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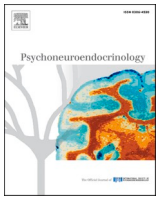

The role of subjective distress in contextualizing testosterone-cortisol coupling

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ABSTRACT

The psychological conditions under which hormonal response profiles predict later distress remain unclear. The present study tested whether subjective distress during a biological stressor moderates the association between testosterone-cortisol coupling and responses to subsequent psychosocial stress. Participants completed a 35% CO_2 Challenge and, a few days later, the Trier Social Stress Test (TSST). Analyses included 115 males and 49 females with hormonal and subjective distress data collected during the stress protocols. Hormonal and subjective responses were quantified using area under the curve with respect to ground (AUC_G), and separate regression analyses were conducted for males and females. Significant interactions between CO_2 Challenge-evoked testosterone, cortisol, and subjective distress emerged for TSST stress in males and females, and TSST fear in males. In males, the dual-hormone hypothesis pattern of higher testosterone coupled with lower cortisol during the CO_2 Challenge predicted higher TSST stress and fear when CO_2 Challenge distress was average or higher. These findings suggest the emotional significance of testosterone-cortisol coupling may depend, in part, on whether the initial stressor is experienced as distressing.

1. Introduction

Individual differences in stress responses shape emotional and physiological functioning, and influence vulnerability to psychopathology. Variability in biological stress responses, particularly activity of the hypothalamic-pituitary-adrenal axis, has been linked to risk for stress-related disorders (Donner and Lowry, 2013; Kessler et al., 1995; Radley et al., 2015). However, biological responses alone do not fully explain variability in stress reactivity. Increasing evidence suggests stress responses reflect coordinated activity across multiple physiological systems (McEwen, 2007; Ulrich-Lai and Herman, 2009) as well as the psychological meaning individuals assign to stressors (Lazarus and Folkman, 1984). In this sense, stress responding reflects an interaction between biological activation and cognitive-emotional processes, such that perceived threat can shape whether physiological responses are experienced as emotional distress (Lazarus and Folkman, 1984). Despite growing recognition that biological activation and psychological appraisal jointly shape stress responding, the conditions under which hormonal response profiles become emotionally consequential remain unclear. Subjective distress during an initial stressor may provide context for interpreting whether hormonal response predicts later emotional responding. In particular, it is not yet known whether distress experienced during a stressor alters the extent to which hormonal coupling predicts distress during a subsequent stressor.

A central feature of the stress response is crosstalk between endocrine axes, particularly the hypothalamic-pituitary-adrenal (HPA) and hypothalamic-pituitary-gonadal (HPG) axes. Cortisol, the primary product of the HPA axis, has been extensively studied and is a key component of the stress response system (McEwen, 2007; Ulrich-Lai and Herman, 2009). Testosterone, a key product of the HPG axis, is relevant in both males and females, with sex-specific differences in its relative biological centrality. Testosterone has received comparatively less attention in stress research despite evidence linking it to affective functioning, stress perception, acute stress responding, and bidirectional

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regulation between the HPA and HPG axes (Domes et al., 2024; Ilkevič et al., 2025; Goldstat et al., 2003; Ngun et al., 2011; Viau, 2002). The term "coupling" describes coordinated activity between these axes (Shirtcliff et al., 2015) and highlights the value of examining hormone interactions rather than individual hormones. In studies of human stress reactivity, this coordinated HPA-HPG activity can be operationalized as testosterone-cortisol coupling. From this perspective, consideration of the joint release of testosterone and cortisol may provide more information about downstream emotional responding than either hormone considered in isolation.

Consistent with this approach, the dual-hormone hypothesis provides an influential framework for interpreting a pattern of testosterone-cortisol coupling. The dual-hormone hypothesis was first proposed in relation to status-relevant behavior, positing testosterone is most strongly associated with dominance when cortisol is low (Mehta and Josephs, 2010). Although the hypothesis has received strong empirical support (Glenn et al., 2011; Tackett et al., 2014; Zilioli et al., 2015), effects vary across contexts, and not all studies observe the dual-hormone hypothesis pattern or find effects in the predicted direction (Dekkers et al., 2019; Donovan and Corpuz, 2025; Rosenfield et al., 2022; Shirtcliff et al., 2015). At the same time, the dual-hormone hypothesis has been applied beyond dominance- and status-relevant outcomes to risk-relevant behavioral, affective, and stress-related outcomes, including aggression, psychopathy, empathy-related outcomes, anxiety symptoms, and subjective distress (Chronister et al., 2021; Glenn et al., 2011; McAfee et al., 2025; Tackett et al., 2014; Zilioli et al., 2015). Consistent with this broader literature (Chronister et al., 2021; McAfee et al., 2025; Tackett et al., 2014; Zilioli et al., 2015), we use the term "dual-hormone hypothesis" to refer to the high testosterone by low cortisol interaction pattern emphasized in this work, rather than as a direct test of dominance or status-relevant behavior. One possible explanation for variability across this literature is that the emotional significance of testosterone-cortisol coupling may depend on the psychological context in which the hormonal response occurs, particularly the degree to which the stressor is experienced as threatening.

Appraisal models of stress indicate individuals exposed to the same standardized event can differ substantially in threat perception, with higher perceived threat associated with stronger cortisol responding under otherwise comparable stressor conditions (Dickerson and Kemeny, 2004; Gaab et al., 2005; Tomaka et al., 1997). From this perspective, testosterone-cortisol coupling may reflect a form of biological sensitivity whose emotional implications are most likely to emerge when the stressor is subjectively experienced as distressing. This interpretation is consistent with broader context-sensitive approaches to testosterone-cortisol coupling, including work suggesting hormonal profiles may have different implications depending on the psychological and situational contexts in which they occur (Josephs et al., 2017; Knight et al., 2022). In this framework, subjective distress is not simply another correlate of stress responding, but an index of the psychological context in which hormonal coupling may become predictive of later emotional outcomes.

The 35% CO_2 Challenge (CO_2 Challenge) provides a useful paradigm for examining whether subjective experience during stress exposure alters the meaning of hormonal responding. This laboratory stressor induces transient respiratory acidosis via inhalation of a 35% CO_2 65% O_2 medical-grade gas mixture and reliably produces interoceptive sensations resembling acute panic symptoms, including breathlessness, racing heart, and trembling (Brown et al., 2003; Schmidt and Zvolensky, 2007; Ziemann et al., 2009). The CO_2 Challenge has also been shown to elicit fear and panic in individuals with bilateral amygdala lesions who otherwise report not experiencing fear, supporting the view that the challenge initially operates through mechanisms distinct from conditioned fears, including social-evaluative threat (Feinstein et al., 2013). However, the subjective response to the challenge is not purely biological: individuals vary in the degree to which CO_2-evoked sensations are perceived as threatening, distressing, or aversive, making the CO_2 Challenge an index of interoceptive threat sensitivity and anxiety propensity (Brown et al., 2003; Schmidt et al., 1994; Telch et al., 2010, 2011; Zvolensky and Eifert, 2001). Thus, the CO_2 Challenge is particularly useful as it exposes participants to a standardized biological stressor while allowing meaningful individual differences in subjective distress and perceived threat to be assessed.

Prior work has demonstrated hormonal responses to the CO_2 Challenge, including testosterone-cortisol coupling, predict subsequent subjective distress during the Trier Social Stress Test (TSST; McAfee et al., 2025). Specifically, McAfee et al. (2025) found CO_2 Challenge-evoked testosterone-cortisol coupling predicted later TSST-evoked subjective distress in males, such that the dual-hormone hypothesis pattern of increasing testosterone in the context of decreasing cortisol was associated with greater subsequent subjective distress. This finding established hormonal coupling during a biological stressor can forecast emotional reactivity during a later psychosocial stressor and provided the direct empirical basis for this study. However, the psychological conditions under which this predictive relationship is most likely to emerge remain unclear. The present study addresses this question by testing whether distress experienced during the CO_2 Challenge alters the predictive value of testosterone-cortisol coupling for later TSST distress. This distinction is theoretically important because it shifts the question from whether hormone coupling predicts later distress to when such coupling becomes emotionally meaningful.

