Maternal Vitamin D levels during pregnancy and offspring eating disorder risk in adolescence

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Abstract

Objective: To determine if maternal vitamin D concentrations at 18 weeks gestation predict offspring eating disorder risk in adolescence. **Method:** Participants were 526 Caucasian mother-child dyads from the Western Australian Pregnancy Cohort (Raine) Study. The Raine Study has followed participants from 18 weeks gestation to 20 years of age. Maternal serum 25(OH)-vitamin D concentrations were measured at 18 weeks pregnancy and grouped into quartiles. Offspring eating disorder symptoms were assessed at ages 14, 17 and 20 years. Core analyses were limited to female offspring (n=308). **Results:** Maternal 25(OH)-vitamin D quartiles were a significant predictor of eating disorder risk in female offspring, in multivariate logistic regression models. Vitamin D in the lowest quartile was associated with a 1.8-fold increase in eating disorder risk relative to concentrations in the highest quartile. This association also accounted for the relationship between offspring season of birth and eating disorder risk. Results were significant after adjusting for sociodemographic characteristics, body mass index and depressive symptoms. **Discussion:** This is the first study to link low gestational vitamin D to increased eating disorder risk in female offspring of Caucasian mothers. Research is needed to extend these findings and to consider how gestational vitamin D may relate to the pathogenesis of eating disorders.

Key words: Eating disorders; Vitamin D; Epidemiology

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Low maternal vitamin D during pregnancy has been identified as a significant predictor of schizophrenia risk in offspring in later life (1, 2). This association was identified after researchers hypothesised that links between season of birth and schizophrenia may be due to underlying differences in gestational vitamin D exposure (1, 3). Season of birth has also been studied in relation to eating disorders, and there is some evidence for over-representation of spring births in anorexia nervosa (AN) (4, 5) and autumn (fall) births in bulimia nervosa (BN) (6). However, results are mixed and some studies have found no season of birth effects for eating disorders (7-10). Research in this area has also lacked a theoretical focus (4). Season of birth effects may stem from variations in gestational vitamin D, exposure to infectious agents, temperature and weather, and/or pregnancy and birth complications, all of which have the potential to influence fetal or infant neurodevelopment (4). Studies have not attempted to link season of birth effects in eating disorders to these possible explanatory factors.

Based on findings with schizophrenia, we propose that season of birth effects in eating disorders may stem from underlying gestational differences in vitamin D exposure. Vitamin D is found in very few foods and production primarily occurs through exposure to sunlight, from which ultraviolet B radiation is synthesised to give 25-hydroxyvitamin D (25[OH]D). There is currently no standard definition of optimal 25(OH)D, but concentrations below 50nmol/L (20mg/ml) are generally considered to indicate insufficiency (11, 12). Vitamin D is responsible for maintaining normal levels of calcium and phosphate in the blood, which in turn facilitate other essential processes, including nervous system activity and neurotransmitter functioning (11). In adults, vitamin D has a protective role during brain inflammation and low levels cross-sectionally predict depressive symptoms (13-15).

The mechanisms through which gestational vitamin D may influence offspring development have not been clearly delineated, but a 2012 meta-analysis identified significant associations between low maternal vitamin D during gestation and offspring birth outcomes, including birth weight (16). Low vitamin D during the second trimester may be particularly relevant to fetal brain development, as human and animal studies have found that the cortical structures critical to behavioral regulation are formed at 14 to 26 weeks gestation (17, 18). Consistent with this possibility, low vitamin D in the second trimester has been linked to offspring language impairment in childhood (19), and maternal illness in the second trimester, another possible disrupter to fetal brain development, has been linked to offspring neurocognitive difficulties in childhood (20, 21).

This study aimed to determine if maternal vitamin D in the second trimester of pregnancy would relate to offspring eating disorder risk. It was hypothesised that that low maternal vitamin D at 18 weeks gestation would predict increased offspring eating disorder risk by age 20 years, and that this relationship would account for any associations between season of birth and eating disorder risk.

Material and methods

Design

Data were drawn from the Western Australian Pregnancy Cohort (Raine) Study, details of which have been published previously (22, 23). In brief, women (N=2,900) were recruited from the antenatal booking clinics at King Edward Memorial Hospital for women (KEMH), the only public maternity hospital in Western Australia, between May 1989 and November 1991. Women were enrolled at 18 weeks gestation and there were 2,868 live birth babies delivered. The current study utilized a subset of 526 mother-offspring dyads who had maternal venous blood collected at 18 weeks gestation and who remained in the study to adolescence.

Participants

Participants were mother-offspring dyads where maternal 25(OH)D was collected at 18 weeks gestation (n=929), the mother identified as Caucasian (n=802; 86% of the venous blood sample), and offspring eating disorder data were available in adolescence (n=526; 66% of the Caucasian sample). Of the resulting 526 mother-offspring dyads, there were 218 male offspring (42%) and 308 female offspring (58%). Non-Caucasian participants (n=127) were excluded due to differences in the absorption and metabolism of vitamin D across Caucasian and non-Caucasian adults (24), and insufficient numbers to examine non-Caucasian participants separately in this sample. However, we provide summary details for non-Caucasian participants online as supplementary information.

The eating disorder group (n=98; 87% female) needed to meet criteria for an eating disorder at ages 14, 17 *or* 20 years. Thus, participants with an eating disorder at ages 14 or 17 were included in analyses even if they withdrew from the study prior to age 20. In contrast, the non-eating disorder group (n=428) needed to fail to meet criteria for an eating disorder at ages 14, 17 *and* 20 years. As a result, the proportion of participants with an eating disorder is inflated relative to the true cohort prevalence. Movement between eating disorder diagnostic categories over time was common and diagnostic categories were considered jointly for analyses.

