Brain, Behavior, and Immunity 130 (2025) 106100

Contents lists available at ScienceDirect

Brain Behavior and Immunity

journal homepage: www.elsevier.com/locate/ybrbi

Salivary cytokines in stress research: Reactivity kinetics in response to placebo-controlled acute psychosocial stress and associations with plasma cytokines and endocrine stress markers

Marvin Fischer [a], Lisa-Marie Walther [a], Angelina Gideon [a], Christine Sauter [a], Christiane Waller [b], Ivano Amelio [c], Petra H. Wirtz [a,d,*]

[a] Biological Work and Health Psychology, University of Konstanz, Konstanz, Germany
[b] Department of Psychosomatic Medicine and Psychotherapy, General Hospital Nuremberg, Paracelsus Medical University, Nuremberg, Germany
[c] Chair for Systems Toxicology, Department of Biology, University of Konstanz, Konstanz, Germany
[d] Centre for the Advanced Study of Collective Behaviour, University of Konstanz, 78457 Konstanz, German

ARTICLE INFO

Keywords:
Salivary cytokines
Plasma cytokines
Stress hormones
Placebo-controlled TSST
Acute psychosocial stress

ABSTRACT

Background/objectives: In stress research, the measurement of cytokines from saliva may provide a non-invasive alternative to blood sampling. However, current research is limited by methodological shortcomings, including lack of placebo-stress control groups, comparison with plasma cytokine stress reactivity, infrequent sampling, and insufficient control for salivary flow rate. The aim of this study was to investigate repeatedly measured salivary cytokine responses to the Trier-Social-Stress-Test (TSST) compared to a placebo-TSST (PlacTSST), and to explore associations with plasma cytokines and endocrine stress markers.

Methods: In this placebo-controlled, single-blind, between-subject study, healthy young men were randomized to a stress condition (TSST;$n = 30$) or a placebo-stress condition (PlacTSST;$n = 20$). Salivary interleukin-(IL)-6, IL-1β, tumor-necrosis-factor-(TNF)-α were measured at baseline and repeatedly up to 30 min post-stress, with correction for salivary flow rate. Plasma cytokines were assessed up to 90 min post-stress. Salivary cortisol, epinephrine, and norepinephrine were assessed to explore potential endocrine mechanisms.

Results: The TSST induced significantly greater increases in salivary IL-6 ($p = 0.024$, $\eta_p^2 = 0.07$) and IL-1β ($p = 0.031$, $\eta_p^2 = 0.07$) compared to the PlacTSST, with peak responses at + 1 min post-stress and return to baseline by + 30 min. TNF-α was not stress-reactive, neither in saliva ($p = 0.35$) nor in plasma ($p = 0.16$). Higher total salivary IL-6 reactivity predicted higher plasma IL-6 reactivity ($\beta = 0.48$, $p < 0.001$, $\Delta R^2 = 0.31$), with salivary responses preceding those in plasma. Higher norepinephrine increases related to higher salivary IL-1β responses ($\beta = 0.45$, $p = 0.018$, $\Delta R^2 = 0.15$), pointing to a potential noradrenergic modulation.

Conclusions: Our findings demonstrate that acute psychosocial stress induces rapid and transient independent increases in salivary IL-6 and IL-1β but not TNF-α that relate to plasma cytokine and endocrine changes. These results support the utility of salivary cytokine assessment as a sensitive and non-invasive and less cost-intensive alternative to plasma sampling. Further research is warranted to elucidate underlying regulatory mechanisms and extend findings to different populations.

1. Introduction

In order to gain insights into the physiological pathways linking mental stress and adverse health outcomes (Schneiderman et al., 2005; Turner et al., 2020), a variety of experimental protocols have been developed to investigate the effects of psychological stress on inflammation as a major immune reaction (Marsland et al., 2017; Segerstrom and Miller, 2004). A reliable and frequently used tool for inducing acute psychosocial stress in a laboratory setting is the Trier Social Stress Test (TSST) (Allen et al., 2014; Kirschbaum et al., 1993). The majority of existing research employing the TSST to induce acute psychosocial stress investigated inflammatory cytokine responses measured in blood plasma and found delayed stress-induced increases, most consistently in Interleukin (IL)-6 and IL-1β, but many questions remain open, e.g.,

* Corresponding author at: Biological Work and Health Psychology, University of Konstanz, Universitaetsstrasse 10, Konstanz 78457, Germany.
E-mail address: petra.wirtz@uni-konstanz.de (P.H. Wirtz).

https://doi.org/10.1016/j.bbi.2025.106100
Received 23 April 2025; Received in revised form 29 July 2025; Accepted 5 September 2025
Available online 10 September 2025

regarding ecological validity (Marsland et al., 2017; Steptoe et al., 2007). In this context, the necessary repeated blood sampling is highly invasive and usually requires venous catheter insertion and thus trained personnel which substantially compromises its applicability e.g., in field studies. In comparison to blood sampling, the collection of saliva offers several advantages: cost- and time-effective, safer, less invasive, and thus more applicable, not only in laboratory studies but also in field studies (Engeland et al., 2019).

So far, only a limited number of studies using the TSST have investigated cytokine responses measured in saliva. To date, we identified a total of eight studies examining salivary cytokine responses to the TSST (Izawa et al., 2013; Newton et al., 2017; Quinn et al., 2020; Rodríguez Medina et al., 2019; Saban et al., 2018; Szabo et al., 2016; Szabo et al., 2019; Tell et al., 2018); for review: (Szabo et al., 2020). Overall, six studies report increases in salivary cytokines following stress (Izawa et al., 2013; Newton et al., 2017; Rodríguez Medina et al., 2019; Saban et al., 2018; Szabo et al., 2016; Szabo et al., 2019) while two did not (Quinn et al., 2020; Tell et al., 2018). Notably, the studies differ substantially in terms of methodology with major shortcomings including the 1) lack of a non-stress control group (seven out of eight studies), 2) lack of repeated post-stress sampling (five out of eight studies), 3) lack of consideration of confounding influences of salivary flow rate on salivary cytokines (four studies), or 4) the lack of comparison with plasma cytokines (seven out of eight studies).

First, to control for secondary physical and cognitive demands of the TSST that do not result from psychosocial stress, an active control group such as the placebo-TSST (PlacTSST) (Het et al., 2009) is required. In this regard, only the study by Quinn et al. (2020) included a placebo-stress condition, but analyzed salivary cytokines only once after stress cessation, at + 45 min following TSST in healthy participants. In that study, there were no stress-induced increases in the salivary cytokines IL-6, IL-1β, or TNF-α + 45 min post-stress as compared to baseline (Quinn et al., 2020). Second, with respect to repeated assessment of salivary cytokines after stress, three studies collected salivary samples at five and more timepoints post-TSST ranging from + 1 min (Izawa et al., 2013) to + 60 min (Izawa et al., 2013; Tell et al., 2018) and + 90 min (Saban et al., 2018) post TSST in order to capture a more detailed salivary cytokine responses. While two studies observed significant increases in IL-6 with highest levels at the first measurement timepoint assessed after TSST at + 1 min (Izawa et al., 2013) and + 15 min (Saban et al., 2018), the other study, notably in female cancer patients, could not observe salivary IL-6 increases at any of the post-stress timepoints (Tell et al., 2018). Third, salivary flow rate was considered as a potential confounder in four studies in healthy participants (Izawa et al., 2013) of which three corrected for salivary flow rate (Newton et al., 2017; Szabo et al., 2016; Szabo et al., 2019). All of these studies observed significant increases in salivary cytokines (IL-1ß, IL-6, TNF-α, and/or IL-10) assessed at + 1 min (Izawa et al., 2013) and between + 35 and + 50 min after TSST (Newton et al., 2017; Szabo et al., 2016; Szabo et al., 2019), as compared to baseline, but without considering a control group. Finally, only the study by Saban et al. (2018) simultaneously measured cytokine responses in both plasma and saliva assessed repeatedly up to + 90 min after stress and did not observe any associations between plasma and salivary IL-6 levels at any measurement timepoint (Saban et al., 2018).