To address this question, the present study employed two laboratory stress paradigms with distinct mechanisms: the CO_2 Challenge, which primarily induces stress via interoceptive and chemosensory processes, and the TSST, which elicits stress through social-evaluative threat (Feinstein et al., 2013; Kirschbaum et al., 1993). The CO_2 Challenge is a biologically driven stressor characterized by intense interoceptive sensations, whereas the TSST is a psychosocial stressor involving social-evaluative performance demands, including an impromptu speech and mental arithmetic task, and is widely regarded as a gold-standard paradigm for eliciting robust cortisol and subjective distress responses (Dickerson and Kemeny, 2004; Kirschbaum et al., 1993). Examining these paradigms across separate laboratory sessions allowed assessment of whether hormonal and subjective responses elicited during a biological stressor predicted emotional reactivity during a later psychosocial stressor. In this way, the design tests whether the joint significance of hormonal coupling and subjective distress generalizes across stressors with different proximal eliciting mechanisms.

Hormonal and subjective responses were quantified using area under the curve with respect to ground (AUC_G; Pruessner et al., 2003). AUC_G was selected because it incorporates both baseline levels and cumulative activation across the stressor period, thereby capturing the overall magnitude of the stress response rather than change from baseline alone. This approach was well suited to the present aims since baseline levels may constrain the extent of further stressor-related change, meaning similar increases over time may reflect different underlying stress responses depending on participants' baseline levels. Thus, AUC_G provided an index of the full baseline-inclusive pattern of stress responding to the CO_2 Challenge, rather than stressor-related change alone, which was important for the present study because both baseline state and stressor-evoked activation can contribute to the predictive significance of hormonal coupling.

To account for individual differences in threat sensitivity, dispositional factors associated with threat perception were also considered. Anxiety sensitivity, assessed with the Anxiety Sensitivity Index−3 (ASI−3), reflects the tendency to interpret physiological sensations as dangerous and has been linked to heightened reactivity to interoceptive stressors such as the CO_2 Challenge (Maller and Reiss, 1992; Schmidt and Richey, 2008; Taylor et al., 2007). More broadly, anxiety sensitivity has been shown to moderate the subjective experience of stress, supporting its inclusion as a dispositional covariate relevant to stress appraisal (Wearne et al., 2019). Sensitivity to social-evaluative threat, assessed with the Appraisal of Social Concerns (ASC), captures

individual differences in responses to socially evaluative contexts and is particularly relevant for TSST responding (Petrowski et al., 2021; Telch et al., 2004). Including these measures allowed evaluation of whether the predictive utility of testosterone-cortisol coupling and subjective distress extended beyond established dispositional vulnerability factors. Importantly, ASI−3 and ASC index relatively stable tendencies related to threat sensitivity, whereas CO_2 Challenge-evoked subjective distress reflects the participant's in-the-moment experience of the experimental stressor. Given well-documented biological sex differences in hormonal functioning, particularly testosterone concentrations, and evidence of heteroscedasticity in the present stress outcomes, males and females were analyzed separately. This approach was especially relevant because the present analyses used AUC_G indices, which incorporate baseline hormone levels and therefore preserve large biological sex differences in testosterone concentrations (Arnold et al., 2017; Chafkin et al., 2022; Handelsman et al., 2018).

The primary aim of the present study was to test whether subjective distress during a biological stressor moderates the association between testosterone-cortisol coupling and later psychosocial distress. Building on McAfee et al. (2025), which found increasing testosterone in the context of decreasing cortisol during the CO_2 Challenge predicted greater later TSST distress in males, we hypothesized higher testosterone coupled with lower cortisol during the CO_2 Challenge would predict higher TSST-evoked subjective distress primarily among participants reporting higher CO_2 Challenge-evoked distress. This hypothesis was tested after accounting for dispositional threat sensitivity indexed by the ASI−3 and ASC. By testing whether distress during the initial stressor alters the predictive significance of testosterone-cortisol coupling, the present study extends prior work and supports a framework in which hormonal response profiles are interpreted as potential context-sensitive indicators of stress sensitivity.

2. Methods

2.1. Participants

Participants were recruited from an introductory psychology participant pool at the University of Texas at Austin for a two-day laboratory study (IRB Protocol #2017–11–0031). Participants completed two laboratory sessions scheduled as close together as feasible (approximately 1 – 6 day interval). The initial dataset included 206 participants (63 females, 143 males) who completed both laboratory sessions. All sessions occurred between 1200 and 1800 hr to minimize diurnal variation in salivary hormone concentrations. Female participants were scheduled during the mid-luteal phase (approximately 17 – 24 days from the onset of last menstruation), consistent with the protocol described previously (McAfee et al., 2025; see Table 1 for demographics and relevant descriptive statistics).

Analyses were conducted in participants with complete hormone and subjective distress data required to compute AUC_G indices for the present models (males n = 115; females n = 49). A brief overview of eligibility procedures, inclusion and exclusion criteria, and study protocol are provided below and described previously (McAfee et al., 2025).

2.2. Inclusion & exclusion criteria

Participants were excluded if they endorsed smoking cigarettes, using illicit substances, or other medications likely to affect hormone concentrations. Female eligibility criteria additionally required a regular menstrual cycle (27–30 days) during the prior 6 months (which was used to reduce cycle-length variability and improve scheduling accuracy for the mid-luteal testing window), no initiation or discontinuation of hormonal birth control within the last 3 months, and no pregnancy or breastfeeding.

2.3. Measures

2.3.1. Hormone sample collection

Saliva samples (~3 mL) were collected in sterile 4 mL VWR tubes and stored at −20 °C within 10 min of collection. Participants were instructed to avoid alcohol and caffeine the evening before and morning of each lab visit. As noted above, sessions occurred between 1200 and 1800 hr to reduce diurnal variability in hormone levels (Dickerson and Kemeny, 2004; Hansen et al., 2008). Samples were shipped to Dresden LabService GmbH and assayed using LC-MS/MS (Chafkin et al., 2022). Assay reliability was acceptable (intra-assay CV = 4.3%; inter-assay CV = 5.1%). Although multiple hormones were assayed, the present analyses focused on cortisol and testosterone (see Table 1 for demographics and relevant descriptive statistics).

2.3.2. Subjective distress and dispositional factors

Subjective distress was assessed using three 100-mm visual analogue scales (VAS) measuring stress, anxiety, and fear, anchored at 0 ("not at all") and 100 ("extreme"), at multiple measurement time points (see Fig. 1). Stress, anxiety, and fear were analyzed as separate outcomes, allowing domain-matched tests across predictors and outcomes. Dispositional factors related to threat perception were also assessed. Anxiety sensitivity was measured using the ASI−3 (18 item; 0 – 4 response scale; Taylor et al., 2007), and sensitivity to social-evaluative threat was measured using the ASC (20 items; 0 – 100 response scale; Telch et al., 2004). Internal consistency in the present analytic sample was excellent for both measures (ASI−3 Cronbach's α =.91; ASC α =.96). ASI−3 and ASC total scores were included as covariates in all models (see Table 1 for demographics and relevant descriptive statistics). These measures were included to account for relatively stable tendencies linked to threat sensitivity, whereas stressor-evoked subjective distress captured participants' acute responses to the laboratory stressors.

