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The Presence of a Pet Dog Is Associated with a More Balanced Response to a Social Stressor

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Abstract: Acute and chronic stress each have physical manifestations in the human body that can lead to many negative health impacts. Today, reported stress levels worldwide are at an all-time high, spurring the search for non-pharmaceutical interventions to maintain healthy stress levels. In this study, we examined whether a pet dog's presence influences healthy adults' acute stress responses as assessed through self-reports, heart rate, plasma cortisol, and salivary alpha-amylase. Participating pet dog owners were randomly assigned to undergo the Trier Social Stress Test either with their pet dog or alone. While there was no group difference in perceived anxiety levels, participants undergoing the acute psychological stressor with their pet dogs present had significantly lower heart rates, lower plasma cortisol responses, and higher salivary alpha-amylase responses than people without their dogs. Those who participated without their dogs had a statistically flat alpha-amylase response, which is typically associated with extreme or pathological stress. These findings extend the potential effects of pet dogs beyond merely lowering their owner's stress levels to maintaining a healthier, balanced response across the sympathoadrenal medullary axis and hypothalamic–pituitary-adrenal axis.

Keywords: Trier Social Stress Test; pet dog; plasma cortisol; alpha-amylase; heart rate; anxiety

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1. Introduction

In 2022, a Gallup poll found that individuals across 142 countries reported higher rates of negative experiences, such as worry, sadness, and stress, and lower rates of positive experiences, like laughter, enjoyment, and feeling well rested, compared to previous years [1]. These findings align with those from a recent American Psychological Association survey in which adult U.S. citizens reported elevated stress levels compared to pre-COVID-19 pandemic levels, with over one third of participants reporting their stress as completely overwhelming on most days [2]. While it is possible that these shifts are due to the decreased stigmatization of admitting to being stressed, it is clear that stress is a widespread challenge in modern society. A growing body of research is documenting the negative consequences of these higher chronic stress levels, which include higher rates of cancer, autoimmune conditions, diabetes, cardiovascular dysfunction, and mental illnesses [3].

Although less well studied, unhealthy responses to acute stress are also correlated with worsened long-term health outcomes [4]. Exaggerated acute stress reactivity predicts shortened telomere lengths, increased risk factors for cardiovascular disease, and higher all-cause mortality [5,6]. Blunted stress reactivity is also associated with negative health outcomes, including a greater progression of physical disability symptoms and increased

depression and anxiety [7–9]. Overall, long-term wellbeing is associated with a balanced, rather than a hyperreactive or hyporeactive, acute stress response [4].

In response to an acute stressor, two primary physiological systems are activated: the sympathoadrenal medullary (SAM) axis and the hypothalamic–pituitary-adrenal (HPA) axis. The SAM responds immediately by releasing catecholamines (epinephrine and norepinephrine), responsible for elevations in heart rate and blood pressure. Salivary alpha-amylase levels are increased by the activation of the SAM axis [10,11]. After the acute stress resolves, the HPA axis triggers the release of cortisol from the adrenal gland, which will help to return the body to homeostasis [11]. The activation of both the SAM and HPA systems is essential to a healthy, normal stress response. These systems have typically been studied separately, but, when examined in tandem, they often exhibit asymmetrical reactions to acute stressors [12]. While the implications of such asymmetries are not fully understood, they have been associated with a poor mental health status [12]. Thus, it is important to examine indicators of both systems' responses to gather a more comprehensive view of stress reactivity and to understand the mechanisms of potential interventions to support healthy stress responses.

Much of what is known about acute stress responses and associated interventions is derived from experiments using the Trier Social Stress Test (TSST), one of the most well-characterized methods to reproducibly induce acute psychological stress in a laboratory environment [13]. The effects of the social evaluation and unpredictability incorporated into the TSST typically include increases in heart rate and self-reported anxiety and a two- to three-fold increase in cortisol levels in approximately 75% of healthy participants, indicating a strong HPA axis response [13]. The TSST also typically induces increases in alpha-amylase in healthy participants, which is frequently used as an indicator of the SAM axis response [10].

As interest has grown in non-pharmaceutical methods to foster and support healthier stress responses, a growing body of research has found that interventions such as physical activity, time in nature, and mindfulness-based activities may be beneficial [14–16]. Among these alternative approaches, dogs may be an effective intervention to help manage stress reactivity. Several studies using the TSST have found dogs to be more protective against acute stress hyperreactivity than romantic partners, parents, or close friends [9,17–22]. For example, Polheber and Matchcok [22] found that university students undergoing the TSST in the presence of a dog had lower cortisol levels and heart rate variables compared to students who performed the TSST without a dog. However, the anxiety levels remained elevated among both the dog and no dog groups, suggesting that the beneficial effect of a dog might not be subjectively noticeable. Research studies thus far have been mostly limited to examining stress levels using self-reported measures and/or markers of HPA axis activation (e.g., salivary cortisol). It is worth noting that some of the studies investigating the effect of a dog on stress levels have focused on children or young adults and therapy dogs [18,19,21], reducing their relevance to pet dogs in adults' daily lives. With one third of global households and approximately 40% of U.S. households including pet dogs [23,24], a rigorous and multi-system examination of these pets' impacts on stress responses could provide insights into how this common and widely accessible intervention impacts physiological processes that are influential to human health, well-being, and longevity.

Toward understanding the detailed physiology by which pet dogs affect human acute stress responses, we conducted a single-blinded randomized controlled trial in which healthy adults participated in the TSST with or without their pet dog present. We measured stress using self-reports, SAM system markers (heart rate and salivary alpha-amylase), and plasma cortisol as an indicator of the HPA system contribution for each participant before (T0), immediately after (T1), and 45 min after (T2) the stressor to examine the impact of the dogs' presence on both peak stress (T1 versus T0) and stress recovery (T2 versus T1). We hypothesized that subjects who participated with their pets would exhibit a lower stress response and faster stress recovery than those who participated without their dogs.