To the best of our knowledge, studies are lacking that combine the use of a non-stress control group, most ideally a placebo-stress control group (Het et al., 2009), with frequent repeated assessment of salivary cytokines after stress, either with or without control for salivary flow rate, especially in healthy participants. Moreover, potential associations between salivary and plasma cytokines in response to acute psychosocial stress as well as underlying endocrine mechanisms remain unclear.

We therefore set out to examine changes in salivary cytokine levels in response to acute psychosocial stress as compared to a control condition. Based on previous cytokine stress research (Marsland et al., 2017), we assessed IL-6, TNF-α, and IL-1β, before and repeatedly after TSST or PlacTSST (up to + 90 min) in both saliva and plasma of healthy participants. Potential confounders and in particular salivary flow rate were considered. Based on the studies with repeated assessment of salivary cytokines following TSST, but lacking control groups (Izawa et al., 2013; Saban et al., 2018), we expected stress-induced increases in salivary cytokines close after stress cessation as compared to placebo-stress. Moreover, to consider potential underlying endocrine mechanisms, we exploratively examined whether psychosocial stress-induced salivary cytokine changes related to increases of the stress hormones epinephrine, norepinephrine, or cortisol.

2. Methods

2.1. Study participants

This study is part of a larger research project examining the psychobiological responses to acute psychosocial stress in healthy young men in a design comprising a stress and a placebo-stress condition (Gideon et al., 2020; Gideon et al., 2022). Due to limited funding, study participation was restricted to healthy men between 18 − 30 years, who were medication-free and non-smoking, with exclusion criteria verified via telephone interview (Gideon et al., 2020; Gideon et al., 2022). The decision to not include both sexes given the granted funding was based on (1) the consideration to allow for maximum methodological rigor (e.g. simultaneous assessment of salivary and plasma cytokines in addition to stress hormones, inclusion of a placebo control group, and frequent sampling of all physiological measures) to close major methodological gaps in current literature and (2) the well-established influences of sex hormones and menstrual cycle phases on physiological stress responses in women, particularly salivary cortisol and potentially also immune parameters (Angstwurm et al., 1997; Gervasio et al., 2022; Kirschbaum et al., 1999; O'Brien et al., 2007; Stephens et al., 2016). By recruiting men only confounding influences of sex hormones and menstrual cycle phases, that require a substantial increase in sample size and thus funding, had not to be accounted for. All participants were enrolled students at the University of Konstanz. Guided by our a priori sample size analysis, we aimed for a sample size of N = 50 eligible participants to investigate salivary and plasma cytokine reactivity to acute psychosocial stress. From a total of N = 70 recruited eligible participants, we included in this part of the study n = 30 in the stress condition and n = 20 in the placebo-stress condition with complete plasma cytokine assessment who did not drop out in the course of the study. From these participants, we then measured salivary cytokine levels. Due to the lack of saliva, salivary cytokine data of one participant in the stress group is missing. The study was approved by the Ethics Committee of the University of Konstanz, Germany, and conducted in alignment with the Declaration of Helsinki. Participants provided written informed consent before participating and received financial compensation.

2.2. Study design and procedure

We applied a placebo-controlled, single-blind, between-subject design (Gideon et al., 2020; Gideon et al., 2022). Before participating in the study, participants were instructed to refrain from: (1) consuming caffeinated, sour, or sweet beverages on the study day; (2) any form of physical activity and consuming caffeine-containing drinks for 24 h prior to the study; (3) engaging in strenuous physical activity and consuming alcohol for 48 h; and (4) receiving vaccinations for four weeks or undergoing dental surgery for two weeks before participation. Additionally, participants were advised to maintain a regular sleep schedule in the days leading up to the study, specifically getting up between 7 and 8 AM and going to bed between 10:30 and 11:30 PM. On the day of the study, all participants, both of the stress and the placebo stress condition, arrived at the laboratory of the Biological Work and Health Psychology group at the University of Konstanz at 11 AM to standardize time of day. Upon arrival, they were provided with a

standardized meal consisting of two bottles of Fresubin® (Fresenius Kabi Germany GmbH, 200 ml and 300 kcal per bottle), one piece of crispbread, and water. Following the meal, body weight and height measurements were taken. Approximately 30 min after arrival, a venous catheter (Vasofix® Safety Braunule green/white G 18, B. Braun, Melsungen, Germany) was inserted into the non-dominant arm.

After a 50 min acclimatization period, baseline blood and saliva samples were analyzed for physiological assessments at −1min. Participants were then assigned, based on randomization, to either undergo the TSST (Kirschbaum et al., 1993)) or the PlacTSST (Het et al., 2009). Following the completion of the TSST or PlacTSST, participants remained seated in a quiet room. Further analyses of blood samples comprised + 1, +10, +30, +45, +60 and + 90 min in addition to saliva samples at + 1, +10, +20, +30, +45, and + 60 min post-cessation. The analyses of stress hormones and inflammatory cytokines during biochemical analyses were conducted at various sampling time points (see Biochemical Analyses).

2.3. Acute psychosocial stress induction

The standard protocol for the TSST (Kirschbaum et al., 1993) was used to induce acute psychosocial stress in participants in the stress condition. Based on previous research, the TSST reliably triggers a psychophysiological stress response, including elevated levels of circulating inflammatory markers (Kuebler et al., 2016; Kuebler et al., 2015; Marsland et al., 2017). The protocol includes a brief introduction, followed by a 3 min preparation phase, a 5 min simulated job interview, and a 5 min mental arithmetic task performed in front of an audience, with both video and audio recording.

The placebo-stress condition underwent the placebo version of the TSST, the PlacTSST (Het et al., 2009). This procedure is similar to the TSST but lacks social evaluative and uncontrollable elements. The PlacTSST also consists of a brief introduction, followed by a 3 min preparation phase, a 5mi speech about a recent positive personal experience, as well as a 5 min simple mental arithmetic task, all conducted without an audience or recording. This design of the PlacTSST allows to control for secondary effects of the task, such as non-stress-related activation due to the physical and cognitive demands of the TSST, including potential orthostatic and posture effects.

2.4. Biochemical analyses

Venous blood was drawn in EDTA-coated monovettes (Sarstedt, Nuembrecht, Germany) for determination of plasma cytokines and plasma catecholamines. Blood samples for determination of plasma cytokine and catecholamine levels were immediately centrifugated at 2000 g and 4 °C for 10 min and stored at − 80 °C until analyses. Saliva samples were collected using Salivettes (Sarstedt, Nuembrecht, Germany). To standardize salivary collection, participants were instructed to chew the Salivettes for exactly one minute. Following, samples were stored at − 20 °C until analysis.