2.4. Procedure

2.4.1. Overview

Participants completed online consent, screening questionnaires, and baseline measures before eligibility review and scheduling by a project coordinator. Male participants were scheduled according to availability, whereas female participants were scheduled during the mid-luteal phase (days 17 – 24 following onset of last menstruation), selected a priori to reduce menstrual-cycle-related variability and because cortisol responses to acute stress during the luteal phase are more comparable to males (Wolfram, Bellingrath, and Kudielka, 2011). Menstrual-cycle phase was determined by self-reported date of last menstruation. The laboratory protocol included the CO_2 Challenge on Day 1 and the TSST on Day 2. Each session began with a standardized 30-minute resting period before baseline sampling. Additional procedural details are reported in McAfee et al. (2025).

CO_2 Challenge (Day 1). Participants inhaled a medical-grade gas mixture of 35% CO_2 65% O_2 using a standardized 10-second repeated-breath procedure (McAfee et al., 2025; Zaizar et al., 2018). Repeated-breath procedures have been used in prior CO_2 Challenge studies with 5%, 7%, 20%, and 35% CO_2 concentrations and reduce avoidance during the exposure (Schmidt and Zvolensky, 2007; McAfee et al., 2025; Zaizar et al., 2018). Participants completed one 10-second CO_2 exposure while wearing a mask connected to the gas mixture and were instructed to breathe normally for the duration of the procedure. Saliva was collected twice (baseline and 30 min post-CO_2 Challenge), and VAS ratings were collected three times (baseline, immediately following the CO_2 Challenge, and 30 min post-CO_2 Challenge; see Fig. 1 for relevant study protocol information).

TSST (Day 2). The TSST was implemented in accordance with standard procedures (Kirschbaum et al., 1993). VAS ratings were collected five times: baseline, anticipatory period, immediately following the TSST, 15 min post-TSST, and 30 min post-TSST (see Fig. 1 for relevant

Table 1

Demographics, descriptive statistics, regression output, and simple slopes. Regression models tested whether CO_2 Challenge–evoked testosterone, cortisol, and subjective distress (stress, anxiety, and fear) predicted TSST-evoked subjective distress in males and females in domain-matched models. ASI−3 and ASC were included as covariates but omitted from the table for space and clarity. *p < 0.05, **p < 0.01; p-values < 0.001 reported as such.

Demographics & Other Descriptive Statistics						
Variable Name	**Females**			**Males**		
Demographic Variables	**N**	**Percentage**		**N**	**Percentage**	
Participants	49	100%		115	100%	
(Hormonal Birth Control)	16	33%		-	-	
Caucasian	24	49%		60	52%	
Hispanic	17	35%		24	21%	
Non-Hispanic	32	65%		91	79%	
Asian/Pacific Islander	18	37%		43	37%	
Black, American Indian/Alaska Native, Other	7	14%		12	11%	
Descriptive Statistics	**N**	**Mean (Range)**	**SD**	**N**	**Mean (Range)**	**SD**
Age	49	18.73 (18−23)	1.02	115	19.15 (18−31)	1.59
ASI−3	49	16.02 (1.00–44.00)	10.21	115	16.70 (0.00–57.00)	12.66
ASC	49	34.80 (0.00–89.00)	23.21	115	31.48 (0.00–94.50)	25.16
Stress pre CO_2	49	26.12 (0.00–80.50)	22.90	115	19.18 (0.00–78.00)	19.87
Stress peak CO_2	49	48.23 (0.00–100.00)	27.11	115	43.78 (0.00–100.00)	29.11
Stress post CO_2	49	20.52 (0.00–69.00)	19.86	115	17.21 (0.00–81.00)	19.25
Stress AUC_G CO_2	49	173.90 (0.00–377.14)	100.06	115	153.18 (0.00–379.18)	100.98
Anxiety pre CO_2	49	20.81 (0.00–72.00)	21.92	115	19.38 (0.00–79.50)	19.92
Anxiety peak CO_2	49	49.11 (0.00–100.00)	29.07	115	43.84 (0.00–100.00)	28.75
Anxiety post CO_2	49	20.12 (0.00–74.50)	22.11	115	15.86 (0.00–87.50)	18.99
Anxiety AUC_G CO_2	49	173.34 (0.00–372.68)	108.18	115	150.51 (0.00–410.21)	101.34
Fear pre CO_2	49	7.32 (0.00–60.50)	12.13	115	6.80 (0.00–68.00)	12.84
Fear peak CO_2	49	34.76 (0.00–100.00)	30.54	115	32.95 (0.00–100.00)	28.28
Fear post CO_2	49	7.14 (0.00–66.50)	11.76	115	7.59 (0.00–62.50)	12.92
Fear AUC_G CO_2	49	104.81 (0.00–335.36)	87.99	115	101.07 (0.00–342.32)	88.99
Stress pre TSST	49	16.31 (0.00–78.50)	18.63	115	14.20 (0.00–74.50)	17.56
Stress anticipatory TSST	49	45.36 (0.00–98.50)	25.57	115	29.43 (0.00–91.00)	24.02
Stress completion TSST	49	45.17 (0.00–100.00)	30.73	115	32.80 (0.00–100.00)	28.18
Stress 15-post TSST	49	21.39 (0.00–84.00)	21.32	115	16.51 (0.00–67.50)	18.23
Descriptive Statistics	**N**	**Mean (Range)**	**SD**	**N**	**Mean (Range)**	**SD**
Stress 30-post TSST	49	14.14 (0.00–78.50)	19.04	115	11.62 (0.00–60.50)	14.63
Stress AUC_GTSST	49	155.43 (4.68–404.52)	97.43	115	123.89 (0.00–388.45)	91.05
Anxiety pre TSST	49	13.88 (0.00–61.50)	16.93	115	11.43 (0.00–78.00)	14.94
Anxiety anticipatory TSST	49	51.44 (0.00–100.00)	29.09	115	40.47 (0.00–100.00)	26.15
Anxiety completion TSST	49	46.05 (0.00–100.00)	33.00	115	30.90 (0.00–100.00)	29.44
Anxiety15-post TSST	49	20.70 (0.00–88.00)	23.50	115	15.52 (0.00–71.50)	18.93
Anxiety30-post TSST	49	15.23 (0.00–80.50)	22.40	115	9.99 (0.00–64.50)	13.95
Anxiety AUC_G TSST	49	161.79 (5.79–412.84)	108.38	115	118.64 (0.68–362.89)	92.23
Fear pre TSST	49	3.64 (0.00–36.60)	6.47	115	5.47 (0.00–67.50)	10.38
Fear anticipatory TSST	49	34.74 (0.00–100.00)	31.08	115	25.72 (0.00–96.00)	26.75
Fear completion TSST	49	23.90 (0.00–99.50)	30.03	115	14.28 (0.00–100.00)	22.53
Fear 15-post TSST	49	8.76 (0.00–59.50)	13.55	115	6.83 (0.00–80.50)	13.82
Fear 30-post TSST	49	4.61 (0.00–47.50)	9.63	115	4.70 (0.00–65.00)	10.06
Fear AUC_G TSST	49	86.05 (0.30–352.52)	85.31	115	62.49 (0.00 – 360.52)	76.65
C (nmol/L) pre CO_2	49	2.48 (0.11–13.13)	2.50	115	2.64 (0.11–12.36)	2.29
C (nmol/L) post CO_2	49	2.22 (0.27–9.62)	2.16	115	1.89 (0.16–8.11)	1.46
Cortisol AUC_G CO_2	49	11.74 (2.00–56.88)	10.74	115	11.32 (1.57–48.71)	8.48
T (pg/mL) pre CO_2	49	7.23 (1.24–40.92)	8.49	115	59.50 (6.84–179.15)	32.32
T (pg/mL) post CO_2	49	13.35 (1.13–105.56)	22.81	115	56.15 (1.89–170.49)	32.43
Testosterone AUC_G CO_2	49	51.46 (8.06–266.99)	64.73	115	289.13 (45.46–874.10)	149.37

