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ORIGINAL ARTICLE ***Psychology and counselling***

Psychosocial stress and ovarian function in adolescent and young adult cancer survivors

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STUDY QUESTION: Is psychosocial stress associated with ovarian function in reproductive-aged survivors of cancer diagnosed as adolescents and young adults (AYA survivors)?

SUMMARY ANSWER: We observed no association between self-reported and biomarkers of psychosocial stress and ovarian function in AYA survivors.

WHAT IS KNOWN ALREADY: Psychosocial stress suppresses hypothalamic-pituitary-ovarian axis, resulting in ovulatory dysfunction, decreased sex steroidogenesis and lower fertility in reproductive-aged women. Many cancer survivors experience high psychosocial stress and hypothalamic-pituitary-adrenal axis dysregulation. The menstrual pattern disturbances and infertility they experience have been attributed to ovarian follicle destruction, but the contribution of psychosocial stress to these phenotypes is unknown.

STUDY DESIGN, SIZE, DURATION: A cross-sectional study was conducted estimating the association between perceived stress, measured by self-report and saliva cortisol, and ovarian function, measured by bleeding pattern, dried blood spot (DBS) FSH and LH, and saliva estradiol. We included 377 AYA survivor participants.

PARTICIPANTS/MATERIALS, SETTING, METHODS: AYA survivor participants were ages 15–35 at cancer diagnosis and ages 18–40 at study enrollment, had completed primary cancer treatment, had a uterus and at least one ovary, did not have uncontrolled endocrinopathy and were not on hormone therapy. Recruited from cancer registries, physician referrals and cancer advocacy groups, participants provided self-reported information on psychosocial stress (Perceived Stress Scale-10 (PSS-10)) and on cancer and reproductive (fertility, contraception, menstrual pattern) characteristics. DBS samples were collected timed to the early follicular phase (cycle Days 3–7) for menstruating individuals and on a random day for amenorrheic individuals; saliva samples were collected three time points within 1 day. FSH and LH were measured by DBS ELISAs, cortisol was measured by ELISA and estradiol was measured by liquid chromatography tandem mass spectrometry.

MAIN RESULTS AND THE ROLE OF CHANCE: The median age of participants was 34.0 years (range 19–41) at a median of 6.0 years since cancer diagnosis. The most common cancer was breast (32.1%). Median PSS-10 score was 15 (range 0–36), with 5.3% scoring $\geq$26, the cut point suggestive of severe stress. Cortisol levels followed a diurnal pattern and cortisol AUC was negatively correlated with PSS-10 scores ($P = 0.03$). Neither PSS-10 scores nor cortisol AUC were associated with FSH, LH, estradiol levels or menstrual pattern. Waking and evening cortisol and the cortisol awakening response also were not related to ovarian function measures.

LIMITATIONS, REASONS FOR CAUTION: Our analysis is limited by its cross-sectional nature, heterogeneity of cancer diagnosis and treatments and low prevalence of severe stress.

WIDER IMPLICATIONS OF THE FINDINGS: The lack of association between psychosocial stress and a variety of ovarian function measures in female AYA cancer survivors suggests that psychosocial stress does not have a significant impact on the reproductive axis of AYA survivors. This finding is important in counseling this population on their menstrual pattern and family building plans.

STUDY FUNDING/COMPETING INTEREST(S): NIH HD080952, South Korea Health Industry Development Institute HI18C1837 (JK). Dr A.D. works for Bluebird Bio, Inc., Dr D.Z. works for ZRT Labs and Dr P.M.S. works for Ansh Labs, which did not sponsor, support or have oversight of this research. Other authors report no competing interests.

TRIAL REGISTRATION NUMBER: N/A

Key words: psychosocial stress/ gonadotropins/ ovarian steroid/ menstrual pattern/ cortisol

Introduction

The hypothalamic-pituitary-adrenal (HPA) axis is one of the primary regulators of reproductive function. At homeostatic levels, cortisol regulates gonadal function at all levels of the hypothalamic-pituitary-ovary (HPO) axis (Whirledge and Cidlowski, 2010). Psychosocial stress activates the HPA axis and stimulates adrenal release of glucocorticoids including cortisol (Whirledge and Cidlowski, 2013). Stress-induced increases in cortisol inhibit GnRH transcription, alter GnRH receptor expression and regulate gonadotropin subunit expression. In turn, LH secretion and pulsatility are reduced and follicle atresia in animal models is increased (Dobson *et al.*, 2012; Whirledge and Cidlowski, 2013; Breen and Mellon, 2014). Beyond central effects, cortisol directly inhibits LH-stimulated steroidogenesis in human granulosa cells in culture (Michael *et al.*, 1993).

Several clinical reproductive sequelae of psychosocial stress have been observed.

High perceived stress has been associated with lower sex steroids and lower fertility (Nepomnaschy *et al.*, 2004; Schliep *et al.*, 2015). Also, both in the general and infertile populations, psychosocial stress has been related to menstrual irregularities (Boivin and Schmidt, 2005; Geraghty and Kaufer, 2015; Schliep *et al.*, 2015). The prospective BioCycle cohort study of 259 premenopausal women in New York with no known reproductive disorders showed that daily perceived stress was associated with lower estradiol, luteal progesterone and LH levels, higher FSH levels and anovulation (Schliep *et al.*, 2015). In contrast, some studies have shown no association between self-reported or biomarkers of stress (i.e. cortisol and alpha amylase) and pregnancy loss (Milad *et al.*, 1998; Lynch *et al.*, 2014).