2. Results

2.1. Cohort Demographics and Baseline Characteristics

The participants' mean trait anxiety score at baseline was 34.0 ± 6.9 (range: 23–56), as assessed by the STAI-AD. Twenty-three participants did not menstruate, 11 were in the follicular phase of their menstrual cycle, and nine were in the luteal phase. There were no significant differences between the experimental and control groups in any of these characteristics or in the baseline self-reported stress, heart rate, plasma cortisol, or salivary alpha-amylase levels (Table 1).

Table 1. Participant characteristics and baseline data. Between-group differences were examined using an independent-samples *t* test (two-tailed) for continuous variables or Fisher's exact test for multinomial variables. *p* values are uncorrected. Data are presented as mean ± standard deviation.

Characteristic	Dog (*n* = 22)	No Dog (*n* = 21)	Significance
Gender	4 (18.2%) Male 18 (81.8%) Female 0 (0%) Non-binary	1 (4.8%) Male 19 (90.5%) Female 1 (4.8%) Non-binary	$\chi^2 = 2.72$, $p = 0.26$
Age (years)	39.0 ± 14.3	35.7 ± 15.1	$t = 0.73$, $p = 0.47$
Race/ethnicity	1 (4.5%) American Indian/Alaska Native 1 (4.5%) Asian 1 (4.5%) Black/African American 1 (4.5%) Hispanic/Latino 18 (81.8%) White/Caucasian	1 (4.8%) Black/African American 2 (9.5%) Hispanic/Latino 18 (85.7%) White/Caucasian	$\chi^2 = 0.08$, $p = 0.78$
Physical activity days/week	3.41 ± 2.02	4.29 ± 2.26	$t = -1.34$, $p = 0.19$
Menstrual cycle phase	13 (59.1%) N/A 4 (18.2%) Follicular 5 (22.7%) Luteal	10 (47.6%) N/A 7 (33.3%) Follicular 4 (19.0%) Luteal	$\chi^2 = 1.51$, $p = 0.47$
STAI-AD trait anxiety	32.9 ± 6.73	34.0 ± 5.63	$t = 0.60$, $p = 0.55$
STAI-AD state anxiety, T0	26.1 ± 4.62	27.6 ± 5.71	$t = -0.97$, $p = 0.34$
Heart rate, T0 (BPM)	75.0 ± 10.1	75.2 ± 7.80	$t = -0.07$, $p = 0.95$
Cortisol, T0 (ng/mL)	73.5 ± 36.1	82.1 ± 38.8	$t = -0.76$, $p = 0.45$
Sal α-amylase, T0 (U/mL)	65.0 ± 71.7	42.5 ± 62.3	$t = -1.0$, $p = 0.31$

Of the twenty-one dogs who participated with their owners, nine (43%) were male and 12 (57%) were female. All but two were spayed or neutered. The dogs' ages ranged from 1 to 13 years old, and thirteen were reported as mixed breeds by their owners. Two dogs were German shepherds, and no other pure breed was reported more than once.

2.2. TSST Response

To determine whether the TSST affected the self-reported anxiety levels, heart rate, plasma cortisol, and salivary alpha-amylase levels, we ran a repeated-measures ANOVA on the aggregated cohort, as well as on each group separately (Table 2).

Table 2. Within-subject ANOVA results with differences between time points and Bonferroni post hoc comparisons. A Greenhouse–Geisser correction was used if Mauchly's test of sphericity was statistically significant. Significant results are bolded for the RM ANOVA and for the post hoc Bonferroni tests.

Measure	Group	Maulchy's Test of Sphericity	Greenhouse–Geisser Correction ε	RM ANOVA Results	Mean Difference (T1–T0) and Post Hoc *p* Value	Mean Difference (T2–T1) and Post Hoc *p* Value	Mean Difference (T0–T2) and Post Hoc *p* Value
STAI-AD state	Aggregate	$\chi^2(2) = 34.8$ $p < 0.001$	0.64	**$F_{1.27,53.45} = 203$ $p < 0.001$ partial $\eta^2 = 0.83$**	**24.7 $p < 0.001$**	**−24.7 $p < 0.001$**	−0.02 $p = 1.0$
	Dog	$\chi^2(2) = 21.7$ $p < 0.001$	0.60	**$F_{1.20,25.27} = 109$ $p < 0.001$ partial $\eta^2 = 0.84$**	**22.6 $p < 0.001$**	**−22.7 $p < 0.001$**	0.14 $p = 1.0$
	No dog	$\chi^2(2) = 13.4$ $p = 0.001$	0.66	**$F_{1.33,26.57} = 98.8$ $p < 0.001$ partial $\eta^2 = 0.83$**	**26.9 $p < 0.001$**	**−26.7 $p < 0.001$**	−0.19 $p = 1.0$
Heart rate (BPM)	Aggregate	$\chi^2(2) = 15.7$ $p < 0.001$	0.76	**$F_{1.52,63.71} = 85.4$ $p < 0.001$ partial $\eta^2 = 0.67$**	**15.0 $p < 0.001$**	**−17.9 $p < 0.001$**	**2.98 $p = 0.01$**
	Dog	$\chi^2(2) = 0.79$ $p = 0.675$	N/A	**$F_{2,42} = 29.6$ $p < 0.001$ partial $\eta^2 = 0.59$**	**10.4 $p < 0.001$**	**−13.2 $p < 0.001$**	2.77 $p = 0.37$
	No dog	$\chi^2(2) = 25.1$ $p < 0.001$	0.58	**$F_{1.15,23.08} = 73.9$ $p < 0.001$ partial $\eta^2 = 0.79$**	**19.7 $p < 0.001$**	**−22.9 $p < 0.001$**	**3.19 $p = 0.005$**
Plasma cortisol (ng/mL)	Aggregate	$\chi^2(2) = 9.70$ $p = 0.008$	0.81	**$F_{1.62,59.86} = 42.1$ $p < 0.001$ partial $\eta^2 = 0.53$**	**29.3 $p < 0.001$**	**−32.1 $p < 0.001$**	2.79 $p = 1.0$
	Dog	$\chi^2(2) = 8.45$ $p = 0.015$	0.72	**$F_{1.44,25.87} = 15.0$ $p < 0.001$ partial $\eta^2 = 0.46$**	**20.4 $p = 0.02$**	**−28.5 $p < 0.001$**	8.12 $p = 0.15$
	No dog	$\chi^2(2) = 1.68$ $p = 0.433$	N/A	**$F_{2,36} = 32.1$ $p < 0.001$ partial $\eta^2 = 0.64$**	**38.2 $p < 0.001$**	**−35.6 $p < 0.001$**	−2.54 $p = 1.0$
Sal α-amylase (U/mL)	Aggregate	$\chi^2(2) = 3.38$ $p = 0.19$	N/A	$F_{2,72} = 2.12$ $p = 0.13$ partial $\eta^2 = 0.06$	17.1 $p = 0.24$	−12.1 $p = 0.32$	−5.03 $p = 1.0$
	Dog	$\chi^2(2) = 1.63$ $p = 0.44$	N/A	**$F_{2,36} = 4.45$ $p = 0.02$ partial $\eta^2 = 0.20$**	**34.6 $p = 0.02$**	−16.7 $p = 0.65$	−17.9 $p = 0.27$
	No dog	$\chi^2(2) = 13.8$ $p = 0.001$	0.63	$F_{1.27,21.56} = 0.29$ $p = 0.65$ partial $\eta^2 = 0.02$	−1.46 $p = 1.0$	−7.13 $p = 0.74$	8.58 $p = 1.0$