Regressions: Males	**SE**	**df**	**t**	**p**	**R^2**
AUC_G CO_2 TxCxS ⇒ AUC_G TSST S	0.01	105	−2.37	0.020*	0.03
AUC_G CO_2 TxCxA ⇒ AUC_G TSST A	0.14	105	−1.16	0.250	0.01
AUC_G CO_2 TxCxF ⇒ AUC_G TSST F	0.12	105	−3.00	0.003**	0.04

Simple Slopes	**- ß 1**	**-p 1**	**ß 1**	**p 1**	**+ß 1**	**+p 1**	**- ß 2**	**- p 2**	**ß 2**	**p 2**	**+ß 2**	**+p 2**	**- ß 3**	**- p 3**	**ß 3**	**p 3**	**+ß 3**	**+p 3**
CO_2 TxCxS ⇒ TSST S	−1.94	0.68	−0.69	0.86	0.55	0.93	6.12	0.05	0.84	0.76	−4.45	0.31	14.18	< 0.001**	2.37	0.53	−9.45	0.12
CO_2 TxCxA ⇒ TSST A	3.70	0.39	3.19	0.41	2.69	0.69	9.13	< 0.001**	5.05	0.07	0.96	0.82	14.57	< 0.001**	6.90	0.08	−0.77	0.90
CO_2 TxCxF ⇒ TSST F	−0.03	0.99	1.49	0.63	3.02	0.54	8.91	< 0.001**	3.06	0.17	−2.79	0.43	17.85	< 0.001**	4.62	0.16	−8.61	0.14

Regressions: Females	**SE**	**df**	**t**	**p**	**R^2**
AUC_G CO_2 TxCxS ⇒ AUC_G TSST S	0.02	38	−2.17	0.037*	0.07
AUC_G CO_2 TxCxA ⇒ AUC_G TSST A	0.02	38	−0.62	0.541	0.01
AUC_G CO_2 TxCxF ⇒ AUC_G TSST F	0.03	38	0.78	0.442	0.01

Simple Slopes	**- ß 1**	**-p 1**	**ß 1**	**p 1**	**+ß 1**	**+p 1**	**- ß 2**	**- p 2**	**ß 2**	**p 2**	**+ß 2**	**+p 2**	**- ß 3**	**- p 3**	**ß 3**	**p 3**	**+ß 3**	**+p 3**
CO_2TxCxS ⇒ TSST S	−1.64	0.83	9.88	0.10	21.40	0.06	2.04	0.66	1.99	0.58	1.94	0.77	5.71	0.53	−5.91	0.29	−17.53	0.02*
CO_2TxCxA ⇒ TSST A	0.31	0.96	2.65	0.64	4.99	0.68	0.19	0.97	−0.55	0.88	−1.29	0.84	0.06	0.99	−3.76	0.50	−7.57	0.29
CO_2TxCxF ⇒ TSST F	1.26	0.83	−3.04	0.43	−7.34	0.40	3.88	0.36	4.10	0.15	4.32	0.35	6.50	0.44	11.24	0.01*	15.98	0.06

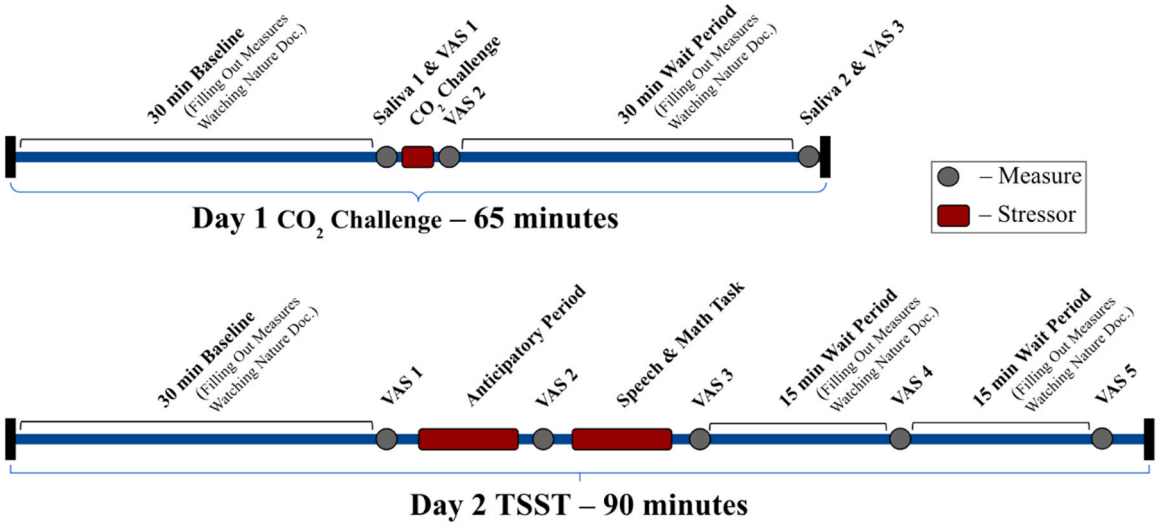

Fig. 1. CO_2 Challenge and TSST Protocol Timeline (adapted from McAfee et al., 2025). The figure illustrates the timing of saliva sample collection and subjective distress ratings during the CO_2 Challenge (Day 1) and TSST (Day 2). Saliva samples were collected at baseline and 30 min post-CO_2 Challenge, and subjective distress ratings were collected at three time points during the CO_2 Challenge and five time points during the TSST, as shown. These repeated measures were used to compute AUC_G indices.

study protocol information).

This sampling structure provided the repeated hormone and subjective distress measurements required to compute AUC_G indices for the CO_2 Challenge predictors and TSST subjective distress outcomes examined in the present study.

2.5. Data analysis plan

Statistical analyses were conducted in R version 4.0.3 (R Core Team, 2020). Given concerns regarding heteroscedasticity, Levene's tests were used to evaluate the main outcome variables. An a priori power analysis indicated 63 participants were required to detect a medium effect size with.80 power (Faul et al., 2009). Levene's tests and Fisher's combined p-value across key TSST subjective distress outcomes supported the presence of heteroscedasticity across males and females, further supporting sex-stratified analyses. Because hormone distributions were right-skewed, testosterone and cortisol were log-transformed prior to regression analyses.

Hormonal and subjective distress indices were computed using area under the curve with respect to ground (AUC_G; Pruessner et al., 2003). AUC_G values were calculated for Day 1 CO_2 Challenge testosterone, cortisol, and subjective distress domains (stress, anxiety, and fear), and for Day 2 TSST subjective distress outcomes in the corresponding domains.