2.5. Endocrine stress response

To evaluate the reactivity of the sympathetic adrenal medullary axis, plasma levels of the catecholamines epinephrine and norepinephrine were measured at four blood sampling time points (−1, +1, +10, +60 min) using high-pressure liquid chromatography with electrochemical detection following liquid–liquid extraction (Ehrenreich et al., 1997) in the Laboratory for Stress Monitoring (LSM, Hardegsen, Germany). The intra-assay coefficients of variance (CVs) for epinephrine and norepinephrine were 3.0 % and 3.7 %, respectively, with a detection limit of 8 pg/ml.

We measured salivary cortisol to assess hypothalamic pituitary adrenal (HPA)-axis reactivity at seven time points (−1, +1, +10, +20, +30, +45, and + 60 min). Cortisol data was missing in three PlacTSST participants due to technical problems at baseline assessment. Prior to analysis, samples were thawed and centrifuged at 2500g at room temperature for 10 min (Megafuge 40R; Heraeus, Thermo Fisher Scientific, Langenselbold, Germany). Cortisol concentrations were determined using a commercial enzyme-linked immunosorbent assay (ELISA) following the manufacturer's instructions (Cortisol Saliva ELISA, RE-52611, IBL International GmbH, Hamburg, Germany). Optical densities were read using a microtiter plate reader (Synergy H1 Multi-Mode Microplate Reader; BioTek Instruments Inc., Bad Friedrichshall, Germany). The inter- and intra-assay coefficients of variance (CVs) were 9.3 % and 7.3 %, respectively, as per the manufacturer's specifications. The detection limit was 0.003 μg/dL.

2.6. Salivary and plasma cytokines

Plasma and salivary levels of the cytokines IL-1β, IL-6, and TNF-α were determined using a high sensitivity sandwich immunoassay (Meso Scale Discovery, Rockville, USA). Based on previous findings (Marsland), we measured plasma cytokines at sampling timepoints up to 90 min after stress cessation (−1,+1,+10,+30,+45,+90 min). To identify their kinetics, salivary cytokines were measured at − 1,+1,+10,+30,+45,+60, and + 90 min after (placebo) stress cessation in a subset of 10 participants per subject group. Based on the obtained results (see results below) and due to financial restrictions, salivary cytokine assessment of the remaining participants were restricted to four assessment timepoints up to 30 min post (placebo) stress (−1,+1,+10,+30 min). Notably, given that plasma IL-1β levels were below detection limit in more than 50 % of the samples, we did not further consider plasma IL-1β measurements in this study. Also, 49 out of 392 salivary IL-6 and TNF-α cytokine levels were undetectable and replaced by half of the lower detection limit (LLOD/2) (Lydersen and Giskeødegård, 2022; Uh et al., 2008).

Detection limits were 0.05 pg/ml for IL-1β, 0.06 pg/ml for IL-6, and 0.04 pg/ml for TNF-α. Intra- and weighted inter-assay coefficients of variance for cytokines were below 4.7 % (IL-6: 3.91 %(plasma)/3.30 % (saliva) and 3.87 %(plasma)/3.11 %(saliva); TNF-α:2.89 % (plasma)/ 4.61 % (saliva) and 2.88 % (plasma)/4.66 % (saliva); IL-1β: 2.30 % (saliva) and 2.26 % (saliva)).

3. Statistical analyses

3.1. Data preparation

3.1.1. Plasma cytokine levels

Plasma cytokine levels within the first 45 min after TSST/PlacTSST cessation were corrected for stress hemoconcentration-related blood volume changes (Dill and Costill, 1974; Matomäki et al., 2018). Moreover, to account for catheter and stress-induced leucocyte redistribution effects including fast leukocyte increases (Beis et al., 2018; Walther et al., 2024) and subsequent decreases (Dhabhar, 2018; Hinterdobler et al., 2021) plasma cytokine levels were standardized to 10^6 leukocytes (LC) per ml. Stress hemoconcentration-corrected *plasma* cytokine levels per 10^6 leukocytes per ml at each timepoint up to 45 min after TSST/PlacTSST cessation ($LC_{t\text{-corrected}}$) were calculated based on changes in hemoglobin from baseline ($Hb_{baseline}$) to the respective sampling timepoint (Hb_t) using the formula $LC_{t\text{-corrected}} = ((LC_t/LC_{baseline}) \times (Hb_{baseline}/Hb_t)) \times LC_{baseline}$ (Matomäki et al., 2018).

3.1.2. Salivary cytokine levels

To address potential confounding effects of variations in salivary flow rates as expected to be induced by (Plac)TSST (Nater et al., 2005), we corrected salivary cytokine concentrations across sampling timepoints using a two-step method. First, we standardized cytokine concentrations of each measurement timepoint by standardizing cytokine levels per ml saliva collected in one minute. Second, we adjusted salivary cytokine levels per ml saliva of each post-(Plac)TSST timepoint (i.

Table 1
Participants' characteristics.

	Total ($n = 50$)	TSST ($n = 30$)	PlacTSST ($n = 20$)	Group differences p
Age (years)	23.3 ± 2.5 (18–28)	23.3 ± 2.6 (18–28)	23.2 ± 2.4 (20–27)	0.89
BMI (kg/m^2)	23.4 ± 2.4 (18.4–29.1)	23.9 ± 2.6 (18.37–29.1)	22.7 ± 2.0 (20.5–28.6)	0.11
Cortisol (nmol/l)	6.3 ± 3.3 (1.7–16.9) $n = 47$	6.1 ± 3.1 (1.7–16.9)	6.5 ± 3.7 (2.0–13.9) $n = 17$	0.85
Epinephrine (pg/ml)	23.3 ± 12.8 (8.0–67.0) $n = 48$	23.0 ± 15.2 (8.0–67.0)	23.8 ± 7.8 (8.3–38.7) $n = 18$	0.32
Norepinephrine (pg/ml)	281.8 ± 101.3 (148.3–623.8) $n = 48$	296.1 ± 94.8 (148.3–605.9)	284.7 ± 113.8 (166.0–623.8) $n = 18$	0.57
Salivary IL-6 (pg/ml)	0.2 ± 0.4 (0.01–1.5) $n = 49$	0.2 ± 0.1 (0.01–1.5) $n = 29$	0.2 ± 0.1 (0.02–1.3)	0.85
Salivary IL-1β (pg/ml)	8.6 ± 1.2 (0.3–38.2) $n = 49$	8.4 ± 1.6 (0.3–30.1) $n = 29$	9.0 ± 2.0 (0.5–38.2) $n = 20$	0.82
Salivary TNF-α (pg/ml)	0.2 ± 0.3 (0.01–1.0) $n = 49$	0.2 ± 0.5 (0.01–1.0) $n = 29$	0.2 ± 0.04 (0.01–0.8)	0.45
Plasma IL-6 (pg/ml)	0.1 ± 0.01 (0.02–0.4)	0.8 ± 0.01 (0.03–0.4)	0.8 ± 0.02 (0.2–0.3)	0.84
Plasma TNF-α (pg/ml)	0.4 ± 0.4 (0.1–1.7)	0.4 ± 0.03 (0.2–0.8)	0.4 ± 0.1 (0.1–1.7)	0.31

Note. Data are presented as mean ± SEM (range); n, number of participants in case of missing data.

e. + 1 min, +10 min, and + 30 min) for percentage changes in salivary flow rate (i.e. ml saliva/min) from pre- (−1min) to the respective post-(Plac)TSST measurement timepoint. This procedure ensures to correct for increases in salivary cytokine levels that result from stress-induced reductions in salivary flow rate. Therefore, the resulting corrected cytokine concentrations reflect the physiological changes induced by psychosocial stress (as compared to placebo stress), independent of confounding variations in saliva production. For reasons of clarity, we present below results using both, uncorrected and corrected salivary cytokine levels.