Many cancer survivors experience high psychosocial stress and HPA dysregulation (Zeltzer *et al.*, 2009; Taylor *et al.*, 2012; Oancea *et al.*, 2014). Blunted cortisol responses and flatter diurnal cortisol slopes have been observed with psychosocial stress and fatigue in female cancer survivors (Bower *et al.*, 2005; Cuneo *et al.*, 2017). The contribution of psychosocial stress to the reproductive function of young cancer survivors is largely unknown. Clinically, reproductive-aged female survivors of cancer diagnosed as AYA between ages 15 and 35 (AYA survivors) exhibit more menstrual pattern disturbances including amenorrhea and infertility compared to women without cancer (Petrek *et al.*, 2006; Barton *et al.*, 2013; Akhtar *et al.*, 2015). Because cancer treatments can be gonadotoxic, these clinical phenotypes have been attributed to ovarian follicle destruction (Pampanini *et al.*, 2019). Recently, Hardy *et al.* (2019) conducted a cross-sectional study with 24 female childhood cancer survivors and described that both perceived stress and salivary cortisol explained part of the variation in anti-Mullerian hormone (AMH), FSH and LH levels.

In this study, we hypothesized that there would be a negative association between psychological stress and ovarian function in AYA survivors. To test this hypothesis, we examined the relationship of self-reported psychosocial stress and diurnal saliva cortisol levels with gonadotropins, ovarian steroids and menstrual pattern.

Materials and methods

Population

We performed a cross-sectional study using biosample and survey data collected at enrollment for the Reproductive Window Study, a prospective cohort study estimating the trajectory of ovarian function after cancer treatment. Full details of the cohort and primary findings have been previously reported (Su *et al.*, 2020). Eligibility criteria included: females with cancer diagnoses between ages 15–35, ages 18–40 at study enrollment, completion of primary cancer treatment, presence of at least one ovary and no uncontrolled endocrinopathies (e.g. thyroid and adrenal disease). AYA survivors could enter the study from 1 day to 25 years post-treatment. The included cancer types were breast, leukemia, lymphoma, gynecologic (cervix, uterus, ovary), gastrointestinal (intestines, gall bladder, pancreas), sarcomas, skin and thyroid. Through mailings and provider referrals, AYA survivors were recruited to a study about ovarian function after cancer treatment from the California and Texas Cancer Registries (36.0%), University of California, San Diego Health System (29.6%), cancer advocacy organizations (10.8%), physician referrals (3.9%) and other sources (19.7%).

For the present analysis, we included participants who completed dried blood spot (DBS) and saliva collection at enrollment. In order to interpret bleeding patterns and gonadotropin and estradiol levels, we excluded those on hormones (menopausal hormone therapy, oral contraceptive pills, GnRH agonist, tamoxifen, aromatase inhibitors, progestin implants, progestin intrauterine systems) in the prior 12 months as well as those with history of hysterectomy. The State of California Committee for the Protection of Human Subjects and the Institutional Review Boards at the University of California, San Diego and the Texas Department of State Health Services approved this study.

Study procedures

Potential eligible participants were mailed recruitment letters with directions to the online study portal. All other potential participants

were also directed to the online study portal via telephone calls or emails. On the secure, web-based study portal, potential participants registered, answered screening questions, reviewed study requirements and completed informed consent documents in order to enroll. Followed for up to 18 months, enrolled participants were asked to complete an online questionnaire through the study portal and self-collect DBS every 6 months (Frank *et al.*, 1997; Worthman and Stallings, 1997). Participants were also asked to collect saliva to measure the cortisol levels (Gettler *et al.*, 2011). The questionnaire collected self-reported information on cancer, reproduction (e.g. fertility, contraception, menstrual pattern), stress (Perceived Stress Scale-10 (PSS-10)), medication use, medical, demographic and lifestyle characteristics using questions derived from previous cancer and reproductive cohort studies (Cohen *et al.*, 1983; Freeman *et al.*, 2005a, b; Groves *et al.*, 2009). Participants were compensated a $30 gift card for the completion of the questionnaire, DBS collection and saliva collection.

Participants completed the Health Insurance Portability and Accountability Act (HIPAA) and medical record release forms that allowed study staff to obtain primary cancer treatment records. Using the Childhood Cancer Survivor Study methods and case report forms (Leisenring *et al.*, 2009), two board-certified pediatric oncologists and one board-certified reproductive endocrinologist abstracted cancer diagnosis and treatment data from participants' primary medical records with high agreement on re-review of 25% of abstracted data.

Perceived Stress Scale

The PSS-10 consists of 10 items with each item rated on a 5-point Likert scale, ranging from 0 'never' to 4 'very often'. The PSS-10 comprises six negative and four positive items (Cohen *et al.*, 1983). The total score of PSS is obtained by reversing the scores on the positive items and then summing across all the items, with a higher score indicating higher perceived stress. Possible total scores for PSS-10 range from 0 to 40. Psychometric properties of PSS-10 in a large national sample of Americans included adequate internal consistency reliability (Cronbach's alpha = 0.78) (Cohen and Williamson, 1988).