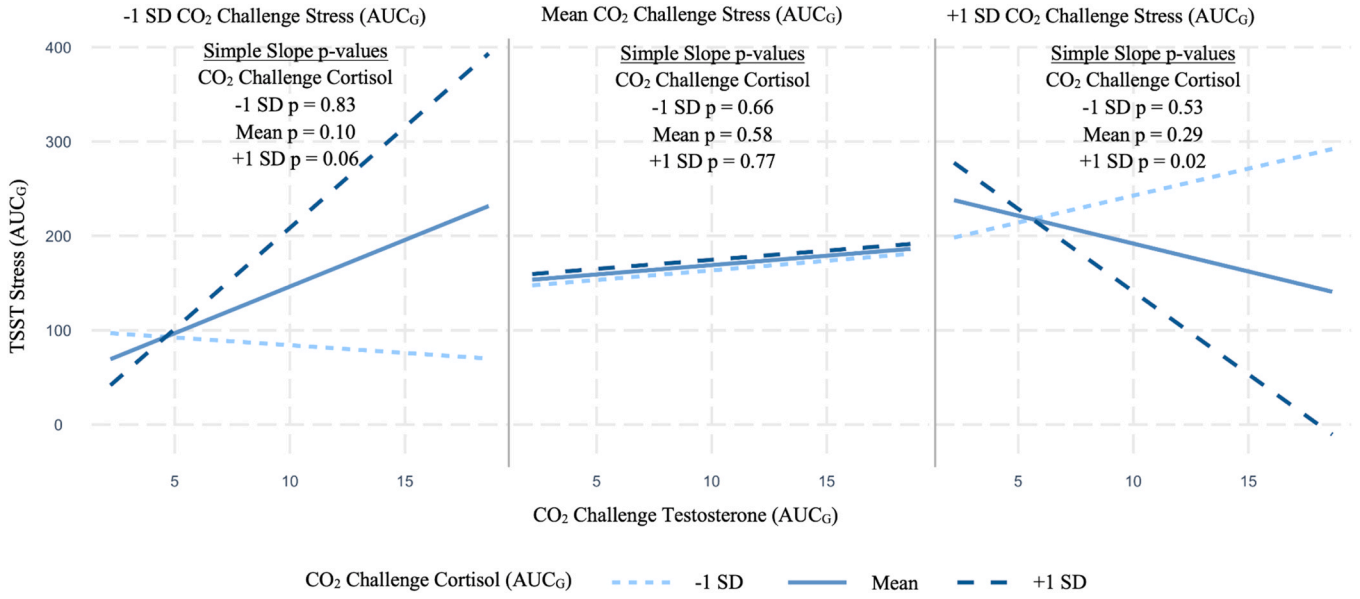

Fig. 2. CO_2 Challenge Testosterone by Cortisol by Subjective Stress Predicting TSST Subjective Stress in Females. The graph illustrates the CO_2 Challenge-evoked testosterone by cortisol by subjective stress interaction predicting TSST subjective stress while controlling for ASI−3 and ASC covariates. The left, middle, and right panels represent low (−1 SD), mean, and high (+1 SD) levels of CO_2 Challenge subjective stress, respectively. Within each panel, lines show the association between CO_2 Challenge testosterone and TSST subjective stress at low (−1 SD), mean, and high (+1 SD) levels of CO_2 Challenge cortisol; the light dashed line represents −1 SD cortisol, the solid line represents mean cortisol, and the dark dashed line represents + 1 SD cortisol. Although the overall three-way interaction was significant, the simple slopes varied across levels of CO_2 Challenge subjective stress, and the pattern should be interpreted cautiously.

For each sex, separate regression models assessed whether Day 1 CO_2 Challenge AUC_G testosterone, cortisol, and domain-matched subjective distress interacted to predict Day 2 TSST AUC_G subjective distress. Domain-matched models were specified a priori such that CO_2 stress predicted TSST stress, CO_2 anxiety predicted TSST anxiety, and CO_2 fear predicted TSST fear. All models included ASI−3 and ASC total scores as covariates. For female models, hormonal birth control status was additionally included as a covariate to account for potential differences in steroid hormone profiles associated with hormonal contraceptive use.

Significant interactions were examined using simple slopes at conventional values (mean and ±1 SD; Aiken and West, 1991), implemented in R with the *interactions* package (Long, 2024). Model assumptions, including residual normality, linearity, and homoscedasticity, were generally acceptable, although residual normality was questionable in some models. Conventional p-values are reported, and findings were further confirmed via bootstrap resampling with 10,000 iterations. Estradiol and progesterone were not included in the present models; therefore, interpretation of the female analyses should consider estradiol- and progesterone-related differences not captured by hormonal birth control status.

3. Results

3.1. Overview

The present analyses included participants with complete hormone and subjective distress data required for the mixed hormone-subjective distress models, yielding an analytic sample of 115 males and 49 females. See Table 1 for demographics and relevant descriptive statistics. Complete regression output for all interaction models is reported in Table 1. Full regression output, including main effects, lower-order interactions, covariates, and model fit statistics, is provided in the Supplemental Materials. Results are presented separately for males and females within each subjective distress domain (stress, anxiety, fear).

3.2. CO_2 challenge testosterone by cortisol by subjective stress predicting TSST stress

For subjective stress reported in response to the TSST, the CO_2 Challenge-evoked testosterone by cortisol by subjective stress interaction was significant in females ($t(38) = -2.17$, $p = 0.037$, $R^2 = 0.07$) and males ($t(105) = -2.37$, $p = 0.020$, $R^2 = 0.03$).

In females, simple-slopes analyses revealed a somewhat variable pattern across the three panels of Fig. 2. For all interaction plots, panels represent −1 SD, mean, and + 1 SD levels of CO_2 Challenge subjective distress, and lines within each panel represent −1 SD, mean, and + 1 SD levels of CO_2 Challenge cortisol, with the solid line representing mean cortisol. In the middle and right panels, the light dashed blue lines slope upward. Although these simple slopes were not statistically significant ($\beta = 2.04$, $p = 0.66$, and $\beta = 5.71$, $p = 0.53$, respectively), the pattern of the slopes suggested that among participants with mean (middle panel) or higher (right panel) CO_2 Challenge subjective stress, higher testosterone in the context of lower cortisol was associated with higher TSST subjective stress. In the right panel, the dark blue line slopes downward. This pattern indicates that among participants with higher CO_2 Challenge subjective stress, higher testosterone in the context of higher cortisol was associated with lower TSST subjective stress ($\beta = -17.53$, $p = 0.02$). In contrast, in the left panel, the dark blue line slopes upward. Although this slope did not reach conventional significance ($\beta = 21.40$, $p = 0.06$), the pattern indicates that among participants with lower CO_2 Challenge subjective stress, higher testosterone in the context of higher cortisol was associated with higher TSST subjective stress. Although the overall pattern of the effect was consistent with our hypothesis, the female simple-slopes results are more variable and should be interpreted with caution (see Fig. 2 for depiction of this interaction).

Simple-slopes analyses in males revealed a pattern consistent with the dual-hormone hypothesis (panels of Fig. 3). In the middle and right panels, the light dashed blue lines slope upward. This pattern indicates that among participants with mean (middle panel) or higher (right panel) CO_2 Challenge subjective stress, higher testosterone in the context of lower cortisol was associated with higher TSST subjective stress ($\beta = 6.12$, $p = 0.05$, and $\beta = 14.18$, $p < 0.001$, respectively). In