3.2. Analyses

Statistical analyses were conducted using SPSS (Version 30.0, IBM SPSS Statistics, Chicago, Il, USA). Data are presented as mean ± standard error of the mean (SEM). Tests were two-tailed with the significance level of $p < 0.05$ and the level of marginal significance set at $p < 0.10$. Effect size parameters (f) were calculated from partial eta squared (η_p^2) using G*Power (Version 3.1.9.6, Heinrich Heine Universität Düsseldorf, Germany) or R^2, respectively, and are reported where appropriate (effect size conventions f:0.10 = small, 0.25 = medium, 0.40 = large) (Cohen, 1988). To test for potential group differences in *baseline levels* of the assessed physiological parameters as well as in age and BMI, we calculated univariate analyses of variance (ANOVAs) with group (TSST vs. PlacTSST) as independent variable. Statistical assumptions for univariate ANCOVA, repeated measures ANCOVA, and multiple regression were assessed. While positive skewness was observed for cytokine data, all analyses were conducted using untransformed percentage change data, given the robustness of ANCOVA and regression analysis against violations of normality, especially in within-subject designs with balanced groups (Blanca et al., 2017; Blanca et al., 2023; Schmidt and Finan, 2018).

3.3. Reactivity to TSST and PlacTSST

To test for group differences in *endocrine stress reactivity*, we calculated repeated measures ANCOVAs with group (TSST vs. PlacTSST) as independent variable and repeated epinephrine, norepinephrine, or cortisol levels as dependent variables (Auer et al., 2024; Het et al., 2012). To test whether the TSST as compared to the PlacTSST induced significantly greater *cytokine responses,* we accordingly calculated repeated measures ANCOVAs with group (TSST vs. PlacTSST) as independent variable and repeated levels of salivary or plasma cytokines as dependent variables. Cytokine levels were calculated as percentage changes in salivary and plasma IL-6, IL-1β, and TNF-α concentrations.

Post-hoc testing comprised testing for group differences at single measurement timepoints of cytokine levels by means of univariate ANCOVAs as well as separate repeated measures reanalyses within each subject group. To control for potential confounding effects of age and body mass index (BMI) on endocrine and cytokine reactivity (Allen et al., 2017; Kühnel et al., 2023; Stamou et al., 2023; Wirtz et al., 2008), we included age and BMI (calculated as body weight in kilograms divided by body height in meters squared) as covariates in all group comparison ANCOVAs.

3.4. Interrelations between cytokines

To examine interrelations between stress-reactive salivary and plasma cytokine responses, we performed linear regression analyses between aggregated measures of salivary and plasma cytokines. We computed total cytokine release from baseline to + 30 min (for saliva) and + 90 min (for plasma) using the area under the curve with respect to the ground (AUCg) as well as the change in cytokine levels the AUC with respect to increase (AUCi) (Pruessner et al., 2003). Post-hoc testing of significant associations between AUCs of salivary and plasma cytokines comprised linear regression analyses at individual measurement timepoints in saliva (up to + 30 min post-stress, i.e. −1min, +1min, +10 min, and + 30 min) and plasma (up to + 90 min post-stress, i.e. −1min, +1min, +10 min, +30 min, +45 min, and + 90 min).

3.5. Interrelations between changes in endocrine markers and stress-reactive salivary cytokines

We examined interrelations between stress-reactive salivary cytokines (IL-6 and IL-1β) and the endocrine stress markers epinephrine, norepinephrine, and cortisol. We computed aggregated measures of epinephrine, norepinephrine (from baseline to + 10 min post TSST/PlacTSST), and cortisol (from baseline to + 30 min post TSST/PlacTSST) using AUCi to capture dynamic changes over time. We used aggregated epinephrine, norepinephrine, and cortisol measures to predict the AUCis of stress-reactive corrected salivary cytokine levels. Post-hoc testing of significant associations between AUCis of endocrine stress markers and

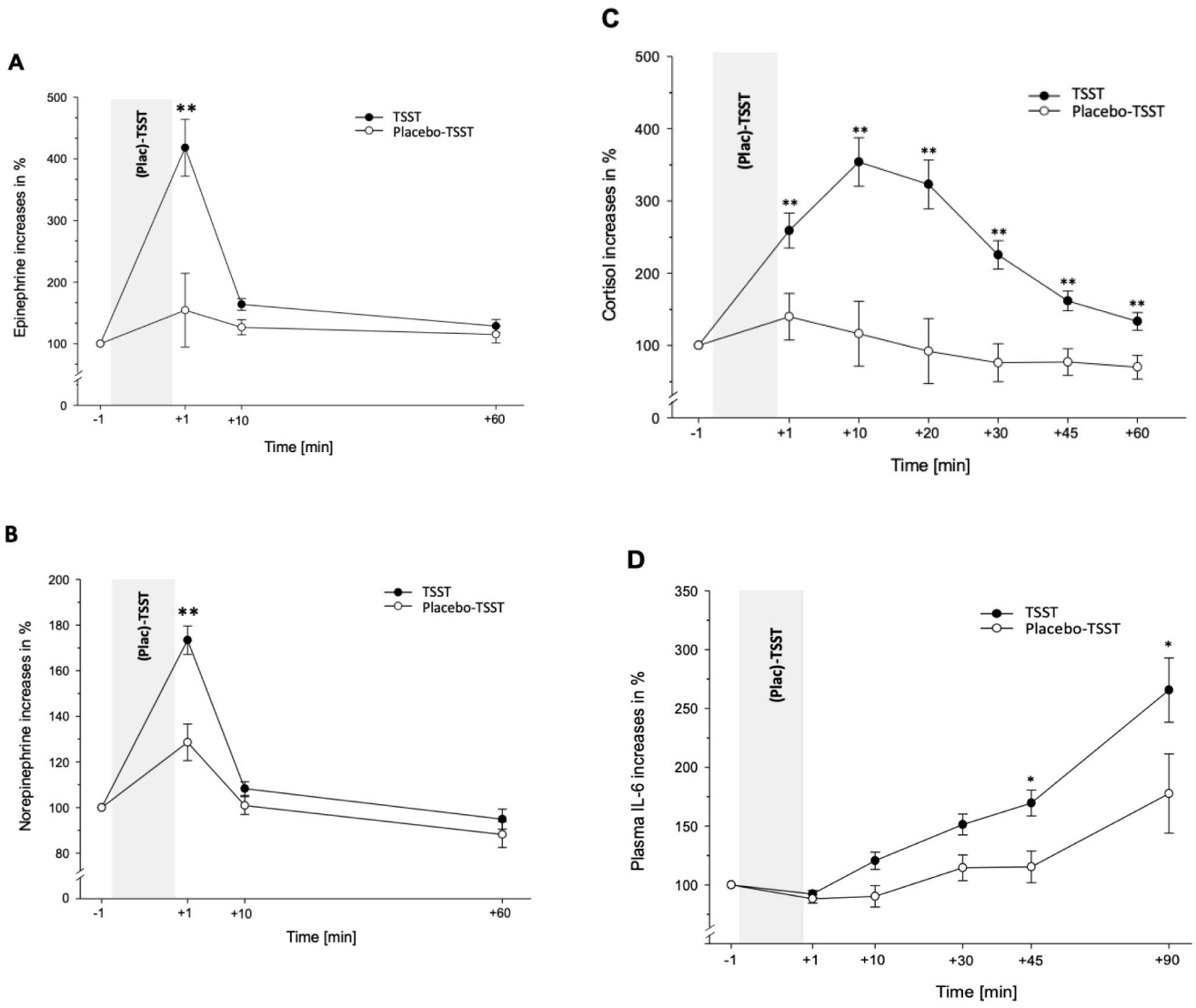

Fig. 1. Endocrine and plasma cytokine responses to (Plac)TSST. Endocrine and plasma IL-6 responses in participants undergoing the Trier Social Stress Test (TSST; black dots) or the Placebo-TSST (PlacTSST; white dots). Panels show increases of (A) plasma Epinephrine, (B) plasma Norepinephrine, (C) salivary Cortisol, (D) plasma IL-6. Asterisks denote significant differences between the TSST and PlacTSST groups ($p < 0.10$; *$p < 0.05$; **$p < 0.001$). Age and BMI were included as covariates. Data are presented as mean ± SEM.

salivary cytokines comprised linear regression analyses at the single measurement timepoints (i.e., −1min, +1min, +10 min, and + 30 min).