DBS collection and FSH, LH and AMH assays

The enrollment DBS collection was timed to the early follicular phase (cycle Days 3–7) for menstruating individuals and on a random day for amenorrheic individuals. Study staff met with participants via telephone or video calls for the first DBS collection for quality control. Following verbal, written and picture instructions, participants punctured their finger pad and applied up to five drops of whole blood to the blood spot filter paper. Participants then allowed samples to dry at room temperature for at least 4 h prior to placement in a gas impermeable plastic bag with desiccant and shipment back to UC San Diego via 2-day mail. Once received, study staff inspected the DBS samples for quality prior to being frozen at −80°C.

DBS were assayed for AMH, LH and FSH levels (limit of detection of 0.03 ng/ml, 0.02 and 0.07 mIU/ml, respectively, and inter- and intra-assay coefficient of variation <10%) using ELISA's designed specifically for the measurement of AMH, LH and FSH in human DBS specimens (Product AL.129, AL-190 and AL-187, respectively, AnshLabs, Webster, TX). These assays were validated previously in women without cancer (Worthman and Stallings, 1994, 1997). DBS AMH concentrations are traceable to the manufacturer's recombinant human AMH standard and are corrected for the DBS dilution factor so that values assigned are relative to the subject's serum levels. DBS LH and FSH concentrations are traceable to WHO preparations 81/535 and 83/575, respectively. The LH and FSH calibrators are corrected for the DBS dilution factor so that values assigned are relative to the subject's serum levels. The dilution recovery of DBS AMH specimens containing 0.319–11.967 ng/ml, DBS LH specimens containing 0.18–8.62 mIU/ml and DBS FSH specimens containing 0.18–22.0 ng/ml were 92–113%, 97–108 and 93–113, respectively. Using 29 matched serum and DBS samples ranging from 0.745 to 16.326 ng/ml AMH in serum, DBS AMH levels measured in these samples have been compared to serum levels measured with the Ansh picoAMH ELISA (Product # AL.124). Passing Bablok analysis of the results yielded the following Regression: DBS AMH ng/ml = −4.404 + 0.065 Serum AMH ng/ml ($r = 0.96$). Using 14 matched serum and DBS samples ranging from 0.085 to 11.2 mIU/ml of LH and 0.97 to 8.5 mIU/ml of FSH in serum, DBS LH and FSH levels were measured and compared to serum levels measured with the Ansh US LH ELISA (Product # AL.188) and Ansh US FSH ELISA (Product # AL.186). Regression analysis of the results yielded the following regression: DBS LH mIU/ml = 0.96 Serum + 0.16 mIU/ml ($r = 0.96$) and DBS FSH mIU/ml = 0.92 Serum + 0.35 mIU/ml ($r = 0.96$), respectively.

Saliva collection and cortisol and estradiol assays

Participants collected saliva samples the day prior to or after their DBS collection, to minimize the impact of finger prick on salivary cortisol. Collection occurred by passive drool at three time points within 1 day: upon awakening before getting out of bed, 30 to 45 min later, and before going to bed. Participants were then instructed to keep samples in a plastic bag in their refrigerator until all three samples were collected with shipment back to UC San Diego via 2-day mail. Once received, study staff inspected saliva samples for quality prior to being frozen at −80°C.

Salivary estradiol was measured by liquid chromatography tandem mass spectrometry (LC-MS/MS) using an AB Sciex Triple Quad 5500 (Keski-Rahkonen *et al.*, 2015). Saliva was processed and assayed for cortisol with a Food and Drug Administration approved direct (non-extracted) salivary enzyme immunoassay cortisol kit (Pantex). Cortisol was measured in 25 µl saliva samples by slight modifications of a previously described method (Du *et al.*, 2013). Inter-assay coefficient of variation for cortisol is 8% at 1 ng/ml, 7.1% at 4 ng/ml and 7.6% at 12.9 ng/ml. The detectable limit is 0.1–30 ng/ml.

Statistical analyses

Stress measures that were considered as primary exposures for analysis included longitudinal salivary cortisol and PSS-10 scores. In order to quantify total cortisol over the period of observations, AUC was calculated using measurements from each of the three timed salivary sample collections (i.e. time of awakening, 30 min after awakening and prior to bedtime) (Khoury *et al.*, 2015). The AUC represents an

approximated integral of the total saliva cortisol concentration over the three measurements, and was calculated as:

$$\text{AUC} = \left(\sum_{i=1}^{2} \frac{(c_i + c_{(i+1)})}{2} \times (t_{(i+1)} - t_i)\right) - \left(c_{\min} \times \sum_{i=1}^{3} t_{(i+1)} - t_i\right)$$

where i indicates time points {1, 2, 3}, c_i indicates cortisol measured at time point i, $c_{\min} = \min(c_1, c_2, c_3)$ and $t_i =$ the time in minutes at time point i. Cortisol AUC was categorized in quartiles for analysis. Secondarily, T1, T3 and the cortisol awakening response (T2-T1 levels) were considered as exposures.

The outcomes were FSH, LH, estradiol and AMH levels and menstrual pattern. FSH, LH, AMH and estradiol were log-transformed to meet the assumptions of normality and analyzed as continuous variables. Menstrual pattern was categorized as regular (10 or more menses in prior 12 months), oligomenorrhea (1–9 menses, >60 days between two consecutive periods or >1 period started 7 or more days from the day expected) and amenorrhea (no menses).