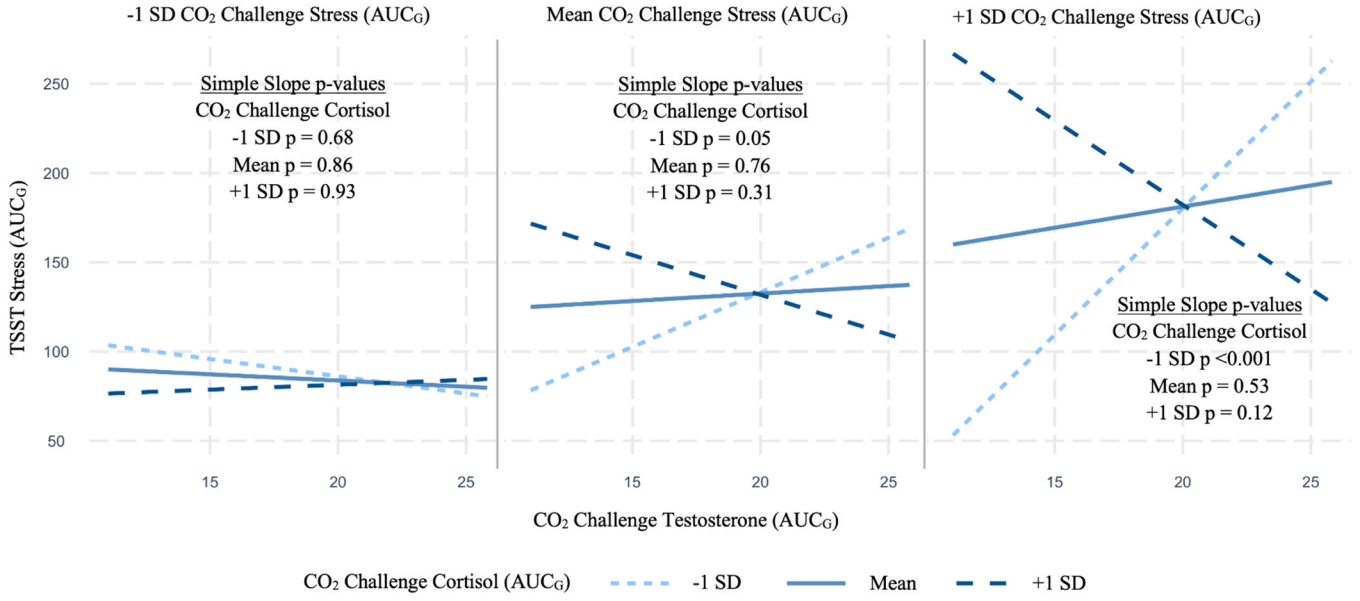

Fig. 3. CO_2 Challenge Testosterone by Cortisol by Subjective Stress Predicting TSST Subjective Stress in Males. The graph illustrates the predictive utility of the CO_2 Challenge-evoked testosterone by cortisol by subjective stress interaction for TSST subjective stress while controlling for ASI−3 and ASC covariates. The left, middle, and right panels represent low (−1 SD), mean, and high (+1 SD) levels of CO_2 Challenge subjective stress, respectively. Within each panel, lines show the association between CO_2 Challenge testosterone and TSST subjective stress at low (−1 SD), mean, and high (+1 SD) levels of CO_2 Challenge cortisol; the light dashed line represents −1 SD cortisol, the solid line represents mean cortisol, and the dark dashed line represents + 1 SD cortisol. Males who had mean or higher subjective stress to the CO_2 Challenge and had higher testosterone and −1 SD cortisol to the CO_2 Challenge experienced higher stress to the TSST.

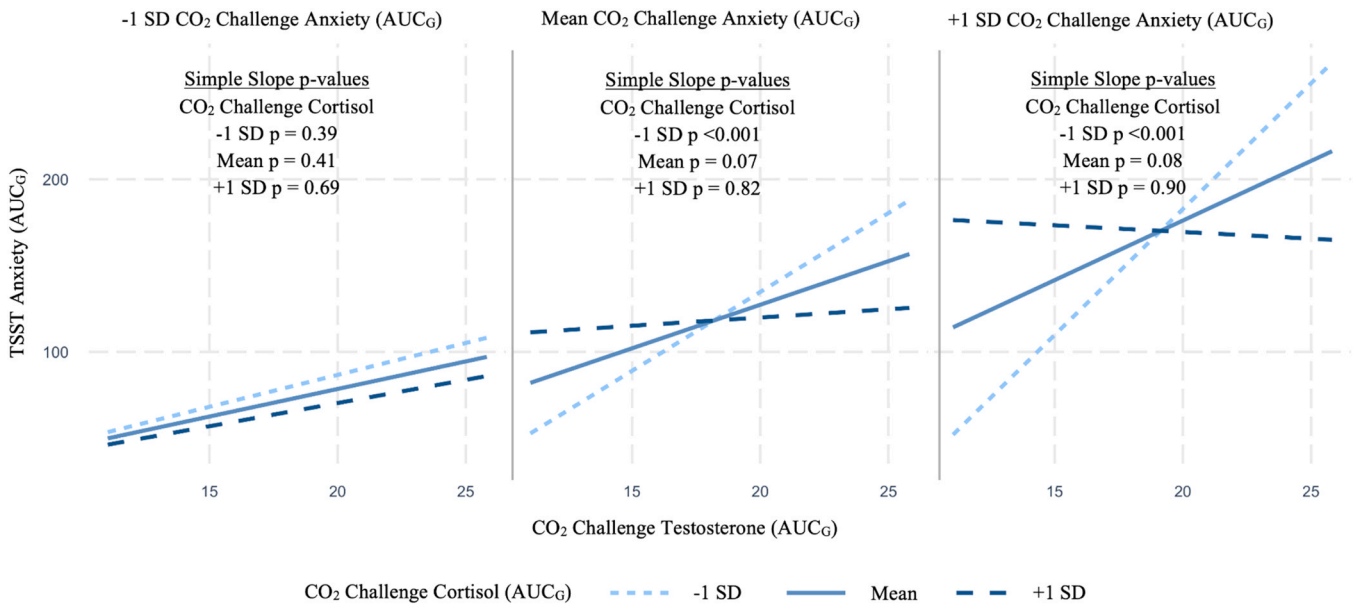

Fig. 4. CO_2 Challenge Testosterone by Cortisol by Subjective Anxiety Predicting TSST Subjective Anxiety in Males. The graph illustrates the CO_2 Challenge-evoked testosterone by cortisol by subjective anxiety interaction predicting TSST subjective anxiety while controlling for ASI−3 and ASC covariates. The left, middle, and right panels represent low (−1 SD), mean, and high (+1 SD) levels of CO_2 Challenge subjective anxiety respectively. Within each panel, lines show the association between CO_2 Challenge testosterone and TSST subjective anxiety at low (−1 SD), mean, and high (+1 SD) levels of CO_2 Challenge cortisol; the light dashed line represents −1 SD cortisol, the solid line represents mean cortisol, and the dark dashed line represents + 1 SD cortisol. The overall three-way interaction was not significant, but the plotted simple slopes show a descriptive pattern in the hypothesized direction.