Measures

Maternal 25(OH)D at 18 weeks gestation. From 1989 to 1991, venous blood was obtained at 18 weeks gestation from 929 randomly selected Raine Study women (86% / n=802 Caucasian), centrifuged, and serum collected and stored at -80^{o}C. In June 2011, serum 25(OH)D levels were measured using an enzyme immunoassay kit from Immunodiagnostic Systems Ltd (Scottsdale, Arizona, USA). Vitamin D concentrations in stored sera have been shown to remain stable for over three decades (25, 26). Twenty-eight samples were also measured using isotope-dilution liquid chromatography-tandem mass spectrometry by RMIT Drug Discovery Technologies (Melbourne Australia) according to published methodology (27). The correlation of 25(OH)D concentrations for samples assayed by the two techniques was strong (r^2 = .87) and confirmed that there were no molecules (vitamin D metabolites or otherwise) in sera of 18-week pregnant women that interfered with the immunoassay of 25(OH)D. The assay of 25(OH)D by isotope-dilution liquid chromatography-tandem mass spectrometry gave concentrations of 25(OH)D that were slightly higher than those measured by immunoassay (slope 0.95 $\pm$ 0.07). Overestimation of 25(OH)D by the former assay has previously been reported (28). For these reasons, the serum 25(OH)D levels were divided into quartiles, which due to the strong correlative value were not influenced by the assay used for the measure of serum 25(OH)D concentration. The use of quartiles is well-established in vitamin D research methodology (1, 11) and has been successfully applied in the Raine cohort previously (19). The fetus is completely reliant on maternal vitamin D, and maternal concentrations of 25(OH)D at 18 weeks gestation provide an accurate measure of fetal exposure during the second trimester (29).

Season of birth. Season of birth was categorised for the southern hemisphere, with September-November as spring, December-February as summer, March-May as autumn, and June-August as winter. As maternal blood was collected at 18 weeks gestation, spring births had 25(OH)D levels assessed during autumn (fall) / early winter; summer births had levels assessed in winter / early spring; autumn births had levels assessed in spring / early summer; and winter births had levels assessed in summer / early autumn. Participants born in spring and summer would be expected to have lower 18 week 25(OH)D levels at 18 weeks gestation than those born in autumn or winter.

Offspring eating disorder symptoms in adolescence. Eating disorder symptoms were assessed at the 14, 17 and 20-year Raine Study assessments, using 24 items adapted from the Child Eating Disorder Examination (ChEDE) (30) and Eating Disorder Examination-Questionnaire (EDE-Q) (31). Items assessed for DSM-IV-TR and DSM-5 diagnostic criteria for AN, BN and BED, and for dietary

restraint and eating, weight and shape concerns more generally. Items also assessed for purging disorder (PD) (32), an example of the eating disorders 'not elsewhere classified' in DSM-5 (33). Eating disorders were classified on the basis of responses to these 24 items, in conjunction with measured height and weight (used to calculate body mass index [BMI]) and self-reported menstruation status for females. We have previously reported on this process (22). With changes in diagnostic criteria from DSM-IV-TR to DSM-5, we refer to both narrowly defined, DSM-IV-TR threshold cases (e.g., binge eating and purging at least 2x week for BN) and more broadly defined, DSM-5 compatible cases (e.g., binge eating and purging at least 1x week for BN). The eating disorder classification process is summarised online as supplementary information.

Covariates. We considered pregnancy, birth and adolescent variables that may impact on the association between gestational vitamin D and offspring eating disorder risk. These are summarised in Table 1. Maternal and family characteristics during pregnancy were assessed via self-report questionnaires administered to mothers at their 18-week antenatal visit. Maternal BMI and health problems during pregnancy (e.g., kidney dysfunction, thyroid dysfunction) and offspring birth weight and gestational age were recorded by KEMH. Adolescent variables were taken from the 14-year adolescent assessment but in all cases correlated highly with data from 17 and 20 years. Family characteristics were reported by mothers and adolescent depressive symptoms were self-reported by adolescents using the Beck Depression Inventory for Youth (BDI-Y) (34). Adolescent's height and weight were measured during a face-to-face assessment and used to calculate BMI (kg / m^2).

Statistical analyses

Preliminary analyses. Participants with an eating disorder by age 20 (n=98) were compared to participants without an eating disorder (n=428) on pregnancy, birth and adolescent covariates, using analysis of variance (ANOVA) and Chi square tests. Participants included in the current study (n=526) were also compared to participants where maternal blood was collected at 18 weeks gestation but offspring were lost to follow-up before adolescence (n=276), and to participants where maternal blood was not collected at 18 weeks gestation but offspring were followed to adolescence (n=1070).

Preliminary screening was undertaken to examine the linearity or otherwise of any relationship between maternal 25(OH)D and offspring eating disorder risk, as the possibility of a J-shaped association has been identified for schizophrenia (i.e., elevated risk at low levels and very high

levels) (1). To achieve this, maternal 25(OH)D concentrations were divided into deciles and eating disorder risk ratios were calculated for each decile.

Associations between maternal 25(OH)D levels and offspring season of birth. Linear-by-linear Chi square tests were used to examine the association between maternal 25(OH)D quartile at 18 weeks gestation and offspring season of birth. Whilst Chi square tests are poorly suited to detecting seasonal *patterns* in risk, they are suited to comparing seasonal proportions across groups (35).

Associations between maternal 25(OH)D levels, offspring season of birth, and offspring eating disorder risk. Two logistic regression models were constructed to examine the effects of (i) maternal 25(OH)D quartile on risk for an eating disorder by age 20 and (ii) season of birth on risk for an eating disorder by age 20, adjusting for covariates. These models were run for male and female participants separately. Given the low prevalence of male eating disorders, results from male participants should be viewed as preliminary. For female participants, a multivariate model was also constructed to examine the joint effects of maternal 25(OH)D quartile and season of birth on risk for an eating disorder by age 20. The sample of 308 female participants provided over 80% power to detect an absolute differential exposure of 15% if non-eating disorder cases had a 20% prevalence of low vitamin D (i.e., quartile one), or 10% if non-cases had a prevalence of 10% ($p < .05$).

Results

Preliminary analyses

Table 1 summarises between-group differences between participants who experienced an eating disorder by age 20 and those who did not. Based on these differences, maternal kidney problems at 18 weeks gestation, family income and whether the biological father was present at age 14 years, and offspring BMI and depressive symptoms at age 14 years were included as covariates in all analyses.