2.2.1. STAI-AD State

The self-reported anxiety levels nearly doubled immediately after the TSST (T1), before returning to the baseline levels at T2. The repeated-measures ANOVA analysis revealed a significant main effect of time ($p < 0.001$) among the aggregate, dog, and no dog groups. Post hoc analysis with Bonferroni corrections showed a significant increase in anxiety from baseline (T0) to immediately after the TSST (T1), as well as a significant decrease from T1 to T2 (recovery). This pattern was similar in the aggregate, dog, and no dog groups (see Table 2 and Figure 1A).

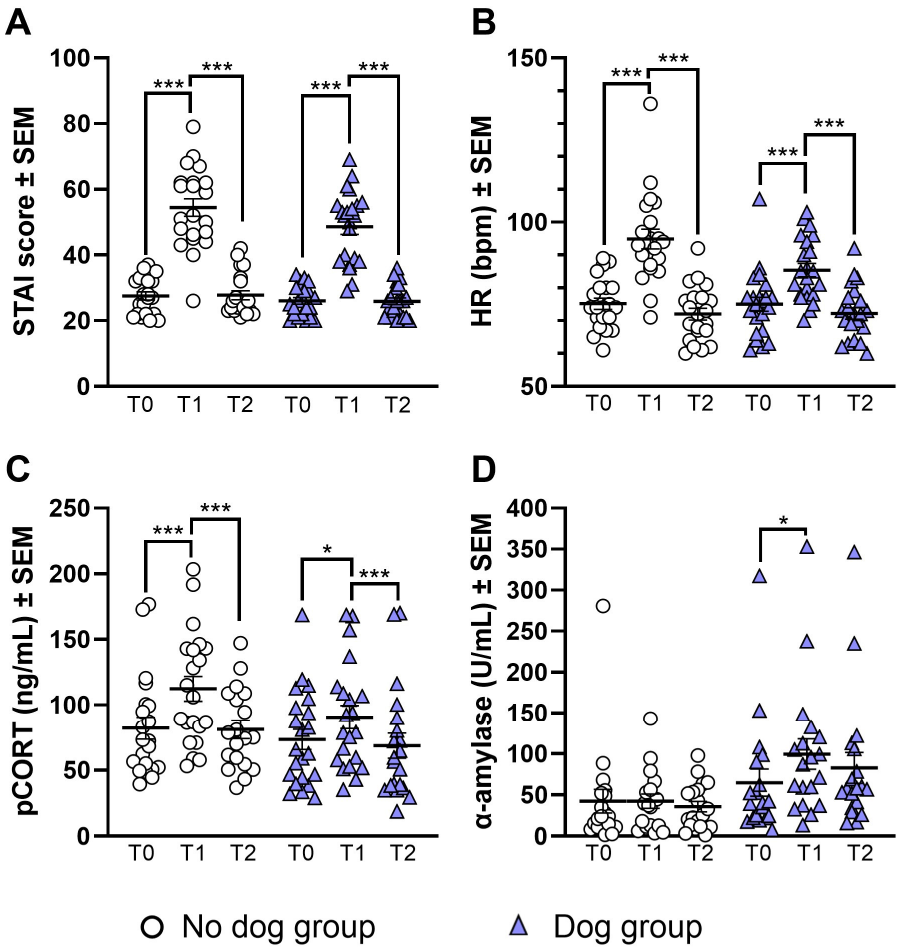

Figure 1. Measures of stress across time in experimental and control groups. (**A**) Anxiety scores (STAI), (**B**) heart rate (beat per minute), (**C**) plasma cortisol levels, and (**D**) salivary alpha-amylase levels. Data are represented as mean ± SEM. (* $p < 0.05$ and *** $p < 0.001$).