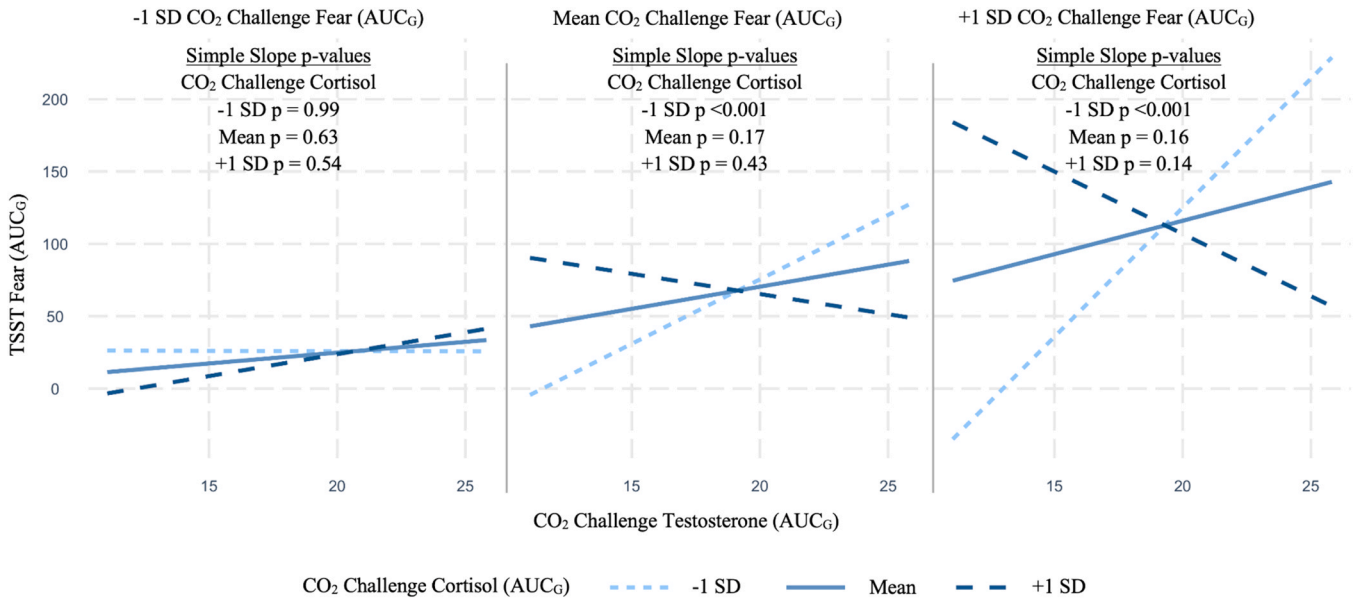

Fig. 5. CO_2 Challenge Testosterone by Cortisol by Subjective Fear Predicting TSST Subjective Fear in Males. The graph illustrates the predictive utility of the CO_2 Challenge-evoked testosterone by cortisol by subjective fear interaction for TSST subjective fear while controlling for ASI−3 and ASC covariates. The left, middle, and right panels represent low (−1 SD), mean, and high (+1 SD) levels of CO_2 Challenge subjective fear, respectively. Within each panel, lines show the association between CO_2 Challenge testosterone and TSST subjective fear at low (−1 SD), mean, and high (+1 SD) levels of CO_2 Challenge cortisol; the light dashed line represents −1 SD cortisol, the solid line represents mean cortisol, and the dark dashed line represents + 1 SD cortisol. Males who had mean or higher subjective fear to the CO_2 Challenge and had higher testosterone and −1 SD cortisol to the CO_2 Challenge experienced higher fear to the TSST.

these same panels, the dark blue lines slope downward. Although these simple slopes were not statistically significant ($\beta = -4.45$, $p = 0.31$, and $\beta = -9.45$, $p = 0.12$, respectively), the pattern of the slopes suggested that among participants with mean or higher CO_2 Challenge subjective stress, higher testosterone in the context of higher cortisol was associated with lower TSST subjective stress. Thus, consistent with our hypothesis, the dual-hormone hypothesis pattern of higher testosterone and lower cortisol predicted higher TSST subjective stress primarily among males reporting average or higher subjective stress during the CO_2 Challenge (see Fig. 3 for depiction of this interaction).

3.3. CO_2 challenge testosterone by cortisol by subjective anxiety predicting TSST anxiety

For subjective anxiety reported in response to the TSST, the CO_2 Challenge-evoked testosterone by cortisol by subjective anxiety interaction was not significant in females ($t(38) = -0.62$, $p = 0.54$) or in males ($t(105) = -1.16$, $p = 0.250$).

Because the three-way interaction was not significant, these simple-slopes results should be interpreted cautiously and are presented as descriptive rather than confirmatory. Within this context, simple-slopes analyses among males revealed a consistent pattern across the panels of Fig. 4 in the hypothesized direction. In the middle and right panels, the light dashed blue lines slope upward. This pattern indicates that among participants with mean (middle panel) or higher (right panel) CO_2 Challenge subjective anxiety, higher testosterone in the context of lower cortisol was associated with higher TSST subjective anxiety ($\beta = 9.13$, $p < 0.001$, and $\beta = 14.57$, $p < 0.001$, respectively; see Fig. 4 for depiction of this interaction).

3.4. CO_2 challenge testosterone by cortisol by subjective fear predicting TSST fear

For subjective fear, the CO_2 Challenge-evoked testosterone by cortisol by subjective fear interaction was not significant in females ($t(38) = 0.78$, $p = 0.442$). In males, the interaction was significant ($t(105) = -3.00$, $p = 0.003$, $R^2 = 0.04$).

Simple-slopes analyses among males revealed a consistent pattern across the panels of Fig. 5 in line with the dual-hormone hypothesis. In the middle and right panels, the light dashed blue lines slope upward. This pattern indicates that among participants with mean (middle panel) or higher (right panel) CO_2 Challenge subjective fear, higher testosterone in the context of lower cortisol was associated with higher TSST subjective fear ($\beta = 8.91$, $p < 0.001$, and $\beta = 17.85$, $p < 0.001$, respectively). In these same panels, the dark blue lines slope downward. This pattern indicates that among participants with mean or higher CO_2 Challenge subjective fear, higher testosterone in the context of higher cortisol was associated with lower TSST subjective fear, though these slopes were not significant ($\beta = -2.79$, $p = 0.43$, and $\beta = -8.61$, $p = 0.14$, respectively). Consistent with expectation and the pattern observed for subjective stress, the dual-hormone hypothesis pattern of higher testosterone and lower cortisol predicting higher TSST subjective fear was primarily observed among males reporting average or higher subjective fear during the CO_2 Challenge (see Fig. 5 for depiction of this interaction).

3.5. Summary of interaction pattern

Across outcomes, the hypothesized three-way interaction pattern was most clearly evident for TSST-related subjective stress and fear in males. In males, the dual-hormone hypothesis pattern of higher testosterone and lower cortisol predicted higher TSST subjective stress and fear primarily when corresponding CO_2 Challenge subjective stress and fear were at the mean or higher. In females, the three-way interaction significantly predicted TSST subjective stress; however, the simple-slopes pattern was more variable and should be interpreted with caution given the smaller, underpowered female analytic sample, despite being broadly consistent with the hypothesized direction. For TSST subjective anxiety, the overall three-way interaction was not significant in either sex, though the male simple slopes were descriptively in the hypothesized direction.

4. Discussion

The present study investigated whether subjective distress during a biological stressor moderates the association between testosterone-cortisol coupling and subsequent psychosocial stress reactivity. Consistent with the dual-hormone hypothesis pattern, significant three-way interactions between CO_2 Challenge-evoked testosterone, cortisol, and subjective distress emerged for subjective stress and fear in males. Specifically, the dual-hormone hypothesis pattern of higher testosterone and lower cortisol predicting later TSST subjective distress was observed only among males reporting average or higher levels of CO_2 Challenge-evoked distress. These findings extend prior stress-related work on the dual-hormone hypothesis pattern of testosterone-cortisol coupling (McAfee et al., 2025) by identifying subjective distress during the CO_2 Challenge as a psychological context in which this coupling pattern becomes predictive of later emotional responding. To our knowledge, this is the first study to examine the dual-hormone hypothesis pattern in relation to the level of subjective distress experienced during a stressor. These findings suggest the association between hormonal coupling and later distress is shaped, in part, by the extent to which an initial stressor is experienced and appraised as threatening or distressing (Lazarus and Folkman, 1984).

The observed interactive effects emerged above and beyond established vulnerability factors, namely anxiety sensitivity and social-evaluative threat, indicating these associations persisted after accounting for dispositional risk factors (Maller and Reiss, 1992; Petrowski et al., 2021; Schmidt and Richey, 2008; Telch et al., 2004). This distinction is important because anxiety sensitivity and social-evaluative threat index relatively stable tendencies linked to threat sensitivity (Maller and Reiss, 1992; Petrowski et al., 2021; Schmidt and Richey, 2008; Telch et al., 2004), whereas CO_2 Challenge-evoked subjective distress captures participants' in-the-moment responses to the laboratory stressor (Schmidt et al., 1994; Telch et al., 2010, 2011; Zvolensky and Eifert, 2001). The use of AUC_G allowed hormonal and subjective responses to be modeled across the full, baseline-inclusive pattern of stress responding, which was important for the present aims because both baseline state and stressor-evoked activation may shape the emotional significance of hormonal coupling (Pruessner et al., 2003). Taken together, these findings suggest the predictive significance of testosterone-cortisol coupling is not reducible to dispositional vulnerability alone and underscore the importance of considering hormonal and subjective responses across the full stress-response profile.