Data were summarized using frequencies for categorical variables, means and standard deviation for continuous variables that were normally distributed, and median (range) for continuous variables that were not normally distributed. ANOVA was used to analyze the differences among group means for continuous variables, and Fisher's Exact for categorical variables. *Pearson* correlation coefficient was calculated to estimate the correlation between two variables. Statistical significance was set at $P < 0.05$. All statistical analyses were performed using SAS version 9.3 (SAS Institute).

Results

For the parent study, 1125 individuals were screened and 1072 completed the PSS-10 scale questions. Among those, the final analysis included 377 participants who completed a saliva and DBS collection and met eligibility criteria for this analysis (Table I). Individuals who did not have DBS (n = 365) were on hormonal therapy (n = 366), and/or did not have a uterus (n = 36) were excluded from analysis. The median age of participants was 34.0 years (range 19–41) at a median of 6.0 years since cancer diagnosis. The most common types of cancer were breast cancer (32.1%) followed by hematologic cancers (32.0%). Half of participants (50.1%) were nulligravida.

While the median PSS-10 score was 15 (range 0–36), 5.3% of participants had scores greater than 26, the cut point suggestive of severe stress (Cohen and Williamson, 1988). Cortisol levels largely followed a diurnal pattern; following wakening, levels rose at 30 min and nadired prior to sleep (Fig. 1). The PSS-10 score was significantly correlated with cortisol AUC (Pearson correlation coefficient = −0.11, $P = 0.03$, Fig. 2). Higher PSS-10 scores were correlated with the lower cortisol AUC. When participants were grouped according to the quartiles (Q) of cortisol AUC, PSS-10 score was highest in Q1 (median 17.0, range 0–34.0) compared to Q2 (median 15.8, min 1.0, max 32.0), Q3 (median 14.4, min 1.0, max 36.0) and Q4 (median 14.6, min 3.0, max 33.0).

By bleeding pattern, 9.6% of participants were amenorrheic, and 47.2% were oligomenorrheic. In addition, 38.4% experienced menses consistently more than or <7 days from prior pattern, and 20.2%

Table I Participant demographic, cancer and reproductive characteristics, stress measures and ovarian function measures (n = 377).

Characteristic	N = 377
Age at enrollment, median (range)	34.0 (19.0, 41.0)
Age at cancer diagnosis, median (range)	28.0 (9.0, 37.0)
Years between cancer treatment and enrollment, median (range)	6.0 (0, 27.0)
BMI (kg/m^2), mean ± SD	27.0 ± 6.9
Marital status, n (%)	
Married	221 (58.6)
Living with partner	45 (11.9)
Other	111 (29.5)
Graduated high school, n (%)	292 (77.5)
Nulligravida, n (%)	189 (50.1)
History of infertility, n (%)	23 (6.1)
Annual income, n (%)	
<$51 000	85 (22.6)
≥$51 000	268 (71.0)
Prefer not to answer	24 (6.4)
Type of cancer, n (%)	
Breast	121 (32.1)
Leukemia or lymphoma	120 (32.0)
Thyroid	1 (0.3)
Cervix, ovary or uterus	23 (6.1)
Bone or soft tissue	20 (5.3)
Intestine	10 (2.7)
Skin	2 (0.1)
Others	80 (21.2)
Cyclophosphamide equivalent dose, mean ± SD	5281.5 ± 6005.7
Treatment gonadotoxicity, n (%)	
Low	110 (29.2)
Moderate	226 (60.0)
High	36 (9.6)
Cancer recurrence, n (%)	27 (7.1)
Stress measures	
PSS-10 score, median (range)	15.0 (0, 36.0)
Cortisol (ng/ml)—Wakening (T1)[a]	5.0 (0.1, 28.3)
Cortisol (ng/ml)—30 min after wakening (T2)[a]	6.3 (0.4, 55.4)
Cortisol (ng/ml)—before sleep (T3)[a]	0.7 (0.1, 14.3)
Ovarian function measures	
AMH[b] (pg/ml), median (range)	1.3 (0, 11.8)
FSH[b] (IU/l), median (range)	7.8 (0, 192.4)
LH[b] (IU/l), median (range)	4.1 (0, 75.3)
Estradiol (pg/ml)[a], median (range)	0.7 (0, 18.8)
Menses past 12 months, n (%)	
10–12	163 (43.2)
1–9	178 (47.2)
0	36 (9.6)

[a]Saliva measurement.
[b]Dried blood spot measurement.
AMH, anti-Mullerian hormone; PSS, Perceived Stress Scale.