2.2.2. Heart Rate

The heart rate increased by 20% between T0 and T1. We found a significant main effect of time ($p < 0.001$) among the aggregate, dog, and no dog groups. Post hoc analysis revealed a significant increase in the heart rate from T0 to T1, followed by a return to baseline levels at T2. This pattern was similar in the aggregate, dog, and no dog groups (Table 2 and Figure 1B).

2.2.3. Plasma Cortisol

We observed an overall 30% increase in cortisol levels immediately after the TSST. The significance of this effect on time was confirmed by the RM ANOVA ($p < 0.001$, Table 2) among the aggregate, dog, and no dog groups. The cortisol levels significantly increased from T0 to T1 and significantly decreased from T1 to T2 (Bonferroni post hoc tests) in the aggregate cohort, as well as in the dog and no dog groups (see Table 2 and Figure 1C).

2.2.4. Salivary Alpha-Amylase

The alpha-amylase levels increased by 53% in the dog group only, and, accordingly, we found a significant main effect of time in the dog group ($p = 0.02$), but not in the aggregate or no dog groups ($p = 0.13$ and $p = 0.70$, respectively; see Table 2). Post hoc analysis confirmed a significant increase in alpha-amylase levels in the dog group from T0 to T1 ($p = 0.02$) but no significant differences between T1 and T2 and T0 and T2 (Table 2 and Figure 1D). The no dog group did not exhibit significant differences between any pair of time points.

Overall, in both the dog and no dog groups, we observed a significant effect of the TSST on the anxiety scores, heart rate, and plasma cortisol levels, with the notable exception of salivary alpha-amylase, for which only the dog group exhibited a significant increase after the TSST. We next examined whether the presence of a pet dog had a significant effect on the parameters that we measured.

2.3. Dog Effect

No demographic covariates had a significant impact on the results. Therefore, only the baseline levels were included as a covariate in the ANCOVA run to evaluate pet dogs' effects.

2.3.1. STAI-AD State

All participants, regardless of the presence of their dogs, exhibited increased anxiety levels immediately after the TSST (Figure 2A). However, there was no statistically significant difference between the dog and no dog groups in the percent change values (Table 3 and Figure 2A), suggesting that the presence of a pet dog did not affect the self-reported anxiety scores.

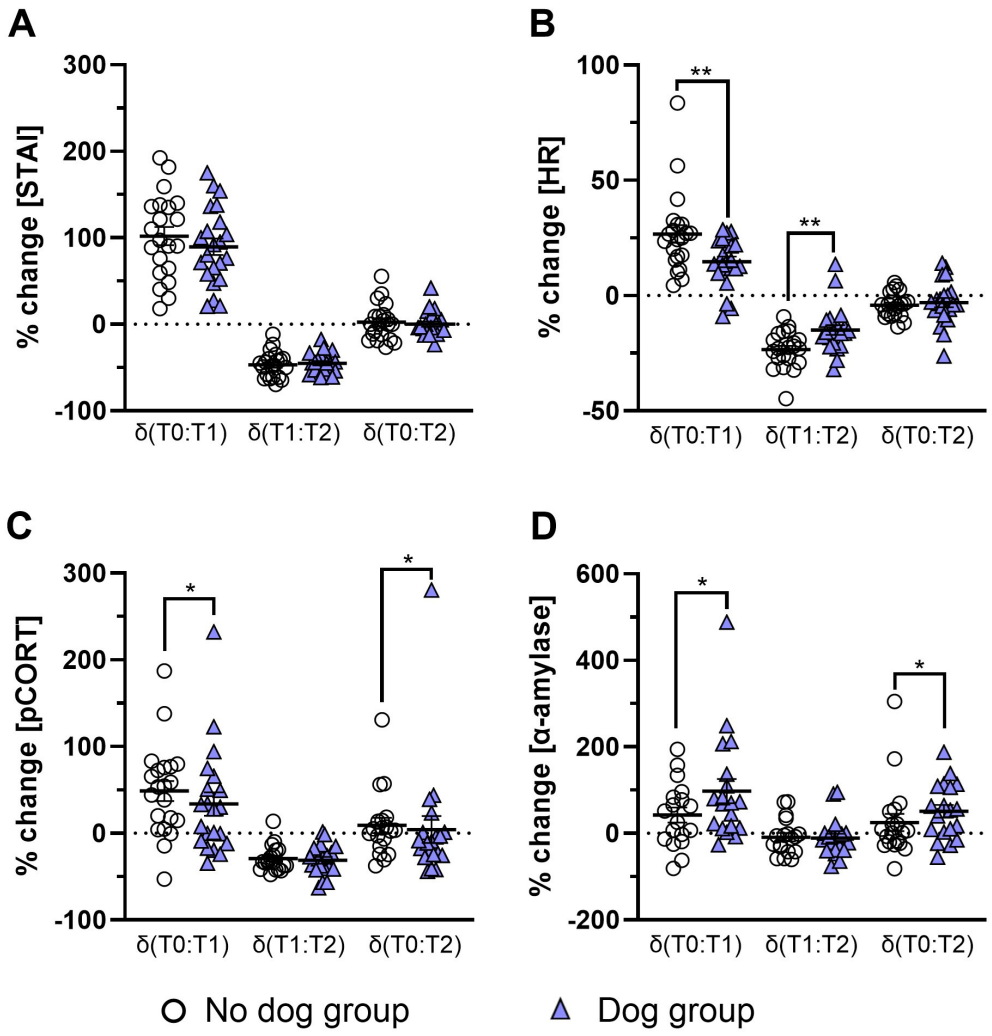

Figure 2. Percentage change in stress response for each pair of time points between groups. (**A**) Percent changes for anxiety scores (STAI), (**B**) percent changes for heart rate, (**C**) percent changes for plasma cortisol levels, and (**D**) percent changes for salivary alpha-amylase levels. Data are represented as mean ± SEM. (* $p < 0.05$ and ** $p < 0.01$).