[TABLE 1]

Differences between the current sample and the broader Raine Study cohort are summarised online as supplementary information. There were no significant differences between participants who met ‘narrow’ (DSM-IV-TR) criteria for an eating disorder and those who met broader DSM-5 criteria (*p*s = .11 - .90)

When examining offspring eating disorder risk by each decile of maternal 25(OH)D, we found no evidence for a J-shaped curve of association. Risk was greatest in deciles 1 and 2, which correspond to the lowest quartile of concentrations.

Associations between maternal 25(OH)D levels and offspring season of birth.

Mean 25(OH)D levels for each vitamin D quartile are summarised in Table 2.

[TABLE 2]

There was a significant association between maternal 25(OH)D quartile and offspring season of birth (χ^2 [1]=19.68, p<.001). Only 6% (n=7/92) of the participants born in winter (i.e., with vitamin D assessed in summer or early autumn) had maternal 25(OH)D levels in the lowest quartile at 18 weeks gestation, whereas 45% (n=67/149) of the participants born in summer (vitamin D assessed in winter or early spring) had maternal 25(OH)D levels in the lowest quartile. Similarly, mean 25(OH)D scores were significantly lower at 18 weeks gestation for participants born in summer (M[SD]=49.36 [15.82]) than for participants born in spring (M[SD] = 58.69 [18.81]) or autumn (M[SD] = 58.93 [18.71]), and were significantly lower for participants born in spring and autumn than for participants born in winter (M[SD]=67.42 [18.96]) (p < .001).

Associations between maternal 25(OH)D levels, offspring season of birth, and offspring eating disorder risk

Univariate analyses. There was a significant association between maternal 25(OH)D quartile and eating disorder risk in females, after adjusting for covariates (χ^2 [8]=38.84, p<.001, Nagelkerke R^2 = .20). This overall effect stemmed from offspring eating disorder risk being over two times greater when maternal 25(OH)D levels were in the first (lowest) quartile compared to when they were in the fourth (highest) quartile (see Table 3). Figure 1 summarises the proportion of female participants within each vitamin D quartile, by eating disorder status.

[FIGURE 1]

There was also a significant association between season of birth and eating disorder risk in females, after adjusting for covariates (χ^2 [8]=35.03, p<.001, Nagelkerke R^2 = .12). This effect stemmed from offspring eating disorder risk being approximately three times higher when female participants were born in spring relative to winter (see Table 3). Figure 2 summarises the proportion of participants born in each season, by eating disorder status.

[FIGURE 2]

There was no significant association between maternal 25(OH)D quartile and eating disorder risk in males (χ^2 [8]=10.85, p=.054, Nagelkerke R^2 = .16). Similarly, there was no significant

association between season of birth and eating disorder risk in males, although the overall model was significant due to the effects of covariates (χ^2 [8]=13.50, p=.019, Nagelkerke R^2 = .19) (see Table 3).

[TABLE 3]

Multivariate analyses. When maternal 25(OH)D quartile and offspring season of birth were jointly entered as predictors of eating disorder risk in females, the multivariate model was significant (χ^2 [11]=43.37 p<.001, Nagelkerke R^2 = .23), but season of birth did not contribute to the model significantly. Maternal 25(OH)D concentrations in the first quartile were associated with a significant two-fold increase in eating disorder risk, relative to concentrations in the fourth quartile (see Table 4). The R^2 statistic was comparable to that for the model with maternal 25(OH)D alone. The presence of maternal kidney difficulties at 18 weeks gestation contributed significantly to all models for females.

[TABLE 4]

Secondary analyses were conducted to examine associations between maternal 25(OH)D quartile, offspring season of birth, and risk for each specific eating disorder diagnosis in females. When comparing female participants with no eating disorder by age 20 (n=223) to female participants with BN by age 20 (n=54), the pattern of results was comparable to that obtained with the full eating disorder sample, and all identified effects remained significant ($p < .05$). When comparing female participants with no eating disorder by age 20 to female participants with AN (n=10), BED (n=23) or PD (n=20), the pattern of results was comparable to that obtained with the full sample, in each instance, but identified effects were not statistically significant (ps = .365 - .937).

Discussion

This study focused on associations between maternal 25(OH)D concentrations at 18 weeks gestation, offspring season of birth, and offspring eating disorder status in adolescence. We hypothesised that low maternal 25(OH)D would predict increased offspring eating disorder risk, and that this association would account for any relationship between offspring season of birth and eating disorders. This study provides initial support for these hypotheses.

When considered individually, maternal vitamin D status at 18 weeks gestation and offspring season of birth were both significant predictors of eating disorder risk in females. Female participants born to mothers with 25(OH)D concentrations in the lowest quartile were significantly more likely to experience an eating disorder at age 14, 17 or 20 years than participants born to mothers with 25(OH)D concentrations in the highest quartile. Female participants born in spring were also significantly more

likely to experience an eating disorder by age 20 years than participants born in winter. In multivariate analyses, however, season of birth was not a significant predictor of eating disorder risk. Instead, only maternal 25(OH)D contributed to the model significantly. The interpretation of these findings is aided by the Nagelkerke R^2 statistic, which provides an approximation of the variance accounted for by each logistic regression model. The value for the multivariate model (=.23) was comparable to that for the model with maternal vitamin D alone (=.20), suggesting that season of birth did not add greatly to the explanatory power of vitamin D. In contrast, the value for the multivariate model was double that for offspring season of birth alone (=.12). This suggests that the effects of season of birth on eating disorder risk may, at least in part, reflect underlying differences in gestational vitamin D.

When we stratified analyses by eating disorder diagnosis, results from the full sample were replicated for women with BN. In contrast, results for AN, BED and PD did not reach statistical significance in univariate or multivariate models. Small group sizes for these eating disorder diagnostic groups make replication important, and we view these diagnosis-specific findings as preliminary, and in need of extension in future studies.