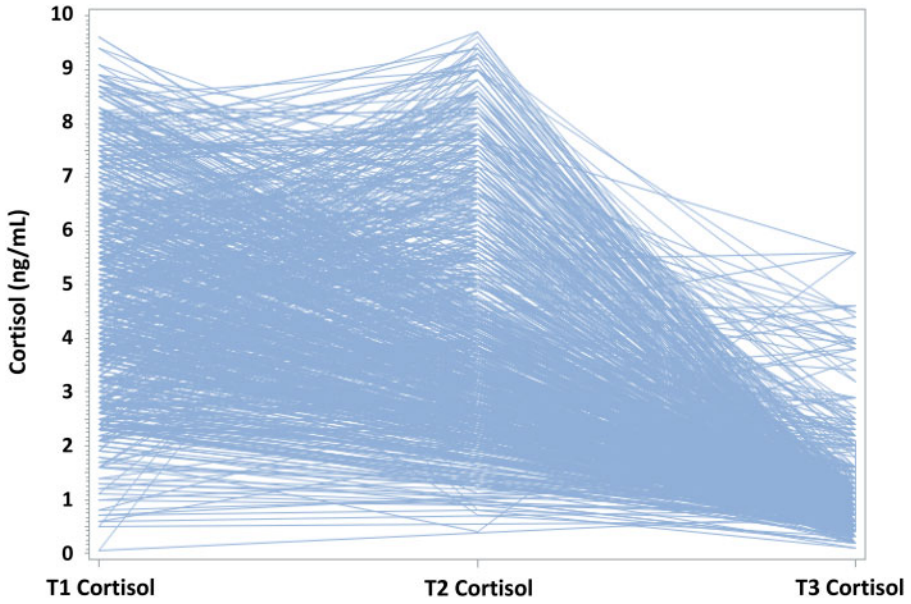

Figure 1. Pattern and distribution of diurnal cortisol levels (cortisol level at the time of awakening (T1 cortisol), 30 min after awakening (T2 cortisol) and before bedtime (T3 cortisol)).

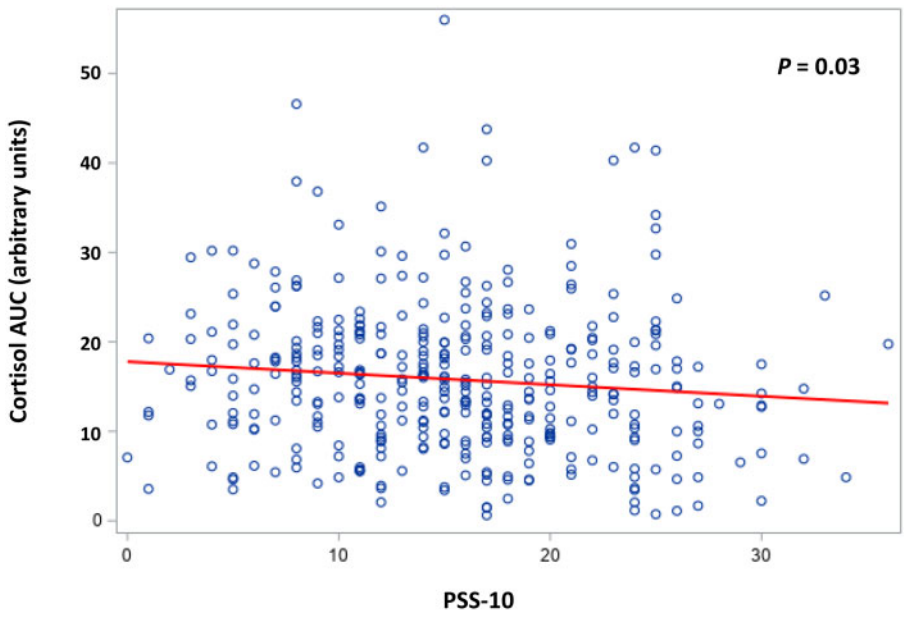

Figure 2. Correlation between diurnal cortisol AUC and Perceived Stress Scale-10 (PSS-10).

experienced menses at least 60 days apart in the prior year. Table I summarizes the distribution of FSH, LH, estradiol and AMH levels.

PSS-10 scores and cortisol AUC were not related to any ovarian function measure, i.e. gonadotropins, estradiol and AMH levels and menstrual pattern (Table II, Figs 3 and 4). When PSS-10 scores and cortisol AUC were compared by menstrual pattern (>9 menses, 1–2 menses or amenorrhea in the prior 12 months), no significant differences were observed (data not shown). Waking cortisol (T1), cortisol

Table II Participant characteristics and ovarian function measures by perceived stress and salivary cortisol quartiles (Q), ordered from lowest to highest perceived stress.