Table 3. Between-subject comparisons of percent changes between time points. Significant results are bolded. Quade's ANCOVAs are marked with an *. All analyses included the baseline levels as a covariate.

Measure	Time	Unadjusted Mean (±SEM)		Adjusted Mean (±SEM)		ANCOVA Results
		Dog	No Dog	Dog	No Dog	
STAI-state percent change	T0–T1	89.6 ± 9.46	102 ± 10.5	86.8 ± 9.07	105 ± 9.29	$F_{1,40} = 1.94$ $p = 0.17$ partial $\eta^2 = 0.05$
	T1–T2	−45.0 ± 2.52	−46.9 ± 3.21	−44.7 ± 2.86	−47.2 ± 2.93	$F_{1,40} = 0.35$ $p = 0.56$ partial $\eta^2 = 0.01$
	T0–T2 *	0.21 ± 2.99	2.36 ± 4.50	−0.73 ± 3.54	3.34 ± 3.62	$F_{1,41} = 0.47$ $p = 0.50$ partial $\eta^2 = 0.01$
Heart rate percent change	T0–T1 *	14.6 ± 2.28	26.7 ± 3.83	14.6 ± 2.90	26.8 ± 2.96	**$F_{1,41} = 7.84$ $p = 0.008$ partial $\eta^2 = 0.16$**
	T1–T2 *	−14.9 ± 2.11	−23.5 ± 1.73	−14.9 ± 1.93	−23.5 ± 1.97	**$F_{1,41} = 10.66$ $p = 0.002$ partial $\eta^2 = 0.20$**
	T0–T2	−3.07 ± 1.99	−4.22 ± 1.16	−3.10 ± 1.52	−4.19 ± 1.56	$F_{1,40} = {}_0.25$ $p = 0.62$ partial $\eta^2 = 0.006$
Cortisol percent change	T0–T1 *	33.7 ± 13.4	48.7 ± 11.5	31.1 ± 11.2	51.3 ± 11.2	**$F_{1,40} = 4.18$ $p = 0.047$ partial $\eta^2 = 0.09$**
	T1–T2	−31.2 ± 4.03	−29.3 ± 3.38	−30.8 ± 3.64	−29.8 ± 3.64	$F_{1,35} = {}_0.04$ $p = 0.84$ partial $\eta^2 = 0.001$
	T0–T2 *	4.28 ± 15.5	9.16 ± 8.83	2.67 ± 12.5	10.9 ± 12.8	**$F_{1,37} = 4.49$ $p = 0.043$ partial $\eta^2 = 0.11$**
Sal α-amylase percent change	T0–T1 *	97.6 ± 28.1	42.4 ± 17.6	103 ± 22.2	36.3 ± 22.8	**$F_{1,35} = 4.55$ $p = 0.040$ partial $\eta^2 = 0.11$**
	T1–T2 *	−11.0 ± 10.0	−8.72 ± 9.69	−11.4 ± 9.92	−8.21 ± 10.2	$F_{1,35} = 0.08$ $p = 0.78$ partial $\eta^2 = 0.002$
	T0–T2 *	50.8 ± 14.8	25.0 ± 19.7	55.1 ± 16.8	20.8 ± 16.8	**$F_{1,36} = 6.14$ $p = 0.018$ partial $\eta^2 = 0.15$**

2.3.2. Heart Rate

There was a significant between-group difference in heart rate response to the TSST ($p = 0.008$, Table 3). On average, the no dog group exhibited nearly double the heart rate increase compared to the dog group in response to the TSST (19.7 and 10.4 beats per minute (BPM), respectively; Table 2). These increases translated into a significantly higher percent increase in heart rate (26.7%) for those who participated without their dog compared to the dog group (14.6%) immediately after the TSST (Table 3 and Figure 2B), suggesting that the presence of a pet dog contributed to lessening the heart rate increase caused by the TSST. It is worth noting that three participants in the dog group, but none in the no dog group, had a lower heart rate immediately after the TSST compared to the baseline, even though they all self-reported increased anxiety levels. There was also a significant

difference between the groups in the T2 vs. T1 percent changes (p = 0.002), with the no dog group showing a larger decrease as both groups returned to baseline. While there was a slight difference at T0–T2 for the no dog groups' heart rates (p = 0.005, Table 2), both groups returned to comparable baseline levels after 45 min of recovery (Figure 1B), with no significant between-group differences at T2 vs. T0 (p = 0.62).

2.3.3. Plasma Cortisol

We noticed a wider spread of values for the percent changes in plasma cortisol between T0 and T1 (Figure 2C), with 11 individuals exhibiting decreased plasma cortisol levels immediately after the TSST compared to the baseline. Of these 11 individuals, eight were in the dog group and only three in the no dog group. There was a significant between-group difference in the cortisol response to the TSST (p = 0.047), with the no dog group exhibiting a more than 50% higher average cortisol response than the dog group (51.3% vs. 31.1% increase, respectively; adjusted means are reported). Furthermore, the dog group did show significantly lower percent changes (2.67%) than the no dog group (10.9%) between T0 and T2 (p = 0.04, see Table 3 and Figure 2C). These results were not affected by the extremely high value in the no dog group. A total of 17 participants had higher cortisol levels at T2 compared to baseline (T0), with 11 of them belonging to the no dog group.

2.3.4. Salivary Alpha-Amylase

There was a significant group difference in the alpha-amylase response to the TSST (p = 0.04), with the dog group showing an increase of 97.6%, compared to a 42.4% increase for the no dog group. Moreover, we found a significant difference for the T0 vs. T2 percent changes, with the dog group exhibiting a larger percent increase (Figure 2D).

