, within the PRIME-AN1MN2 group, it is possible that individuals experiencing primary amenorrhea prior to AN diagnosis may have had a subthreshold diagnosis or undiagnosed/unreported symptoms, which may have limited our ability to observe a masculinization effect in this proxy sample.

Third, menarche occurs later in the pubertal process, on average 2 years after breast development begins (Marshall & Tanner, 1969). This means that gonadal hormone levels begin to increase approximately 2 years prior to menses; therefore, menarche is a rough measure of hormonal influences, and our cut-off age of 16 years for primary amenorrhea may still be within the sensitive period during which women are exposed to gonadal hormones. A study design utilizing a more significant delay in menarche, for example, age 18 years or older, may be necessary to delineate the organizational effects of gonadal hormones in humans—although achieving adequate sample sizes would become increasingly challenging. For example, within our sample, only 24 participants reported an age of menarche at age 18 years or older. When completing the analyses using this definition for primary amenorrhea, only novelty seeking remained significant. Importantly, however, results remained in the same direction. Further, our definition of primary amenorrhea was unable to take into account secondary sexual characteristics and to be confirmed through hormone measurements.

Fourth, organizational effects at puberty may be phenotype specific. The animal literature provides convincing data showing an organizational effect on sex differences in anxiety. Whereas many phenotypes appear to be characteristically masculine or feminine in nature, the driving force behind this difference may not be attributable to organizational effects at puberty and may be the result of prenatal/perinatal hormone activation. Moreover, there are likely many biological and environmental factors that contribute to gender-typical behaviours and characteristics, especially in humans. For example, in humans, sociocultural factors such as parenting or gender role beliefs may play a part in the development of masculine and feminine traits. Thus, defeminization effects might be phenotype specific and only observed in characteristically masculine and feminine traits that are minimally influenced by environmental factors. Further, a delayed pubertal onset would impact additional developmental processes (e.g. growth, thyroid and appetite hormones) that we were unable to capture in this sample, which may also play a role in behaviour.

The phenotypes included in our study and the assessment methods used may also not be the ideal phenotypes and measurement methods to detect a defeminization effect. We were able to identify differences in symptom ratings (e.g. state anxiety). However, no differences emerged for diagnosis suggesting that symptomatology in general may be altered, but specific diagnoses are not. Our sample also included both acutely ill and recovered women with AN, and we were unable to determine the specific criteria for AN fulfilled or amenorrhea status at the time of assessment, which may have contributed to sample heterogeneity. However, we found no significant difference between the groups for current illness status or current BMI and controlled for current illness status in the analyses; therefore, effects of this heterogeneity are likely decreased.

Finally, decreased scores on novelty seeking and impulsivity measures are characteristic features of AN, and several significant differences were observed for eating disorder features based on the presence of primary amenorrhea. Women with primary amenorrhea had a significantly younger age at low weight, longer duration of illness and lower lifetime lowest BMI. Effect sizes for these differences indicated that all are likely clinically meaningful as they clustered around a medium effect. Together, this suggests that women with AN experiencing primary amenorrhea may reflect more prototypic cases of AN and that group differences in eating disorder features may also be due to the primary amenorrhea group being more severe, overall, across the course of their illness.

In sum, in regard to sex-typical behaviours, our findings do not provide significant evidence for attenuated feminization in women with AN who experienced primary amenorrhea—which may be due to the limitations of design described earlier. However, our study is one of the first to examine the organizational effects of gonadal hormones at puberty on characteristic masculine and feminine traits in humans without genetic disorders. More definitive conclusions could be reached using a prospective study design that assesses hormone levels at regular intervals in a large sample of girls beginning in childhood. This type of developmental, longitudinal approach would further clarify hormonal effects on sex-specific behaviours in both healthy women and women with AN.

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