We also provide preliminary data on associations between maternal 25(OH)D, season of birth, and risk for eating disorders in males. In this sample, no significant associations were identified between gestational vitamin D and male eating disorder risk, or between season of birth and male eating disorder risk. Given the small number of male participants with an eating disorder, we recommend extension in future studies. There are sex differences in fetal cortical development (36), allowing for the possibility that low gestation vitamin D will have a different effect on male and female offspring, but further research is required to investigate this possibility.

Implications for the aetiology of eating disorders

As this is the first study to investigate associations between gestation vitamin D and offspring eating disorder risk, our findings need to be interpreted as preliminary. Nonetheless, the possibility that gestational vitamin D levels relate to eating disorder risk in later life is of significance to psychiatry in general and the eating disorder field in particular. Our results highlight the importance of attending to biological as well as psychosocial factors when considering the pathogenesis of eating disorders, a position that has gained favour over the past three decades (e.g., 37, 38). Gestational vitamin D may be a new variable of interest for biologically focused investigation.

Without repeated assessment of gestational vitamin D, it is difficult to draw firm conclusions regarding the importance of vitamin D at 18 weeks pregnancy relative to other time points during pregnancy. The second trimester of pregnancy is recognised, however, as a key period for offspring neurodevelopment. Cortical structures related to behavioral regulation are formed during the second trimester (10) and disruptions to fetal development during this trimester (through low vitamin D, maternal illness, or other stressors to the developing fetus) have been linked to neurocognitive difficulties during childhood (19-21). Given this, it is interesting that increased schizophrenia risk is linked to spring and winter births (vs. spring and summer in eating disorders) and to low vitamin D at the time of birth, reflecting low maternal 25[OH]D3 during the *third* trimester (1). As different genetic and neurobiological factors are associated with different psychiatric disorders, it seems likely that disruptions to fetal development at different time points during gestation will give rise to different psychiatric consequences. Studies that assess maternal vitamin D at multiple time points in pregnancy would help to assess this possibility directly. With additional research, it may be possible to formulate a model of how gestational vitamin D impacts fetal and offspring neurocognitive development, and how this, in turn, serves to increase eating disorder or other psychiatric disorder risk in later life.

To complement direct research on gestational vitamin D and offspring outcomes, research in this area could also be extended if studies on eating disorder prevalence rates included information on average sunshine exposure and/or vitamin D levels in the geographic region/s under investigation.

Study limitations

This study has a number of strengths. It is the first to test the hypothesis that gestational 25(OH)D levels relate to later eating disorder risk, and to consider the possibility that this relationship may account for season of birth effects in eating disorders. Our use of prospective data collected from 18 weeks gestation to 20 years of age is unique in this area. We were able to adjust for relevant covariates, including maternal kidney dysfunction, which may have contributed to low 25(OH)D levels in mothers of offspring who developed an eating disorder. There are also a number of limitations, including the use of a subset of Raine Study participants, the use of self-report eating disorder data, and the modest sample size. Our focus on a subset of the full Raine Study cohort was unavoidable, due to the collection of gestational venous blood in 929 of the original 2900 mothers. Structured self-report questionnaires have been shown to compare well to interview assessment in the detection of eating disorders (39), which lends support to the validity of our self-report data. Regarding sample size, power

was sufficient for analyses with female participants but we can provide only preliminary data on associations between gestational vitamin D and risk for an eating disorder in males. Similarly, we provide preliminary data on possible diagnostic differences in the effects of vitamin D on female eating disorders. Previous research has shown stronger season of birth effects in AN than other eating disorders, and AN-specific research would thus be valuable. The magnitude of our effects also needs further investigation, as relatively large confidence intervals were observed for odds ratios in the logistic regression models. Lastly, it is important to acknowledge the possibility of reverse causation. The observed effects of gestational vitamin D may relate to differences in maternal behaviour during pregnancy (e.g., greater outdoor activity) as well as biological differences in 25(OH)D levels.

In summary, this study has provided new data to link low gestational 25(OH)D to increased eating disorder risk in female offspring of Caucasian mothers. This association may account for the season of birth effects observed in eating disorder groups previously. Ongoing research is required to extend our findings and to clarify the role of vitamin D in the pathogenesis of eating disorders. We recommend that our findings are viewed as preliminary, and as a basis for further research in this area.

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Declaration of interest

The authors have no declarations of interest with regard to the study design, results, or outcomes.

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Figure 1. *The proportion of female offspring within each maternal vitamin D quartile (reflecting maternal vitamin D levels at 18 weeks gestation), by eating disorder status.*

Figure 2. *The proportion of female offspring born in each season, by eating disorder status.*

Table 1

Baseline and adolescent characteristics (% [N] unless indicated) for participants with (n=98) and without (n=428) an eating disorder by age 20 years.

	Raine Study participants followed to adolescence	
	Eating disorder by age 20 (n=98)	No eating disorder by age 20 (n=428)
Pregnancy variables (at 18 weeks gestation unless otherwise specified):		
Maternal age (M[SD])	28.58 yrs (5.81)	29.37 yrs (5.68)
Maternal BMI (M[SD])	22.60 kg/m^2 (4.46)	21.87 kg/m^2 (3.77)
Mother drinking alcohol	12.3% (n=12)	19.4% (n=83)
Mother smoking cigarettes	26.5% (n=26)	21.1% (n=90)
Mother completed secondary education	42.9% (n=42)	45.1% (n=193)
Maternal serum 25(OH)D level (M[SD])	55.00 nmol/L (17.10) $_a$	58.60 nmol/L (16.36) $_b$
Maternal kidney disease or dysfunction	9.2% (n=9) $_a$	3.7% (n=16) $_b$
Maternal urinary tract infection	7.1% (n=7)	3.3% (n=14)
Maternal thyroid dysfunction	1.0% (n=1)	1.9% (n=8)
Low family income	16.3% (n=16)	14.0% (n=60)
Biological father living at home	87.8% (n=86) $_a$	93.9% (n=402) $_b$
Offspring gestational age at birth (M[SD])	38.72 wks (2.31)	38.71 wks (2.00)
Offspring birth weight (M[SD])	3.29 kg (0.64)	3.26 kg (0.58)
Preterm birth (<37wks)	10.2% (n=10)	8.6% (n=37)
Offspring sex - % male	13.3% (n=13) $_a$	47.9% (n=205) $_b$
Adolescent variables (at 14 years):		
Offspring age at 14-year assessment (M[SD])	14.03 yrs (0.14)	13.99 yrs (0.20)
Low family income	27.6% (n=27) $_a$	15.2% (n=65) $_b$
Biological father living at home	52.0% (n=51) $_a$	66.6% (n=285) $_b$
Offspring BMI (M[SD])	22.27 kg/m^2 (4.80) $_a$	20.80 kg/m^2 (3.78) $_b$
Offspring BDI-Y score (M[SD])	10.89 (8.18) $_a$	5.71 (6.24) $_b$

Note. Columns with different subscripts are significantly different at $p < .05$. BMI = Body Mass Index, BDI-Y = Beck Depression Inventory-Youth.