3. Discussion

We successfully elicited an acute stress response in the study participants, as demonstrated by the significant increases in the self-reported state anxiety scores, heart rate, and plasma cortisol levels in the aggregated cohort. Interestingly, we found no significant change in the total cohort when examining the salivary alpha-amylase levels across time, and only the dog group had significantly higher alpha-amylase levels immediately after the TSST. We also found that 45 min was sufficient for recovery in our participants, as demonstrated by the lack of non-negligible differences in any stress markers.

The significant reduction in stress reactivity in the dog group as assessed by the heart rate and plasma cortisol levels, with the self-reported state anxiety levels showing a similar but not significant trend, supports our hypothesis that the presence of a pet dog could elicit a lower stress response. Further, the presence of a pet dog was associated with lower T2 vs. T0 cortisol levels, providing some support for the hypothesized faster stress recovery response. The difference in findings between self-reported and physiologically assessed stress levels has been previously reported in the literature in about half of the studies examining the impact of canine-assisted interventions on stress [25]. Furthermore, significant correlations between cortisol or heart rate reactivity and perceived stress have only been found in about 25% of TSST studies [26]. These differences could be due to factors including cognitive coping strategies, varying speeds of reactivity across stress response types, or social desirability bias impacting survey responses [26].

In contrast, the presence of a dog was associated with a significant increase in salivary alpha-amylase levels following the TSST, while the no dog group showed a statistically flat, or blunted, alpha-amylase response. However, it remains unclear whether a heightened increase in salivary alpha-amylase is healthier than a blunted response. This is further shown in a 2020 meta-analysis of the health effects of acute stress reactivity, which found negative health effects associated with both exaggerated and blunted SAM axis responses, which are often assessed through salivary alpha-amylase levels [4]. Blunted responses were associated with a greater illness frequency, higher body mass index (BMI) and waist circumference, poorer self-reported health, greater progression of disability, more depression

and anxiety symptoms, and poorer cognitive functioning [4]. Similarly, while the current literature is still inconclusive and predominantly focused on children and adolescents, several studies have found that high HPA–low SAM stress responders—most similar to the no dog group in our study—exhibited more emotional and behavioral problems than those with the types of symmetric responses that we observed in the dog group [27–29].

3.1. Limitations

The timing of this study—during the COVID-19 pandemic, in which many adults were experiencing social isolation and poor mental health [30,31]—may explain the blunted alpha-amylase response in the control group, which does not align with most literature on TSST stress responses among healthy adults [32,33]. Typically, the blunted alpha-amylase response that we observed is associated with psychological disorders including psychopathy [34], borderline personality disorder [35], anorexia nervosa [36], and post-traumatic stress disorder (PTSD) [37], and it has also been observed among chronically stressed individuals [38]. However, because we did not ask the participants about life stressors or the impact of COVID-19 on their social interactions, we can only speculate about the implications of these findings.

In addition, while we controlled for several key factors relevant to TSST responses, we did not control for night shift workers, pregnant or breastfeeding individuals, or BMI, which may impact the TSST results [39]. We also did not ask menstruating participants about the standard length of their cycles, and so the categorization of their cycle phase was only an approximation. Our participants were also overwhelmingly White and female, and so our findings may not be generalizable to more diverse groups. Further, while the participants were pre-screened to ensure that they were comfortable having their blood drawn multiple times over the course of the experiment, it is possible that stress related to blood draws may have impacted the overall results. In addition, there is a risk that, because we ran multiple tests on the same sample, there may be an elevation in the alpha error.

3.2. Future Directions

Due to the complexity of the stress response systems, it would be valuable to replicate this study with a greater range of stress markers, ideally with a more diverse participant pool and a sample size large enough to enable sub-group analyses. Specifically, investigating the stress responses of males, young adults, and older adults would add to our current findings for a mostly middle-aged female participant pool. It could also be valuable to compare the stress responses between participants with their pet dogs and those with another dog (e.g., a therapy dog) to help determine to what extent the relationship with the dog impacts stress mitigation. While a crossover design is inappropriate to use with the TSST due to the habituation of the stress response over repeated exposure, it might also be worthwhile to conduct a crossover trial with a different stressor to further explore the impacts of dogs on the stress response [13].

4. Materials and Methods

4.1. Participants

This study was approved by the University of Denver's Institutional Review Board (IRB protocol number 1664556) and Institutional Biosafety Committee (IBC protocol 1773388), and all methods were performed in accordance with their guidelines and regulations. Because the participating dogs were not owned by the university, Institutional Animal Care and Use Committee (IACUC) approval was not required. Each dog's legal owner provided permission for their participation in the study. This study is reported in accordance with the ARRIVE guidelines.

All participants provided written informed consent and were recruited through local therapy dog and dog training organizations, as well as through the campus community. All participants were required to be healthy and at least 18 years old. Exclusion criteria included (1) taking anti-coagulant medication, (2) being a current smoker, or (3) taking

psychotropic medication. Exclusion criterion (1) was in place for participant safety during the multiple blood draws. Exclusion criteria (2) and (3) were in place to limit external factors that are known to impact TSST results [39]. Participants were also asked to respond to a questionnaire to assess their dogs' suitability for the study conditions (for example, whether the dog would be comfortable taking an elevator to reach the floor where the study took place and whether he or she was likely to remain calm while in the presence of strangers). Potential participants who were unable to confidently answer any of the questions spoke with the study facilitator to obtain further clarity and mutually determine whether their dog was likely to become excessively distressed during the experiment. Participants understood that they would be undergoing a stressful situation to investigate whether the presence of their dog impacted their stress response. However, they did not know the details of what they would experience.