	PSS-10 quartile					Cortisol AUC quartile				
	Q 1 (n = 92)	Q 2 (n = 77)	Q 3 (n = 105)	Q 4 (n = 102)	*P*-value	Q 4 (n = 95)	Q 3 (n = 94)	Q 2 (n = 93)	Q 1 (n = 95)	*P*-value
Median PSS score or cortisol AUC[a] (range)	17.1 (3.5, 46.6)	16.3 (2.1, 105.5)	13.7 (0.6, 56.0)	14.2 (0.7, 41.7)	0.22	17.0 (0, 34.0)	16.0 (1.0, 32.0)	14.0 (1.0, 36.0)	14.0 (3.0, 33.0)	0.03
Age at enrollment, median (range)	34.0 ± 4.5	33.0 ± 4.7	34.0 ± 4.5	34.0 ± 4.6	0.73	33.2 ± 4.6	33.7 ± 4.2	33.5 ± 4.5	33.3 ± 4.9	0.89
Age at cancer diagnosis, median (range)	26.8 ± 6.2	27.1 ± 5.5	26.8 ± 5.6	26.9 ± 5.5	0.99	26.9 ± 5.2	27.2 ± 5.4	26.5 ± 5.7	26.8 ± 6.4	0.84
Years between cancer treatment and enrollment, median (range)	6.9 ± 5.0	5.9 ± 4.4	6.8 ± 4.8	6.7 ± 4.8.	0.54	6.3 ± 4.9	6.5 ± 4.7	7.1 ± 5.0	6.5 ± 4.4	0.72
BMI (kg/m^2), mean ± SD	26.5 ± 6.5	26.6 ± 5.9	27.9 ± 7.2	27.1 ± 7.6	0.51	26.0 ± 7.6	26.5 ± 5.8	28.7 ± 7.0	26.9 ± 6.8	0.05
Having a partner (%)	66 (71.7)	58 (75.3)	73 (69.5)	68 (66.7)	0.72	65 (68.4)	70 (75.3)	68 (72.3)	63 (66.3)	0.54
Graduated high school, n (%)	90 (97.8)	76 (98.7)	103 (98.1)	102 (100)	0.71	95 (100)	92 (98.9)	92 (97.9)	93 (97.9)	0.53
Annual income, ≥$51 000 (%)	77 (83.7)	59 (76.6)	81 (77.1)	74 (72.6)	0.44	77 (81.0)	69 (74.2)	75 (79.8)	71 (74.7)	0.58
Nulligravida, n (%)	44 (47.8)	39 (50.7)	54 (51.4)	50 (49.0)	0.86	46 (48.4)	44 (47.3)	49 (52.1)	49 (51.6)	0.89
History of infertility, n (%)	5 (5.4)	7 (9.1)	6 (5.7)	5 (4.9)	0.81	5 (5.3)	7 (7.5)	8 (8.5)	3 (3.2)	0.42
Type of cancer, n (%)	29 (31.5)	38 (49.4)	29 (27.6)	25 (24.5)	0.01	34 (35.8)	29 (31.2)	26 (27.7)	32 (33.7)	0.23
Breast	32 (34.8)	21 (27.3)	37 (35.2)	29 (28.4)		36 (37.9)	27 (29.0)	34 (36.2)	23 (24.2)	
Leukemia or lymphoma	6 (6.5)	2 (2.6)	4 (3.8)	11 (10.8)		5 (5.3)	1 (1.1)	10 (10.6)	7 (7.4)	
Cervix, ovary or uterus	1 (1.1)	2 (2.6)	2 (1.9)	7 (6.9)		4 (4.2)	4 (4.3)	1 (1.1)	3 (3.2)	
Bone or soft tissue	24 (26.1)	14 (18.2)	33 (31.4)	30 (29.4)		16 (16.8)	32 (34.4)	23 (24.5)	30 (31.6)	
Others										
Cyclophosphamide equivalent dose, mean ± SD	5283.7 ± 5521.5	5547.1 ± 7121.2	5108.6 ± 6130.8	5055.5 ± 5098.2	0.98	4426.2 ± 3021.2	5582.8 ± 6245.4	5496.5 ± 6385.1	5589.4 ± 7366.0	0.81
Treatment gonadotoxicity, n (%)	24 (26.7)	18 (23.4)	35 (34.0)	33 (32.7)	0.10	23 (24.5)	33 (35.9)	23 (24.7)	31 (33.3)	0.70
Low	31 (34.4)	14 (18.2)	31 (30.1)	25 (24.8)		34 (36.2)	22 (23.9)	31 (33.3)	15 (16.1)	
Moderate	35 (38.9)	45 (58.4)	37 (35.9)	43 (42.6)		37 (39.4)	37 (40.2)	39 (41.9)	47 (50.5)	
High										
Recurrence, n (%)	7 (7.6)	4 (5.2)	11 (10.5)	5 (4.9)	0.55	4 (4.2)	6 (6.5)	9 (9.6)	8 (8.4)	0.50

Increased PSS-10 scores correlated with lower cortisol AUC. N = 377.

[a]Arbitrary unit.

PSS, Perceived Stress Scale.