Table 2

Maternal serum 25(OH)D concentrations at 18 weeks gestation, for all participants and by 25(OH)D quartile. Data are presented in nmol/L with corresponding ng/ml below. Values below 50nmol/L (20ng/ml) are considered insufficient.

	Mean (SD)	Range
Full sample (n=526)	59.55 (19.98) nmol/L	18.30 - 154.29 nmol/L
	23.42 (7.30) ng/ml	6.13 - 61.82 ng/ml
Quartiles:		
Quartile 1 (n=186)	36.13 (6.99) nmol/L	18.30 - 45.98 nmol/L
	14.48 (2.80) ng/ml	6.13 - 18.42 ng/ml
Quartile 2 (n=205)	52.52 (3.85) nmol/L	46.17 - 59.06 nmol/L
	21.04 (1.54) ng/ml	18.50 - 23.66 ng/ml
Quartile 3 (n=208)	64.83 (3.49) nmol/L	59.12 - 71.39 nmol/L
	25.97 (1.40) ng/ml	23.69 - 28.60 ng/ml
Quartile 4 (n=200)	83.44 (11.64) nmol/L	71.44 - 154.29 nmol/L
	33.43 (4.66) ng/ml	28.62 - 61.82 ng/ml

Table 3

Univariate logistic regression models for the effects of maternal serum 25(OH)D concentrations at 18 weeks gestation and season of birth on eating disorder risk in male and female offspring, adjusting for covariates

	OR	95% CI	*p*
Female participants			
Season of birth (reference = winter)			
Spring	3.05	1.10 - 8.04	.034*
Summer	2.48	0.98 - 6.13	.060
Autumn	1.74	0.65 - 4.62	.269
Maternal 25(OH)D at 18 weeks gestation (reference = quartile 4)			
Quartile 1 (lowest levels)	2.44	1.04 - 5.88	.037*
Quartile 2	1.67	0.72 - 3.90	.234
Quartile 3	1.09	0.44 - 2.74	.849
Male participants [1]			
Season of birth (reference = winter)			
Spring	0.94	0.15 - 5.91	.949
Summer	0.31	0.05 - 1.82	.196
Autumn	0.14	0.01 - 1.40	.094
Maternal 25(OH)D at 18 weeks gestation (reference = quartile 4)			
Quartile 1 (lowest levels)	1.15	0.25 – 5.00	.850
Quartile 2	0.99	0.10 - 3.98	.998
Quartile 3	0.16	0.01 - 1.99	.178

[1] As only 13 male participants met criteria for an eating disorder, these results should be viewed as preliminary.

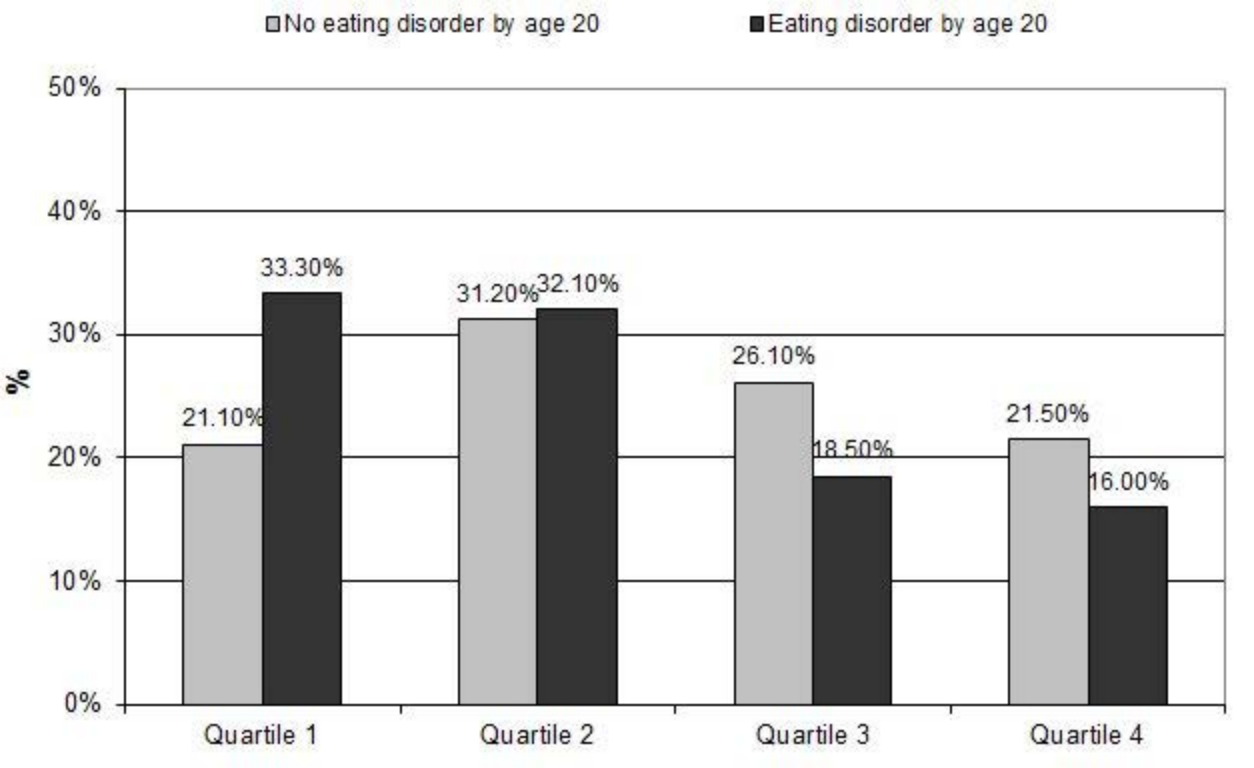
Note. OR = odds ratio, CI = confidence intervals. Models are adjusted for the presence of maternal kidney dysfunction at 18 weeks gestation, family income and whether the biological father was living at home at age 14, and offspring body mass index and depressive symptoms at age 14.