Out of the one hundred and seven participants who were initially screened, 53 were deemed eligible for participation in the study. Blood collection was not possible for seven participants due to fainting or the absence of suitable antecubital veins. One person withdrew from the study during the TSST. Two other participants were removed from the analysis—one reported knowing one of their panelists and one reported an extremely stressful event just prior to participation to the study. Forty-three participants (37 women, five men, and one non-binary individual) were included in the study, which allowed for two groups whose sizes aligned with other studies of the TSST and dogs that have found significant results [18,19,21,40,41]. The participants' mean age was 37.4 $\pm$ 14.6 years (range: 22–75), and they reported a mean of 3.9 $\pm$ 2.2 physically active days in the past week.

4.2. Procedure

Data collection took place between September 2021 and February 2022. Eligible participants were stratified by sex and randomized by coin flip to participate in the experiment with (n = 28) or without (n = 27) their pet dogs. The sample size was determined by an a priori calculation with data from a meta-analysis of cortisol responses among 186 studies that used the TSST [42]. This meta-analysis found an average effect size of d = 0.925 (SEM = 0.043). Assuming a 2-sided t-test at α = 0.05, a sample size of 40 was predicted to provide 80% power to detect a difference in change between the cohorts. Participants were informed of their group assignment ahead of the TSST and asked to leave their dogs at home if they were placed in the no dog group. To account for diurnal fluctuations in cortisol and alpha-amylase levels, each experiment began between 1:00 and 3:00 pm [39,43]. Consistent with best practices for the implementation of the TSST, participants were given email and phone reminders of the expectations prior to arrival, which included instructions not to consume alcohol or take non-pharmaceutical drugs within 24 h prior to the experiment and not to brush or floss their teeth, engage in physical exercise, or eat or drink anything but water within one hour before the experiment [44].

Upon arrival at the laboratory, each participant was verbally guided through and signed an informed consent document. They were then fitted with a heart rate monitor and asked to sit quietly for 30 min in an intake room with neutral reading materials. This rest period was incorporated to mitigate the impacts of any stressful events that may have occurred prior to the start of the experiment [39,45]. Following the resting phase, participants responded to the State-Trait Anxiety Inventory for Adults (STAI-AD) [46]; and then provided blood and saliva samples (T0, baseline). Thereafter, participants were taken to another room for the TSST. Immediately after completing the TSST, the participants again responded to the STAI-AD and provided saliva and blood samples (T1, stressor). They were then taken back to the intake room, where they were instructed to sit quietly for 45 min. After this recovery period, participants filled out the STAI-AD and provided blood and saliva samples for the final time (T2, recovery).

Participants who were randomly assigned to the experimental (dog) group were required to keep their dogs on a 6-foot leash under their control at all times except during the blood draws, during which the study facilitator held the leash for participant and

phlebotomist safety. Participants were otherwise encouraged to interact naturally with their dogs. The study coordinator was trained in canine behavioral observation. If, at any time, the dog became excessively distressed (as determined by the Spectrum of Fear, Anxiety and Stress Chart, Fear Free Pets; https://fearfreepets.com/fas-spectrum/, accessed on 22 September 2022), the study facilitator would end the experiment early. Dog behavior was monitored continuously outside of the rest and recovery periods and logged at T0, T1, and T2. All other elements of the study were consistent between the experimental and control groups.

4.3. Task and Materials

4.3.1. The Trier Social Stress Test (TSST)

Standard TSST protocols were carefully adhered to, with particular attention given to processes that would promote study comparison and replicability [39,42,44,45]. Although protocol variations are common in some elements of the TSST, this team intentionally selected protocol options that have been found to result in a greater cortisol response (e.g., using a three-person, mixed-gender panel rather than a two-person panel of homogenous gender) [39]. Due to the ongoing COVID-19 pandemic, all participants and research personnel wore face masks throughout each experiment.

At the beginning of the TSST, participants were led by the study coordinator into a formal conference room with a mixed-gender panel consisting of three panelists, all of whom were wearing white lab coats and carrying clipboards on which to take notes. Participants were then given three minutes to prepare a speech about why they would be the ideal candidate for their dream job. Next, they were instructed to present their speech to the panelists, whom the participants had been told would be evaluating their performance (e.g., vocal patterns, body language). To further increase the stress responses, a video camera was set up in the room, and participants were falsely told that they would be recorded so that their performance could be more thoroughly evaluated later. Immediately after the five-minute speech, participants were instructed to verbally subtract the number 17 from 2023 and to continue subtracting from the remainder until they were told to stop, five minutes later. Throughout the TSST, only the participant and a single panelist spoke. This lead panelist followed a predetermined script to instruct the participant through the various phases of the TSST. Outside of these instructions, no panelists gave verbal or nonverbal feedback to the participants. The TSST protocol lasted approximately 13 min, after which the participant was escorted out of the room by the study coordinator.

4.3.2. Questionnaires

Immediately after providing consent and being fitted with a heart rate monitor, participants filled out an intake form that gathered demographic information, their rate of recent physical activity, and the date of their last menstrual cycle, all of which have been shown to impact physiological responses to the TSST [34,37]. Participants were not asked about the average length of their menstrual cycle, so the categorization of their cycle was based on a rough estimation of 28 days. Physical activity was assessed by asking participants, "In the past week, on how many days have you done a total of 30 min or more of physical activity, which was enough to raise your breathing rate?" This single question has been found to be a valid method to determine physical activity levels when compared to accelerometer data [47,48]. The phase of the menstrual cycle was determined by asking participants the first date of their most recent menstrual cycle, with participants classified into three groups: N/A (those who do not menstruate), follicular (days 0–14), and luteal (days 15–28). Participants were also asked to sign a form verifying that they had followed the instructions regarding tasks to avoid prior to participating in the experiment (e.g., limiting exercise and alcohol consumption; see "Procedure" for further details).