4. Results

4.1. Participants' characteristics and physiological parameters at baseline

Baseline levels of physiological parameters and participants' characteristics are depicted in Table 1. There were no significant differences between the experimental and control groups in terms of age ($p = 0.89$), BMI ($p = 0.11$), baseline stress hormone levels (salivary cortisol, epinephrine, norepinephrine; p's ≥ 0.69), or baseline cytokines levels (salivary IL-6, IL-1β, TNF-α; p's ≥ 0.45; plasma IL-6, TNF-α; p's ≥ 0.31).

4.2. Endocrine and plasma cytokine reactivity to TSST and PlacTSST

Fig. 1 depicts percent changes of stress-reactive endocrine and plasma cytokine measures in the groups in addition to aggregated absolute stress reactivity in terms of AUCs. As anticipated, participants in the TSST group demonstrated greater reactivity compared to the PlacTSST group with respect to epinephrine (interaction group-by-time: $F(1.23, 54.10) = 10.93$, $p < 0.001$, $\eta p^2 = 0.20$, $f = 0.50$, Fig. 1A), norepinephrine (interaction group-by-time: $F(2.34, 104.00) = 13.73$, $p < 0.001$, $\eta p^2 = 0.24$, $f = 0.56$, Fig. 1B), and salivary cortisol reactivity (interaction group-by-time: $F(2.40,102.88) = 11.40$, $p < 0.001$, $\eta p^2 = 0.21$, $f = 0.52$, Fig. 1C), confirming effective stress induction. Similarly, regarding plasma cytokines, the TSST induced borderline greater increases in plasma IL-6 as compared to the PlacTSST over the total period of + 90 min after TSST/PlacTSST cessation (interaction effect group-by-time: With correction for both leukocyte counts and hemoconcentration $F(1.58, 72.61) = 3.23$, $p = 0.057$, $\eta_p^2 = 0.07$, $f = 0.27$, see Fig. 1D, with correction for hemoconentration $F(1.56, 71.65) = 3.02$, $p = 0.068$, $\eta_p^2 = 0.062$, $f = 0.26$). Regarding plasma TNF-α, there were no significant reactivity differences between groups (p's > 0.12) (Table 2).

4.3. Salivary cytokine reactivity to TSST and PlacTSST

4.3.1. Identification of relevant salivary cytokine measurement timepoints

In order to identify relevant measurement timepoints given our limited funding resources, we first investigated the general kinetics of cytokine responses in saliva in a subset of participants ($n = 10$ per group) from both the TSST and the placebo condition over a time frame of 2 h

Table 2
Summary of cytokine and neuroendocrine responses to (Plac)TSST.

Marker	Specimen	Peak Time	TSST-group Δ Baseline/ peak (% ± SEM)	PlacTSST Δ Baseline/ peak (% ± SEM)	*p*
IL-6 (adjusted)	Saliva	+1min	321.6 ± 47.8	163.6 ± 58.0	$p = 0.005$
IL-6 (unadjusted)	Saliva	+1min	361.0 ± 62.4	144.8 ± 25.0	$p = 0.039$
IL-1β (adjusted)	Saliva	+1min	219.2 ± 26.7	116.9 ± 32.4	$p = 0.007$
IL-1β (unadjusted)	Saliva	+1min	225.6 ± 33.0	108.6 ± 12.6	$p = 0.007$
TNF-a (adjusted)	Saliva	+1min	187.5 ± 29.7	199.2 ± 55.1	$p = 0.74$
TNF-a (unadjusted)	Saliva	+1min	187.5 ± 29.7	200.6 ± 54.9	$p = 0.66$
IL-6 (adjusted)	Plasma	+90 min	265.7 ± 27.4	177.7 ± 33.7	$p = 0.057$
IL-6 (corrected for hemoconc.)	Plasma	+90 min	292.9 ± 35.6	218.0 ± 27.8	$p = 0.068$
TNF-a (adjusted)	Plasma	+90 min	89.9 ± 2.2	81.4 ± 4.0	$p = 0.16$
TNF-a (corrected for hemoconc.)	Plasma	+90 min	96.6 ± 2.1	95.7 ± 3.0	$p = 0.71$
Epinephrine	Plasma	+1min	418.0 ± 46.1	154.4 ± 59.8	$p < 0.001$
Norepinephrine	Plasma	+1min	173.4 ± 6.2	128.6 ± 8.0	$p < 0.001$
Cortisol	Saliva	+20 min	354.0 ± 33.5	116.5 ± 44.8	$p < 0.001$

Note. Data are presented as mean ± SEM; Δ Baseline to Peak Response in % relative to baseline. p-values refer to the group-by-time interaction effect from the respective repeated measures ANCOVA. Adjusted: controlled for age, BMI, salivary flow rate (salivary cytokines) or leukocyte count and hemoconcentration (plasma cytokines).

following TSST/PlacTSST cessation at all measured timepoints (See Fig. 2). These data revealed that cytokine responses in the TSST group were observed during the first 30 min post-stress, with little additional information at later time points. We therefore measured cytokine levels from saliva samples assessed at −1min, +1min, +10 min, and + 30 min relative to (Plac)TSST.