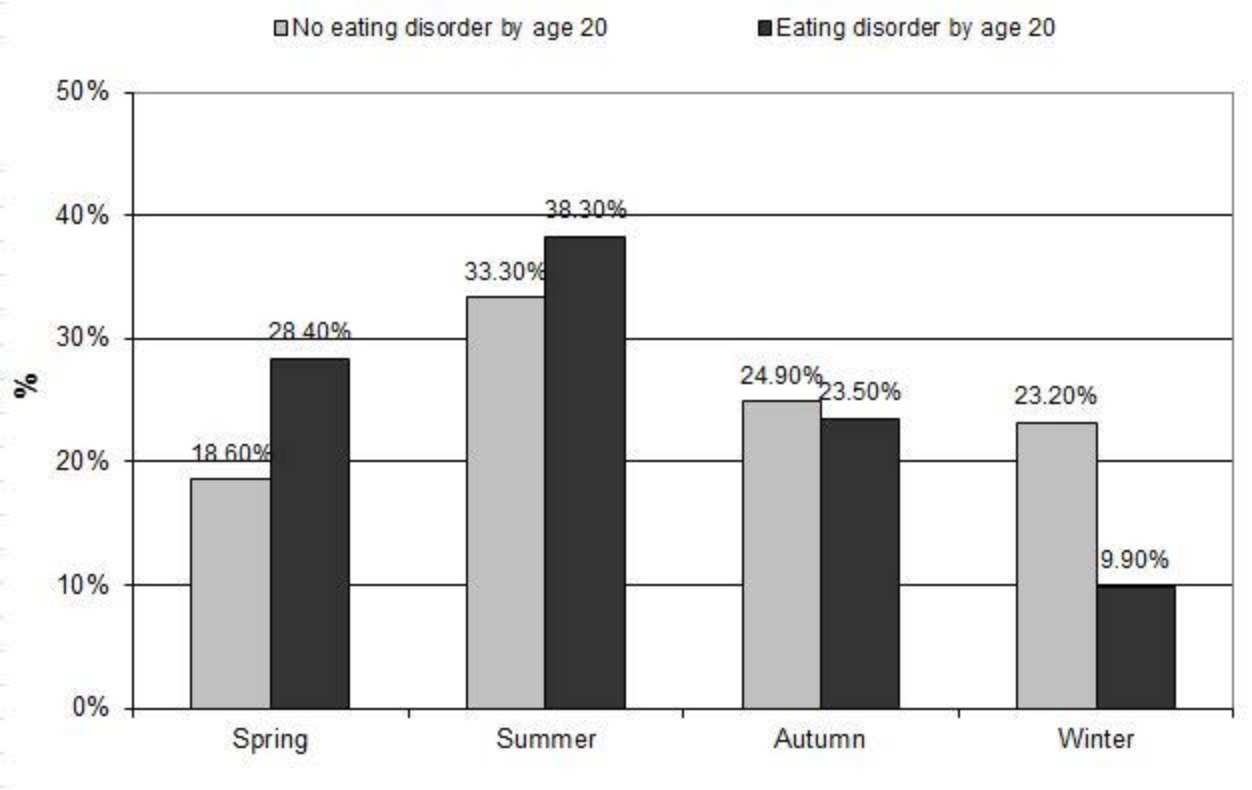
Table 4

Multivariate logistic regression model for the joint effects of maternal serum 25(OH)D concentrations at 18 weeks gestation and season of birth on eating disorder risk in female offspring, adjusting for covariates

	OR	95% CI	*p*
Season of birth (reference = winter)			
Spring	2.70	0.86 - 7.33	.091
Summer	1.99	0.77 - 4.35	.292
Autumn	1.60	0.59 - 3.85	.454
Maternal 25(OH)D at 18 weeks gestation (reference = quartile 4)			
Quartile 1 (lowest levels)	2.09	1.03 – 5.27	.046*
Quartile 2	1.58	0.67 - 3.76	.296
Quartile 3	1.06	0.42 - 2.70	.901

Note. OR = odds ratio, CI = confidence intervals. Models are adjusted for the presence of maternal kidney dysfunction at 18 weeks gestation, family income and whether the biological father was living at home at age 14, and offspring body mass index and depressive symptoms at age 14.


No eating disorder by age 20
Eating disorder by age 20
50%
40%
30%
20%
10%
0%
%
33.30%
31.20%
32.10%
26.10%
21.10%
18.50%
21.50%
16.00%
Quartile 1
Quartile 2
Quartile 3
Quartile 4


No eating disorder by age 20
Eating disorder by age 20
50%
40%
30%
20%
10%
0%
%
18.60%
28.40%
33.30%
38.30%
24.90%
23.50%
23.20%
9.90%
Spring
Summer
Autumn
Winter

Supplementary information for online publication

Associations between gestational vitamin D and offspring eating disorder risk in non-Caucasian participants.

Gestational vitamin D was collected from 127 non-Caucasian mothers, representing 14% of the venous blood sample. Consistent with previous research, the mean 25(OH)D concentration for non-Caucasian mothers (M = 44.60 nmol/L; SD = 18.47) was significantly lower than the mean 25(OH)D concentration for Caucasian mothers (M = 59.95 nmol/L; SD = 19.98) (t [928] = 8.13, $p < .001$).