After the 30 min rest period, participants completed the State-Trait Anxiety Inventory for Adults (STAI-AD), a Likert-scale self-report instrument used to assess both state (i.e., current, momentary) and trait (i.e., temperamental, general) anxiety [39]. "State" items

include "I feel calm" and "I am jittery", whereas "Trait" items include "I worry too much over something that really doesn't matter" and "I make decisions easily". Each of the twenty items is rated on a four-point scale, with total possible scores ranging from 20 to 80. After integrating reverse coding, higher scores on each subtest indicate greater state anxiety or stress. The STAI-AD exhibits excellent internal consistency (αs > 0.89) and test–retest reliability at multiple time intervals (average r = 0.88) [49]. Participants responded again to the State portion of the STAI-AD at T1 and T2, but they only responded to the Trait portion at T0.

4.3.3. Heart Rate

Immediately after providing consent, each participant was fitted with a continuous heart rate monitor (Polar Verity Sense; Polar Electro, Kempele, Finland) on their wrist, which they wore for the duration of the experiment. The T0 and T2 heart rates were assessed by calculating the average heart rate over the final five minutes of each rest period. The T1 heart rate was assessed by calculating the average heart rate during the 13 min of the TSST.

4.3.4. Blood Collection and Processing

Venous blood was collected from a suitable lower arm vein into EDTA-coated Vacutainer tubes. The tubes were inverted three times and stored at 4 °C pending centrifugation after the last blood collection. The tubes were then centrifuged at 1500× *g* at 4 °C for 15 min to separate plasma from other blood components. Plasma was collected in fresh tubes, mixed by inversion, and then aliquoted into microtubes and stored at −80 °C until analysis.

4.3.5. Salivary Alpha-Amylase

Saliva samples were taken using laboratory-grade collection swabs (SalivaBio Oral Swabs, Salimetrics, State College, PA, USA) according to the manufacturer's instructions. Briefly, swabs were placed under the tongue for up to 2 min to collect unstimulated passive drool and then placed in collection tubes before centrifugation at 1500× *g* for 15 min at 4 °C. Extracted saliva was aliquoted and stored at −80 °C. Salivary alpha-amylase levels were assessed using a kinetic enzyme assay kit (Salimetrics, State College, PA, USA) according to the manufacturer's instructions. All samples were run on one occasion in duplicate, along with the controls provided in the assay kit. One participant's sample was mishandled and not included in the analysis.

4.3.6. Plasma Cortisol

The levels of plasma cortisol were measured using a cortisol competitive enzyme immunoassay (Cat. #KGE008B, R&D Systems, Minneapolis, MN, USA) according to the manufacturer's instructions. All samples were run in duplicate on one occasion.

4.4. Statistical Analysis

All statistical analyses were performed using SPSS v.27. All reported *p* values are two-sided. For completeness, we ran the statistical analyses with and without excluding extreme outliers and found that the results were not affected. Therefore, because the number of participants was limited, we chose to carry out statistical analysis regardless of the presence of outliers.

4.4.1. Demographics and Baseline Characteristics

We conducted independent-sample *t* tests to determine whether there were any differences between the groups in the continuous demographic variables or baseline markers of stress. For non-continuous variables, we used Fisher's exact tests to assess differences between groups.

4.4.2. TSST Response

To test whether the TSST had a significant impact on the participants' stress responses, we assessed the data for normality of distribution (Shapiro–Wilk) and sphericity and then conducted one-way repeated-measures ANOVA analyses on each dependent variable. We then carried out post hoc analyses with Bonferroni adjustments to examine within-group differences between each pair of time points. These analyses were conducted on the entire cohort and again on the dog and no dog groups separately, in order to identify any distinct patterns between the groups.

4.4.3. Dog Effect

To further examine the between-group differences, we conducted ANCOVA analyses, comparing the percent change between each pair of time points for the STAI, heart rate, cortisol, and alpha-amylase. We chose this approach because the percent change has been shown to be a more effective means of examining the stress response than using absolute levels and has a lower correlation with the baseline value [50,51]. In all cases, the T0 levels were included as a covariate. We considered the participant age, gender, race/ethnicity, physical activity, and menstrual cycle phases as covariates, as these factors may have significant impacts on physiological responses to the TSST [44,52]. These covariates were only included if they were found to have a main effect on the results.

We assessed the data for homoscedasticity, homogeneity of regression slopes, normality (Shapiro–Wilk), and homogeneity of variance (Levene's test). Where assumptions were met, we performed independent ANCOVA analyses; otherwise, we carried out the non-parametric equivalent, Quade's test [53,54].

5. Conclusions

Our findings align with, while also adding important nuance to, the existing literature indicating that the presence of a pet dog may support a healthy stress response. By examining markers of both the HPA and SAM axes, this study is the first to suggest that dogs may support a balanced, intermediate stress response capacity, as opposed to just reducing stress hyperreactivity. Given the prevalence of pet dog ownership globally, this potential effect has important public health implications. These findings, which highlight the importance of conducting nuanced research on human-animal interactions that accounts for both major physiological systems involved in the human stress response, should be replicated with additional measures and time points to assess the potential long-term impacts on human health and welfare.

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Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki and approved by the University of Denver's Institutional Review Board (IRB protocol number 1664556) and Institutional Biosafety Committee (IBC protocol 1773388). All methods were performed in accordance with their guidelines and regulations. Because the participating dogs were not owned by the university, Institutional Animal Care and Use Committee (IACUC) approval was

not required. Each dog's legal owner provided permission for their participation in the study. This study is reported in accordance with the ARRIVE guidelines.

Informed Consent Statement: Written informed consent was obtained from all subjects involved in the study.

Data Availability Statement: To protect participant privacy, the data collected in this study cannot be shared publicly. Upon reasonable request, the data may be made available by the authors.

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