4.3.2. Salivary IL-6 and IL-1β

In a first step, we compared percentage changes in salivary cytokines without any corrections (i.e., for salivary flow rate or further confounding variables) between the TSST group and the PlacTSST group. Here, we observed significantly greater increases in both, salivary IL-6 and IL-1β levels in the TSST group compared to the PlacTSST group (interaction effect group-by-time: IL-6: $F(2.21,104.05) = 3.22$, $p = 0.039$, $\eta_p^2 = 0.06$, $f = 0.26$; IL-1β: $F(2.50,117.41) = 4.57$, $p = 0.007$, $\eta_p^2 = 0.09$, $f = 0.31$). As the main analysis, we compared percentage changes in salivary cytokines between the TSST group and the PlacTSST group for cytokine concentrations that were corrected for salivary flow, without and with age and BMI as covariates. Again, the TSST group showed significantly greater increases in salivary IL-6 and IL-1β levels compared to the PlacTSST group (interaction effect group-by-time: IL-6: $F(2.46,115.53) = 4.61$, $p = 0.005$, $\eta_p^2 = 0.10$, $f = 0.33$, with covariates age and BMI: $F(2.60,117.20) = 3.45$, $p = 0.024$, $\eta_p^2 = 0.07$, $f = 0.28$; IL-1β: $F(2.50,117.54) = 7.97$, $p = 0.007$, $\eta_p^2 = 0.15$, $f = 0.41$, with covariates age and BMI: $F(2.62,117.80) = 3.23$, $p = 0.031$, $\eta_p^2 = 0.07$, $f = 0.27$, see Fig. 3a and b). Post-hoc analysis revealed significantly higher salivary IL-6 and IL-1β levels in the TSST group at + 1 min post-stress (p's≤.045) but at no later time point (+10 min, +30 min: p's ≥ 0.27). Further post-hoc testing within groups separately revealed significant increases in salivary IL-6 and IL-1β levels over time only in reaction to the TSST (main effect time: IL-6: $F(1.93, 54.02) = 11.60$, $p < 0.001$, $\eta_p^2 = 0.29$, $f = .64$; IL-1β: $F(2.25, 63.03) = 10.63$, $p < 0.001$, $\eta_p^2 = 0.28$, $f = .54$) but not the PlacTSST (p's≥.28). In TSST participants, salivary IL-6 and IL-1β levels were increased as compared to baseline levels immediately at + 1 min (p's < 0.001) and (marginal) significantly increased at + 10 min after TSST cessation (IL-6: p's≤.09; IL-1β: p's≤.032), with values returning to baseline at + 30 min (p's≥.19).

4.3.3. Salivary TNF-α

There were no significant changes in salivary TNF-α levels in

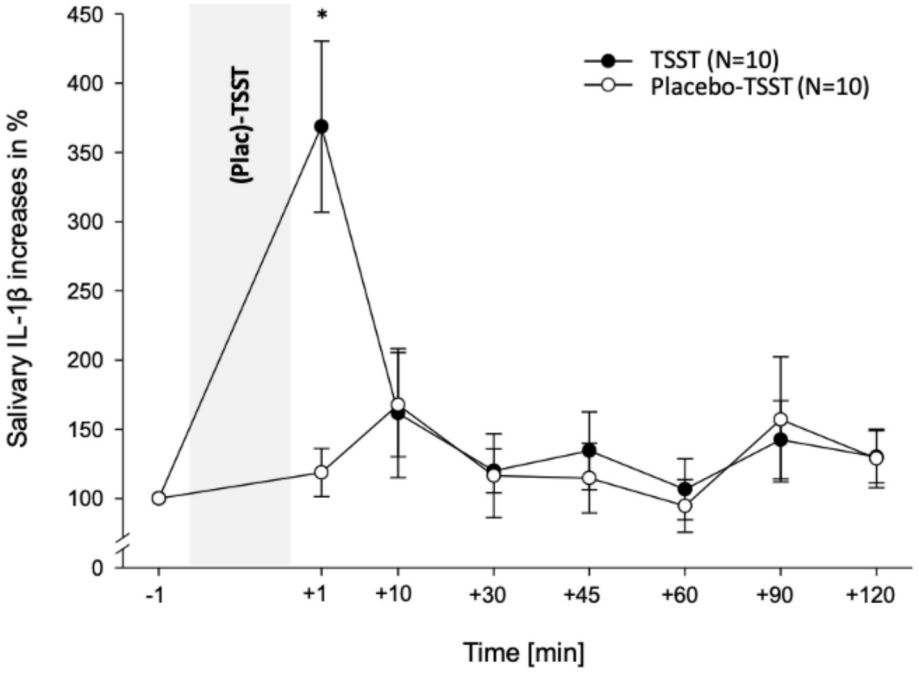

Fig. 2. Identification of relevant salivary cytokine measurement timepoints. Examination of salivary IL-1β in a subset of $n = 10$ participants per group up to + 120 min post-(Plac)TSST. Results indicated that cytokine responses in the TSST group reached baseline levels within 30 min post-stress. Consequently, cytokines were analyzed at −1min, +1min, +10 min, and + 30 min relative to (Plac)TSST cessation. Data are presented as mean ± SEM.

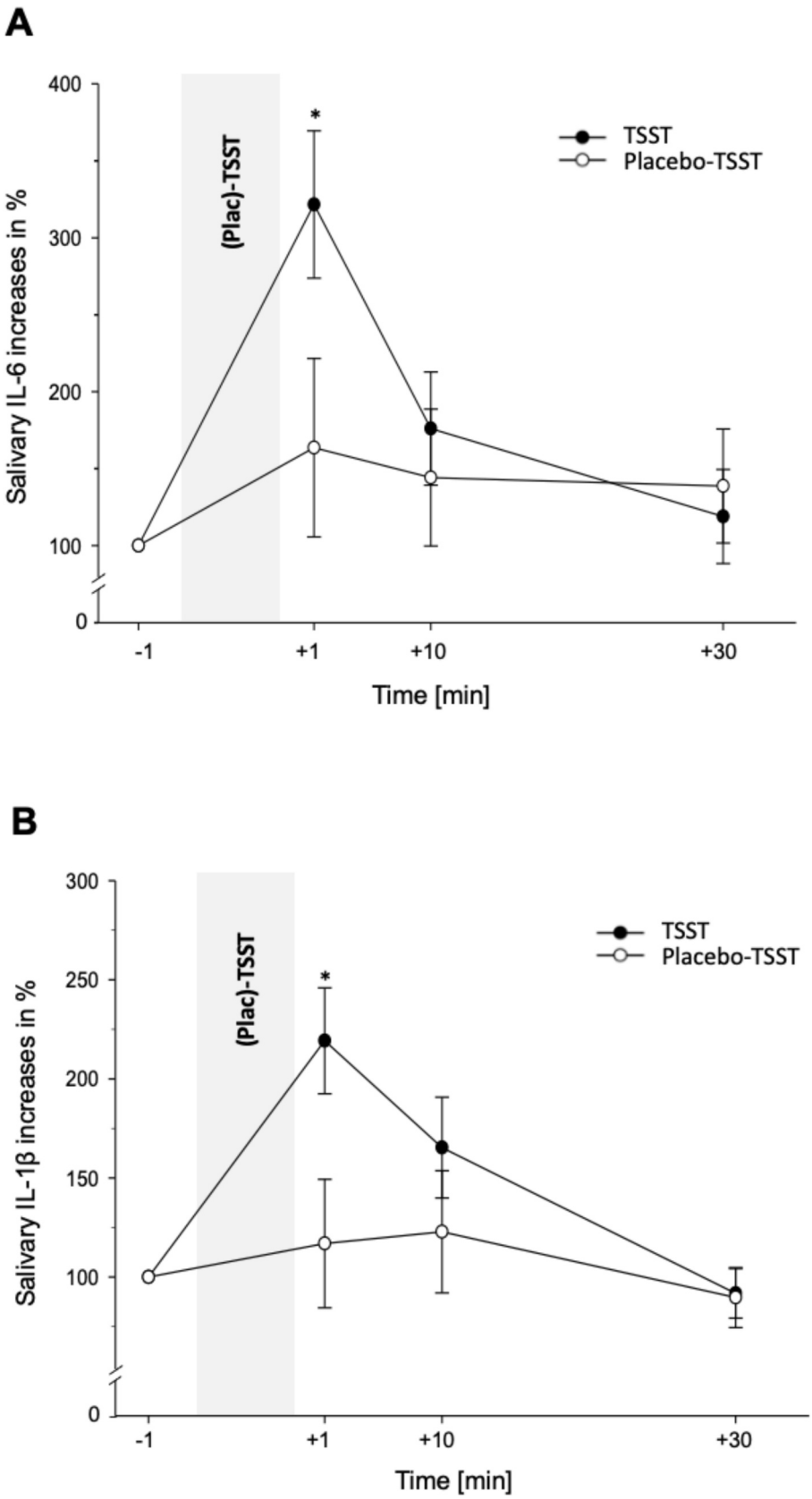

Fig. 3. Salivary Cytokine responses to (Plac)TSST. Salivary IL-6 and IL-1β responses in participants undergoing the Trier Social Stress Test (TSST; black dots) compared to Placebo-TSST (PlacTSST; white dots) (interaction effects group-by-time: A: $p = 0.024$; B: $p = 0.031$). Panels show increases of (A) salivary IL-6 and (B) salivary IL-1β. Asterisks denote significant differences between the TSST and PlacTSST groups ($p < 0.10$; *$p < 0.05$; **$p < 0.001$). Age and BMI were included as covariates. Data are presented as mean ± SEM.

response to the TSST or the PlacTSST (interaction group-by-time: p's $\geq$ 0.66; main effects of time: p's $\geq$ 0.35).