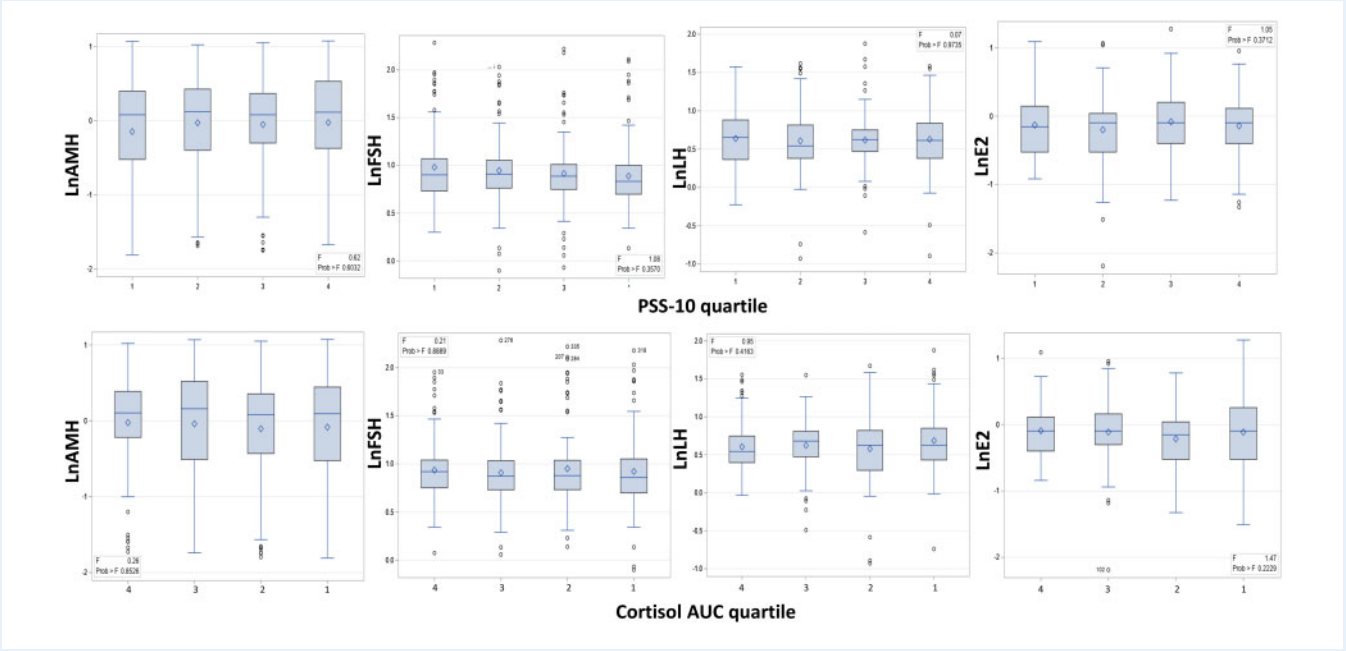

Figure 3. Box and whiskers plots of natural log-transformed (Ln) anti-Mullerian hormone, FSH, LH, and estradiol levels by cortisol AUC and PSS-10 quartiles. Increased PSS-10 scores are correlated with lower cortisol AUC. PSS-10, Perceived Stress Scale-10.

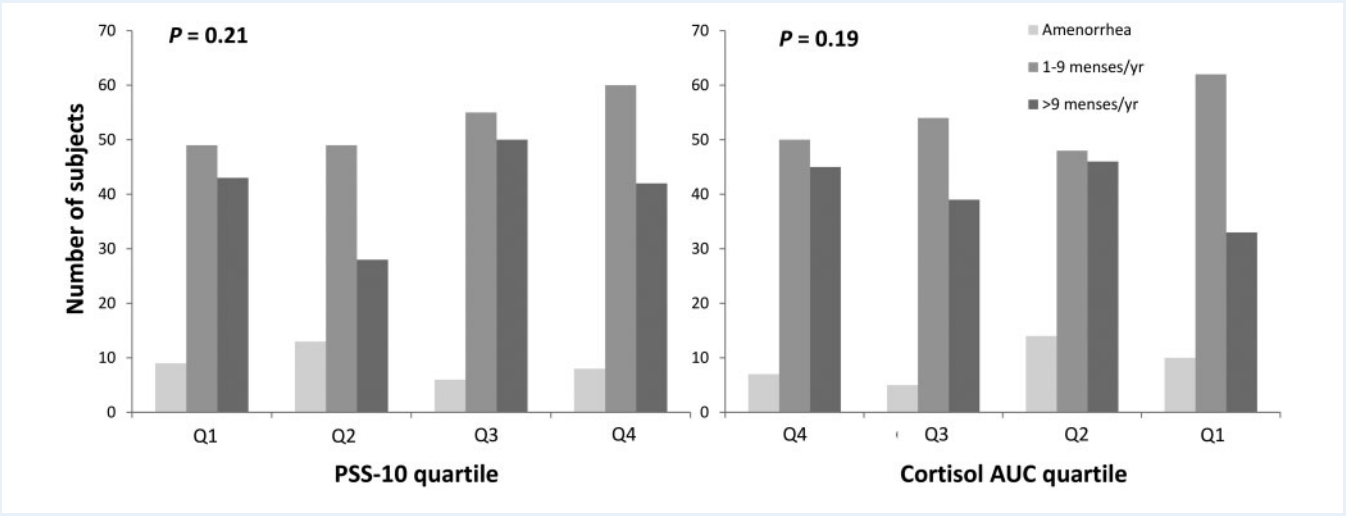

Figure 4. Bar graphs of participants' menstrual pattern by PSS-10 (left) and cortisol (right) quartiles. PSS-10, Perceived Stress Scale-10.

before bed (T3) and the cortisol awakening response also were not related to ovarian function measures (data not shown).

We undertook several sensitivity analyses. First, we restricted to 363 participants with low salivary estradiol level ≤ 1 pg/ml to limit the impact of negative feedback on gonadotropin levels and saw similar findings as in the overall cohort (data not shown). Stratified by gonadotoxicity of cancer treatment (low, moderate and high), there remained no significant association between PSS-10, cortisol AUC and ovarian function outcomes (data not shown). As recent gonadotoxic treatment may induce transient hypergonadotropic hypogonadism, we restricted analyses to 311 participants who were >2 years post-treatment, and there remained no significant association between stress and ovarian function outcomes (data not shown).

Discussion

We investigated if psychosocial stress as measured by self-reported perceived stress and salivary cortisol is associated with suppressed ovarian function in female AYA cancer survivors. Measuring psychosocial stress by self-report and saliva cortisol collected three times in 1 day, we observed that perceived stress and saliva cortisol level are strongly correlated. However, no associations were found between stress and ovarian function measures.