Retention of non-Caucasian participants to adolescence was comparable to that for the Caucasian sample, meaning that eating disorder data were available for approximately two-thirds of the non-Caucasian offspring (n=63). Of these, 36 participants were female (57.1%) and 27 were male (42.9%). There were 5 female, non-Caucasian offspring with an eating disorder at age 14, 17 or 20 years. No male non-Caucasian offspring were identified as experiencing an eating disorder by age 20 years.

The 5 non-Caucasian females who experienced an eating disorder by age 20 years had a mean gestational 25(OH)D level of 26.58 nmol/L (SD 8.47), with a range of 17.08 to 33.33 nmol/L. This compared to a mean 25(OH)D level of 48.48 nmol/L (SD 18.71; range 18.44 – 84.68 nmol/L) for the 31 females who did not experience an eating disorder by age 20. The difference between the two groups was not statistically significant (t [22] = 1.97, $p = .062$). A logistic regression model examining the relationship between 25(OH)D quartile and eating disorder risk in non-Caucasian females also failed to reach significance (X^2 [3] = 4.59, $p = .204$), as did a model examining the association between season of birth and eating disorder risk (X^2 [3] = 2.81, $p = .422$). Given the lack of statistical power available for these analyses, further research is needed before conclusions may be drawn about gestational vitamin D and offspring eating disorder risk in non-Caucasian groups.

Supplementary figure

Classification of eating disorders. *AN = anorexia nervosa, BN = bulimia nervosa, BED = binge eating disorder and PD = purging disorder. Note that some participants met criteria for different eating disorders at different time points in adolescence. Participants diagnosed with a 'broad' form of eating disorder needed to meet the 'narrow' diagnostic threshold for at least one of the listed criteria.*

Supplementary table.

Baseline and adolescent characteristics (% [N] unless indicated) for Raine Study participants in the current study (n=526) and those with maternal 25(OH)D3 levels at 18 weeks gestation but subsequently lost to follow-up (n=276), or excluded from analyses due to the absence of maternal 25(OH)D3 data (n=1070).

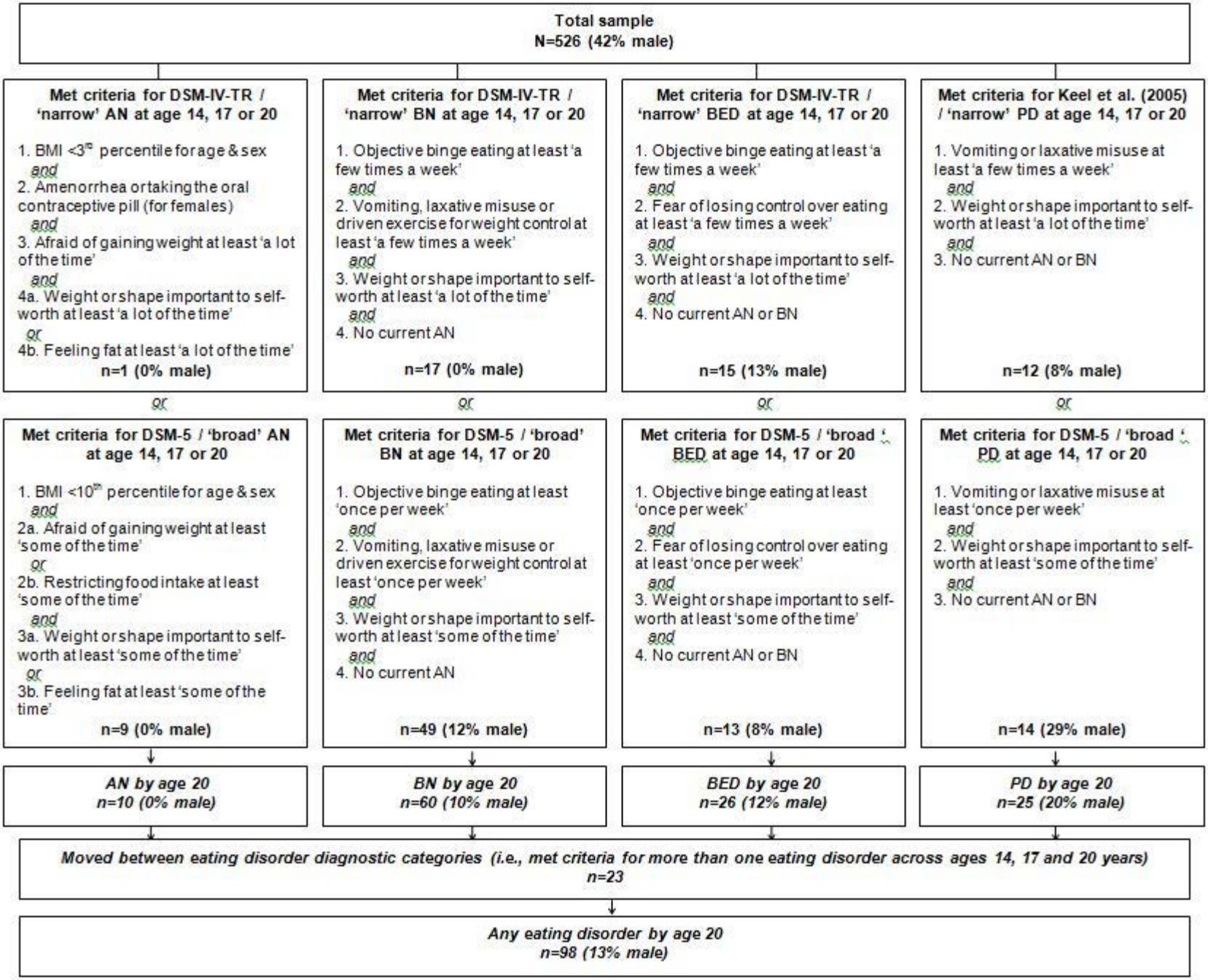
	Raine Study participants with Caucasian mothers and maternal 25(OH)D3 at 18 weeks gestation (n=802)	
	Current sample - participation to adolescence (n=526)	Lost to follow-up (n=276)
Pregnancy variables (at 18 weeks gestation unless otherwise specified):		
Maternal age (M[SD])	28.96 (5.68) [a]	27.16 (5.76) [b]
Maternal BMI (M[SD])	22.26 (4.05)	21.84 (3.91)
Mother drinking alcohol	18.0% (n=95)	16.3% (n=45)
Mother smoking cigarettes	22.1% (n=116) [a]	37.3% (n=103) [b]
Mother completed secondary education	44.7% (n=235)	39.8% (n=110)
Maternal serum 25(OH)D3 level (M[SD])	57.52 (19.02)	58.42 (19.38)
Low family income	14.5% (n=76) [a]	20.3% (n=56) [b]
Biological father living at home	93.7% (n=488) [a]	79.0% (n=218) [b]
Offspring gestational age at birth (wks) (M[SD])	38.73 (2.20)	38.74 (2.22)
Offspring birth weight (kg) (M[SD])	3.28 (0.60)	3.26 (0.61)
Preterm birth (<37wks)	8.9% (n=47)	7.6% (n=21)
Offspring sex - % male	41.4% (n=218)	40.0% (n=110)
	Raine Study participants followed to adolescence	
	Current sample - maternal 25(OH)D3 available (n=526)	No maternal 25(OH)D3 available (n=1070)
Pregnancy variables (at 18 weeks gestation unless otherwise specified):		
Maternal age (M[SD])	28.96 (5.68) [a]	29.13 (5.84)
Maternal BMI (M[SD])	22.26 (4.05)	22.41 (4.30)
Mother drinking alcohol	18.0% (n=95)	22.2% (n=238)
Mother smoking cigarettes	22.1% (n=116) [a]	22.1% (n=237)