4.4. Interrelations between salivary cytokines

Regarding interrelations between *total* salivary IL-6 and IL-1β from baseline to + 30 min ($AUC_{g\ baseline\text{-}30min}$), we found higher total salivary IL-6 to be significantly associated with higher salivary IL-1β ($\beta = 0.56$, $p < 0.001$, $\Delta R^2 = 0.31$, $f^2 = 0.44$). However, when examining the amount of *change* from baseline to + 30 min, salivary IL-6 and IL-1β levels ($AUC_{i\ baseline\text{-}30min}$) were unrelated ($p = 0.42$). Post-hoc analysis revealed (marginal) significant associations between salivary IL-6 and IL-1β levels at baseline (−1min) ($p < 0.001$), as well as + 1 min ($p < 0.001$), +10 min ($p = 0.068$) and + 30 min ($p < 0.001$). For all associations see Appendix A Table A1.

4.5. Interrelations between stress-reactive saliva and plasma cytokines

TNF-α was not stress-reactive, neither in saliva nor in plasma, and IL1β was stress-reactive in saliva but was not reliably measurable in plasma. The only stress-reactive cytokine with reliable assessment both, in saliva and plasma was IL-6. Examining the interrelations between salivary and plasma IL-6 levels, we found higher *total* salivary IL-6 from baseline to + 30 min (sIL6 $AUC_{g\ baseline\text{-}30min}$) to significantly predict higher plasma IL-6 levels from baseline to + 90 min (pIL6 $AUC_{g\ baseline\text{-}90min}$) ($\beta = 0.37$, $p = 0.009$, $\Delta R^2 = 0.16$, $f^2 = 0.19$). Moreover, when examining the amount of *change,* we found a significant positive association between salivary IL-6 levels (sIL6 $AUC_{i\ baseline\text{-}30min}$) and plasma IL-6 (pIL6 $AUC_{i\ baseline\text{-}90min}$) ($\beta = 0.48$, $p < 0.001$, $\Delta R^2 = 0.31$, $f^2 = 0.45$. Post-hoc analysis revealed significant associations between salivary and plasma IL-6 levels at baseline (−1min) ($p = 0.022$), as well as + 1 min ($p = 0.041$), +10 min ($p = 0.011$), and + 30 min ($p = 0.006$) with consistently higher levels in saliva. However, peak levels of saliva IL-6 (+1min) did not predict peak levels of plasma IL-6 (+90 min) ($p = 0.19$). For all associations see Appendix A Table A2.

4.6. Interrelations between changes in endocrine markers and stress-reactive salivary cytokines

A higher amount of change in epinephrine concentration from baseline to + 10 min (EPI $AUC_{i\ baseline\text{-}10min}$) predicted a marginally significantly higher increase in salivary *IL-6* (IL-6 $AUC_{i\ baseline\text{-}30min}$) ($\beta = 0.28$, $p = 0.053$, $\Delta R^2 = 0.16$, $f^2 = 0.18$). However, this association was not independent of epinephrine and cortisol responses ($p = 0.16$). Further, post-hoc analysis could not reveal significant associations between epinephrine and salivary IL-6 at any measurement timepoint, either without or with control for norepinephrine and cortisol (p's > 0.19). Moreover, there were no associations between norepinephrine and salivary IL-6 (p's>.18). Lastly, we observed increases in cortisol from baseline to + 60 min post-stress ($AUC_{i\ baseline\text{-}60min}$) to be independently (i.e., independent of epinephrine and norepinephrine) marginally significantly associated with IL-6 $AUC_{i\ baseline\text{-}30min}$ ($\beta = 0.32$, $p = 0.085$, $\Delta R^2 = 0.22$, $f^2 = 0.29$). Post-hoc analysis could not reveal significant associations between cortisol and IL-6 at the same measurement timepoints, either without or with controlling for epinephrine and norepinephrine ($ps \geq .15$).

When considering *IL-1β*, increases in norepinephrine concentration from baseline to + 10 min ($AUC_{i\ baseline\text{-}10min}$) significantly predicted higher salivary IL-1β increases ($\beta = 0.37$, $p = 0.015$, $\Delta R^2 = 0.13$, $f^2 = 0.15$), also when controlling for epinephrine and cortisol responses in terms of the respective AUC_i ($\beta = 0.45$, $p = 0.018$, $\Delta R^2 = 0.15$, $f^2 = 0.18$). Post-hoc analysis revealed a significant positive association between norepinephrine and salivary IL-1β levels at peak reactivity + 1 min ($p = 0.025$) but no other timepoint ($ps \geq 0.63$). Epinephrine changes however did not relate to salivary IL-1β changes ($p = 0.58$). There were no associations between cortisol change and salivary IL-1β ($ps \geq .51$).

5. Discussion

To address gaps in current literature, we investigated the kinetics of acute psychosocial stress-induced changes in salivary cytokines as compared to a placebo-control group using a repeated sampling protocol in healthy men. To gain insights with respect to validity, we simultaneously assessed salivary and plasma cytokines. Specifically, we examined IL-6, TNF-α, and IL-1β, before and repeatedly after TSST or placebo-TSST while accounting for salivary flow rate and other potential confounders. In addition, we included the measurement of key stress hormones – epinephrine, norepinephrine, and cortisol – to explore potential endocrine mechanisms underlying stress-induced changes in salivary cytokines.

5.1. Effects and kinetics of acute stress on salivary IL-6 and IL-1β

Our main finding was that acute psychosocial stress, as compared to an active placebo-psychosocial stress control condition, induced significantly greater increases in the salivary cytokines IL-6 and IL-1β, but not TNF- α with peak concentrations immediately after the TSST cessation. These effects were of medium to large statistical effect sizes and were observed both with and without correction for salivary flow rate, as well as with and without controlling for age and BMI as confounding variables. Notably, in line with previous literature (Nater et al., 2005), salivary flow rate was reduced in the TSST group as compared to the control group (see Appendix A) which underlines the need to correct for salivary flow rate. Our findings are in line with previous salivary IL-6 findings in healthy and obese participants at risk for cardiovascular disease (CVD) (Izawa et al., 2013; Saban et al., 2018) but extend these studies in two ways. First, by incorporating a placebo-stress control group we can attribute the observed increases in salivary cytokines to the psychosocial stress components of the TSST rather than secondary physical and cognitive demands of the task. Second, although Izawa et al. (2013) considered correcting salivary cytokines for salivary flow rate, they ultimately decided against it, while Saban et al. (2018) explicitly stated that they did not apply any corrections for salivary flow (Izawa et al., 2013; Saban et al., 2018). Therefore, our findings demonstrate that stress-induced increases in salivary IL-6 persist even after controlling for salivary flow rate and other confounding variables and can be extended for the first time to salivary IL-1β. Notably, a previous study by Quinn et al. (2020) included a placebo-stress control condition, but did not find any significant differences in salivary IL-6, IL-1β and TNF-α levels between groups (Quinn et al., 2020). This discrepancy might be explained by the timing of sample collection in that study at + 45 min after TSST which most likely missed earlier peak salivary cytokine stress responses. Our study, similar to previous research (Izawa et al., 2013; Saban et al., 2018), found peak salivary cytokine responses immediately after TSST cessation with levels returning to baseline after + 30 min.