The lack of relationship between psychosocial stress and ovarian function in our study was opposite of our hypothesis. *In-vitro* cell line and *in-vivo* animal studies suggest that corticosteroids and psychological stress not only suppress the HPO axis centrally through the hypothalamus and pituitary (Whirledge and Cidlowski, 2013), but also directly regulate sex steroid synthesis (Michael *et al.*, 1993). The prospective BioCycle cohort study of 259 premenopausal women in New York with no known reproductive disorders showed that daily perceived stress was associated with lower estradiol, luteal progesterone and LH levels, higher FSH levels and anovulation (Schliep *et al.*, 2015). A secondary analysis of the Effects of Aspirin in Gestation and Reproduction (EAGeR) trial, a multicenter trial on the effect of low-dose aspirin on reproductive outcomes in women with 1–2 pregnancy losses, revealed that the women in the highest quartiles of daily preconception perceived stress and urinary cortisol had lower urinary estrone, higher urinary pregnanediol-3-glucuronide and marginally higher risk of anovulation compared to women in lower quartiles (Schliep *et al.*, 2019). In a study of infertile women, chronic life time psychosocial stress rather than 'current' stress was significantly associated with a diagnosis of decreased ovarian reserve (FSH $>$ 10IU/l or poor response to ovarian stimulation) (Pal *et al.*, 2010). To our knowledge, only one study in cancer survivors has been reported. In a cross-sectional study of 24 female childhood cancer survivors, Hardy *et al.* observed that 42%, 39%, 33% and 0%, respectively, of the variation in FSH, LH, AMH and estradiol levels were explained by diurnal cortisol (acute stress), hair cortisol (chronic stress) and perceived stress scale (chronic stress) (Hardy *et al.*, 2019). Hence, we expected to observe lower gonadotropins and estradiol and more evidence of oligo- or anovulation with perceived stress or increased cortisol.

There may be several explanations for why we show disparate results with prior studies. First, psychosocial stress may not significantly modulate sex steroid synthesis or HPO function. Second, psychosocial stress is difficult to measure, with significant variation among studies and no gold standard. Both the BioCycle and EAGeR studies assessed daily stress, which would be more sensitive in capturing variations, in contrast to our PSS-10 measure of stress over the prior month, i.e. more chronic (Cohen *et al.*, 1983). In addition, the proportion of our participants with severe stress was small, similar to studies of populations without cancer, which limited power (Prior *et al.*, 2016). In healthy adults, the median or mean scores of PSS-10 were mostly between 8 and 20, similar to the scores of our AYA survivors (median 15.0) (Prior *et al.*, 2018). Considering that the median years between cancer diagnosis and study enrollment is 6 years, our finding suggests that chronic stress was not high and did not affect ovarian function in AYA survivors. However, we cannot exclude the possibility that acute stress, measured daily, would adversely impact ovarian function. We also speculate that in ovaries that have undergone exposure to a variety of cancer treatments, response to psychosocial stress might be blunted.

Saliva cortisol level has been used to measure both acute and chronic stress (Hannibal and Bishop, 2014; Glenk *et al.*, 2020) and can be conveniently sampled. As part of normal HPA axis function, cortisol levels follow a predictable diurnal pattern, high at awakening, increasing within the first hour after awakening and declining throughout the day to nadir before bedtime (Adam *et al.*, 2017). Leveraging this pattern as well as the variability in levels in a given day, studies have shown that larger cortisol awakening response or an attenuated diurnal slope of cortisol have been related to psychosocial stress (Schlotz *et al.*, 2004; Adam *et al.*, 2006). Cortisol AUC is also a widely used measure of psychosocial stress (Khoury *et al.*, 2015). For our primary analysis, we used salivary cortisol AUC for two reasons: (i) the AUC would account for both the cortisol awakening response and the diurnal slope and (ii) the AUC was significantly correlated with the PSS-10 score. Beyond the Hardy study (Hardy *et al.*, 2019), we do not have a similar population with which to compare our negative results of cortisol levels on ovarian function, motivating the need for future studies.

Our study has several strengths. First, it is well-designed cross-sectional study. DBS collections were timed to the early follicular phase (cycle Days 3–7) for menstruating individuals, and on a random day for amenorrheic individuals, excluding individuals on treatments that would impact gonadotropins, estradiol and menstrual pattern. Detailed instruction was provided via telephone or video calls with study staff during first DBS and saliva collection for quality control. Second, the sample size enabled further subset analysis. Third, reliable information on cancer diagnosis and treatment was collected allowing subset analysis in survivors who did not receive gonadotoxic treatment.

Few limitations warrant discussion. Although this is the largest study so far investigating the relationship between stress and fertility in AYA survivors, our data are still limited by its cross-sectional nature. Also, the questionnaire data were collected via self-report, which may be subject to recall bias. As we observed no association between our exposure and outcomes of interest, we did not further evaluate for effect modification or mediation. Finally, because of the heterogeneity of cancer diagnosis and treatments in our subjects and low prevalence of severe stress, larger future studies are warranted to validate our findings with more power.

In conclusion, we found no association between psychological stress and ovarian function measures in AYA cancer survivors. This important finding can be used to counsel AYA cancer survivors on their reproductive health.

Data availability

The data underlying this article will be shared on reasonable request to the corresponding author.

Authors' roles

B.W.W., A.D., K.S., S.A.D.R., L.N. and H.I.S. formulated the overarching research goals and aims. B.W.W., D.Z., P.M.S., A.D., K.S., S.A.D.R., L.N. and H.I.S. developed the methodology and conducted the research. J.K., B.W.W., B.K. and L.N. performed statistical analysis, and all authors interpret the results and joined in the writing of the article.

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Conflict of interest

Dr A.D. works for Bluebird Bio, Inc., Dr. D.Z. works for ZRT Labs and Dr. P.M.S. works for Ansh Labs, which did not sponsor, support or have oversight of this research. Other authors report no competing interests.

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