More broadly, these results may help account for some of the variability in the dual-hormone literature (Dekkers et al., 2019; Josephs et al., 2017; Shirtcliff et al., 2015). Although emotional responses to stressors differ from the status-relevant outcomes emphasized in the original dual-hormone hypothesis, as noted in the introduction, the dual-hormone hypothesis pattern has also been examined in broader affective and stress-related outcomes, including empathy-related outcomes, anxiety symptoms, and subjective distress (Chronister et al., 2021; McAfee et al., 2025; Zilioli et al., 2015). If testosterone-cortisol coupling carries different implications depending on how a stressor is experienced, then inconsistent findings across studies may reflect differences in participants' subjective responses to ostensibly similar stressors. From this perspective, the dual-hormone hypothesis pattern may not be uniformly associated with negative emotional or behavioral outcomes across contexts; rather, its implications may depend on the psychological meaning of the stressor and the outcome being examined. In the present study, participants were exposed to the same laboratory challenge, yet the predictive value of testosterone-cortisol coupling varied according to the level of distress reported during the challenge. Accordingly, hormonal responses may be most informative when interpreted within a broader stress-response context rather than as stand-alone indicators of vulnerability to later stress-related distress or psychopathology (Burke et al., 2005; Josephs et al., 2017; Wichmann et al., 2017; Zorn et al., 2017). Together, the present findings extend prior work by suggesting the predictive significance of testosterone-cortisol coupling depends not only on the biological response itself, but also on the subjective meaning of the initial stressor.

Despite these overall patterns, several findings warrant cautious interpretation. Although the hypothesized three-way interaction

predicting TSST subjective anxiety in males was not statistically significant, the observed pattern was directionally consistent with the hypothesis and is therefore worth noting cautiously. In females, the three-way interaction predicted TSST subjective stress and accounted for the largest proportion of variance among the interaction models ($R^2 = 0.07$). This finding is notable because the overall response profile resembled the male pattern, although it was less statistically stable. In addition, several models suggested a broader directional tendency: under conditions of higher CO_2 Challenge distress, slopes associated with higher testosterone coupled with higher cortisol tended to be negative, indicating lower TSST distress. This tendency was evident for TSST stress and fear among males and for TSST stress among females, although not all corresponding simple slopes were statistically significant. These patterns are therefore best interpreted as descriptive rather than confirmatory. Although the present study focused on the dual-hormone hypothesis pattern of high-testosterone by low-cortisol, the descriptive high-testosterone by high-cortisol patterns observed in some models suggest other forms of testosterone-cortisol coupling may also be relevant to stress-related subjective distress and should be examined directly in future work. Other coupling profiles, including low-testosterone by low-cortisol patterns, may also carry distinct implications depending on the stress context and outcome being examined (Josephs et al., 2017). This possibility aligns with broader developmental and human-biological approaches to HPA-HPG axis coordination, which emphasize testosterone-cortisol coupling may vary across contexts rather than reflecting a single uniformly meaningful profile (Black et al., 2022; Glass et al., 2025; Harden et al., 2016; Shirtcliff et al., 2015). It is also consistent with recent work linking testosterone-cortisol interactions to stress perception and context-sensitive responding (Ilkevič et al., 2025; Knight et al., 2022).

One possible mechanism for future work is variation in hormone receptor sensitivity. Altered glucocorticoid receptor sensitivity has been observed in several stress-related conditions, including post-traumatic stress disorder and depression (Fitzgerald et al., 2006; Holsboer, 2000; Labonté et al., 2014; Somvanshi et al., 2020), and testosterone has also been shown to influence glucocorticoid receptor functioning during stress (Rohleder et al., 2002, 2003). Accordingly, heightened subjective distress during the CO_2 Challenge may index individual differences in receptor sensitivity that amplify the emotional significance of hormonal responses (Kern et al., 2014; Rohleder et al., 2003). However, receptor sensitivity was not directly measured in the present study, so this possibility remains speculative and should be regarded as a hypothesis for future research rather than an explanation established by the current data.

4.1. Limitations and future directions

Several limitations should be considered when interpreting these findings. First, although the results suggest subjective distress moderates the association between testosterone-cortisol coupling and later distress responses, the biological mechanisms underlying this interaction remain uncertain. Future studies incorporating a broader range of potential biomarkers (e.g., cytokines) may help clarify the role of receptor sensitivity in these processes (Liu et al., 2017; Rohleder et al., 2003; Wolkow et al., 2015). Second, as in McAfee et al. (2025), the clearest and most robust findings were observed in males. Although the female subjective stress interaction reached conventional significance and accounted for 7% of the variance ($R^2 = 0.07$), the corresponding hypothesized simple slopes were not statistically significant. Because the female analytic sample was smaller and underpowered relative to the male analytic sample, these findings should be interpreted cautiously. This caution is further warranted because, although hormonal birth control status was included as a covariate in female analyses, estradiol and progesterone were not included in the present analyses. Accordingly, the female models did not account for estradiol- and progesterone-related differences among participants, including variability that may remain with mid-luteal scheduling. Therefore, replication in larger samples that directly model estradiol, progesterone, and hormonal contraceptive status will be important for clarifying the female findings. More broadly, the sex-specific pattern observed here highlights the need for additional research on sex differences in hormonal regulation and stress responding (Arnold et al., 2017; Donner and Lowry, 2013; Zorn et al., 2017). Third, the present study represents a theory-driven extension of findings derived from the same broader dataset reported previously in McAfee et al. (2025), rather than an independent replication. Additional studies will be needed to determine whether the present moderation pattern replicates in new samples and across other stress paradigms. Fourth, although the CO_2 Challenge and TSST are well-validated laboratory stressors, they do not fully capture the complexity of stress responses in naturalistic environments or across diverse populations (Kirschbaum et al., 1993; Schmidt and Zvolensky, 2007). The undergraduate psychology sample may further limit generalizability, and the TSST's reliance on social-evaluative performance demands, neutral evaluators, and biological sampling may not translate equally across populations and stress contexts (Dickerson and Kemeny, 2004; Henrich et al., 2010; Kirschbaum et al., 1993).

4.2. Conclusion

The present findings are the first to our knowledge to suggest the emotional significance of hormonal coupling may depend, in part, on whether the stressor is experienced as distressing. Among males in this sample, testosterone-cortisol coupling predicted later distress responses among those reporting elevated distress during the CO_2 Challenge, indicating effects of the dual-hormone hypothesis pattern may emerge primarily under conditions of perceived threat (Dickerson and Kemeny, 2004; Gaab et al., 2005; Mehta and Josephs, 2010; Tomaka et al., 1997). These findings support a more context-sensitive interpretation of the dual-hormone hypothesis pattern, suggesting hormonal coupling may reflect context-sensitive biological responding rather than a uniformly negative or risk-conferring profile (Mehta and Josephs, 2010; Dekkers et al., 2019; Knight et al., 2022). More broadly, the results highlight how biological stress responses and threat perception may interact to shape emotional reactivity across distinct stress experiences (Lazarus and Folkman, 1984).

CRediT authorship contribution statement

McAfee Ciara: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Josephs Robert:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization. **Champagne Frances:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition. **Annabelle DiVita:** Writing – review & editing, Project administration, Investigation, Data curation. **Telch Michael:** Writing – review & editing, Methodology, Conceptualization.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.psyneuen.2026.107954.

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