Continued below

Supplementary table cont.

	Raine Study participants followed to adolescence	
	Current sample - maternal 25(OH)D3 available (n=526)	No maternal 25(OH)D3 available (n=1070)
Pregnancy variables (at 18 weeks gestation unless otherwise specified) (cont.):		
Mother completed secondary education	44.7% (n=235)	40.9% (n=438)
Low family income	14.5% (n=76) $_{a}$	11.2% (n=110)
Biological father living at home	93.7% (n=488) $_{a}$	89.4% (n=957)
Offspring gestational age at birth (wks) (M[SD])	38.73 (2.20)	38.81 (2.16)
Offspring birth weight (kg) (M[SD])	3.28 (0.60)	3.34 (0.59)
Preterm birth (<37wks)	8.9% (n=47)	10.0% (n=107)
Offspring sex - % male	41.4% (n=218)	55.0% (n=589)
Adolescent variables (at 14 years unless otherwise specified)		
Offspring age at 14-year assessment (M[SD])	14.00 (0.20)	14.02 (0.19)
Low family income	17.5% (n=92)	18.5% (n=198)
Biological father living at home	63.9% (n=336)	66.5% (n=712)
Offspring BMI (M[SD])	21.32 (4.24)	21.26 (4.08)
Offspring BDI-Y score (M[SD])	6.81 (6.96)	6.34 (6.79)
Eating disorder by age 20	18.6% (n=98)	16.1% (n=173)

Note. Columns with different subscripts are significantly different at $p < .05$.


Total sample
N=526 (42% male)
Met criteria for DSM-IV-TR / 'narrow' AN at age 14, 17 or 20
1. BMI <3rd percentile for age & sex
and
2. Amenorrhea or taking the oral contraceptive pill (for females)
and
3. Afraid of gaining weight at least 'a lot of the time'
and
4a. Weight or shape important to self-worth at least 'a lot of the time'
or
4b. Feeling fat at least 'a lot of the time'
n=1 (0% male)
Met criteria for DSM-IV-TR / 'narrow' BN at age 14, 17 or 20
1. Objective binge eating at least 'a few times a week'
and
2. Vomiting, laxative misuse or driven exercise for weight control at least 'a few times a week'
and
3. Weight or shape important to self-worth at least 'a lot of the time'
and
4. No current AN
n=17 (0% male)
Met criteria for DSM-IV-TR / 'narrow' BED at age 14, 17 or 20
1. Objective binge eating at least 'a few times a week'
and
2. Fear of losing control over eating at least 'a few times a week'
and
3. Weight or shape important to self-worth at least 'a lot of the time'
and
4. No current AN or BN
n=15 (13% male)
Met criteria for Keel et al. (2005) / 'narrow' PD at age 14, 17 or 20
1. Vomiting or laxative misuse at least 'a few times a week'
and
2. Weight or shape important to self-worth at least 'a lot of the time'
and
3. No current AN or BN
n=12 (8% male)
or
or
or
or
Met criteria for DSM-5 / 'broad' AN at age 14, 17 or 20
1. BMI <10th percentile for age & sex
and
2a. Afraid of gaining weight at least 'some of the time'
or
2b. Restricting food intake at least 'some of the time'
and
3a. Weight or shape important to self-worth at least 'some of the time'
or
3b. Feeling fat at least 'some of the time'
n=9 (0% male)
Met criteria for DSM-5 / 'broad' BN at age 14, 17 or 20
1. Objective binge eating at least 'once per week'
and
2. Vomiting, laxative misuse or driven exercise for weight control at least 'once per week'
and
3. Weight or shape important to self-worth at least 'some of the time'
and
4. No current AN
n=49 (12% male)
Met criteria for DSM-5 / 'broad' BED at age 14, 17 or 20
1. Objective binge eating at least 'once per week'
and
2. Fear of losing control over eating at least 'once per week'
and
3. Weight or shape important to self-worth at least 'some of the time'
and
4. No current AN or BN
n=13 (8% male)
Met criteria for DSM-5 / 'broad' PD at age 14, 17 or 20
1. Vomiting or laxative misuse at least 'once per week'
and
2. Weight or shape important to self-worth at least 'some of the time'
and
3. No current AN or BN
n=14 (29% male)
AN by age 20
n=10 (0% male)
BN by age 20
n=60 (10% male)
BED by age 20
n=26 (12% male)
PD by age 20
n=25 (20% male)
Moved between eating disorder diagnostic categories (i.e., met criteria for more than one eating disorder across ages 14, 17 and 20 years)
n=23
Any eating disorder by age 20
n=98 (13% male)