5.2. Interrelations between stress-reactive saliva and plasma cytokines

Regarding the relationship between stress-reactive salivary and plasma cytokines, the only stress-reactive cytokine with reliable assessment both in saliva and plasma was IL-6. We observed that higher *total* aggregated salivary IL-6 levels within the first 30minpost-stress (sIL-6 $AUC_{g\ baseline–30min}$) significantly predicted higher *total* aggregated plasma IL-6 levels up to 90 min post-stress (pIL-6 $AUC_{g\ baseline–90min}$). Similarly, when examining changes in aggregated salivary and plasma IL-6 levels over the same timeframes, we also found a significant positive interrelation. Moreover, we observed significant associations between salivary and plasma IL-6 levels at baseline and across multiple time points post-stress. However, peak salivary IL-6 levels immediately post-stress did not predict later peak plasma IL-6 levels. In sum, despite the observed associations in aggregated salivary and plasma IL-6, the reactivity kinetics to acute stress induction differed

with an earlier onset and recovery in salivary cytokines. To the best of our knowledge, only one other study using the TSST has investigated interrelations between salivary and plasma IL-6 levels but did not find any associations (Saban et al., 2018). Therefore, our results deviate from those observed by Saban et al. (2018), which can most likely be explained by differences in participant characteristics. While we investigated healthy young men, Saban et al. (2018) examined obese women at risk for CVD. Notably, both higher BMI and increased CVD risk have been linked to altered levels of salivary and plasma cytokines, which may have influenced the cytokine dynamics observed in their study (Kosaka et al., 2014; Lehmann et al., 2020; Lehmann-Kalata et al., 2018; Moghbeli et al., 2021; Ostrowska et al., 2023; Wang and He, 2018). When considering other forms of psychological stress, our results align with those of La Fratta et al. (2018), who investigated associations between several salivary and plasma cytokines, including IL-6, in male students 30 min after an academic exam (La Fratta et al., 2018). Similar to our findings, La Fratta et al. (2018) observed a significant positive association between salivary and plasma IL-6 levels, albeit in a field setting. Our study extends these findings to a controlled laboratory environment. Furthermore, our results are in line with a study examining the relationship between salivary and plasma cytokines in non-stressed but otherwise healthy older adults that observed a significant positive correlation between salivary and plasma IL-6 levels (Parkin et al., 2023). We hypothesize that the observed associations between salivary and plasma IL-6 responses to acute psycho-social stress in our study may reflect a shared underlying inflammatory response across multiple biological compartments. However, variations in the production and release of IL-6 from different cell types including salivary gland endothelial cells and peripheral immune cells might explain the differing stress reactivity kinetics between salivary and plasma IL-6 (for comprehensive discussion see Appendix B) (Dhabhar, 2018; Holmberg and Hoffman, 2014; Marsland et al., 2017; Mathison et al., 1994; Proctor and Carpenter, 2007; Tanda et al., 1998; Zhang et al., 2021). Notably, we cannot rule out that confounding influences may have added to the observed differences in stress reactivity kinetics between salivary and plasma cytokines after TSST. For example, dental hygiene, gingivitis, periodontal disease, or oral cancer have been shown to influence salivary cytokines levels (Belstrøm et al., 2017; Slavish et al., 2015).

5.3. *Interrelations between changes in stress hormones and stress-reactive salivary cytokines*

With respect to associations between stress-induced changes in salivary cytokines and sympathetic markers, we found that increases in norepinephrine from baseline to 10 min post-stress significantly predicted increases in salivary IL-1β within 30 min post-stress, independent of epinephrine and cortisol responses. Post-hoc analyses revealed a significant association immediately post-stress, with no associations observed at other measurement time points. For salivary IL-6, we could not observe any independent associations with stress hormones.

To the best of our knowledge, there is only one study that directly examined the relationship between salivary cytokine responses to acute stress induced by the TSST and cortisol. Deviating from our findings, that study observed a negative correlation between cortisol and salivary IL-6 (Izawa et al., 2013). Notably, Izawa et al. included both male and female participants without correction for salivary flow rate, whereas our study focused exclusively on young men and we corrected for salivary flow rate. Existing literature indicates that cortisol and IL-6 responses to stress can vary significantly by biological sex. (Kirschbaum et al., 1992; Kudielka and Kirschbaum, 2005; Lockwood et al., 2016; Rohleder et al., 2001). Finally, as no previous study has examined the association between stress-induced salivary cytokine responses to TSST and catecholamines (epinephrine and norepinephrine), our findings represent novel evidence indicating differential regulation of cytokine responses to stress in saliva. In line with our salivary IL-1β findings, previous in vitro research demonstrated that norepinephrine can stimulate the release of IL-1β from immune cells, especially monocytes and macrophages, through α1-adrenergic receptors (Grisanti et al., 2011a; Grisanti et al., 2011b; Horstmann et al., 2016). In contrast, salivary IL-6 is likely released primarily from local salivary gland epithelial cells, where secretion can be directly triggered by sympathetic neural activation independently of immune cell adrenergic receptor activation (Holmberg and Hoffman, 2014; Mathison et al., 1994; Proctor and Carpenter, 2007). For discussion of interrelations between salivary cytokines see Appendix B.

Strengths of our study include the use of a placebo-controlled, single-blind, between-subject design that allows to address current gaps in literature. Additionally, the implementation of rigorous exclusion criteria as well as the statistical control for age, BMI, and salivary flow rate reduced the potential influence of confounding variables. Lastly, additional consideration of endocrine stress markers allows a broader understanding of the potential physiological mechanisms underlying the observed salivary cytokine responses to acute psychosocial stress. Nevertheless, our study has several limitations. First and foremost, a major limitation of our study is that we included only healthy young male participants. This substantially restricts the generalizability of our findings to women, older participants, or clinical populations. Second, we did not control for dental hygiene, which may influence salivary cytokine levels due to variability in oral health. However, none of the participants reported oral inflammation and participants were assigned randomly to the TSST or placebo-TSST groups. Third, while we used a randomized group assignment procedure, we did not additionally control for further potential confounders such as testosterone levels, which have been shown to relate to both the regulation of stress hormones as well as inflammatory markers (Bianchi, 2019; Mohamad et al., 2019).

6. Conclusion

Taken together, our study demonstrated that acute psychosocial stress induces rapid and transient increases in the salivary cytokines IL-6 and IL-1β, with peak levels occurring immediately after stress cessation. These stress-specific effects were absent in the placebo-stress control group and remained robust after controlling for potential confounding variables, including salivary flow rate. Moreover, we observed associations between salivary and plasma IL-6 responses as well as differential associations with endocrine stress markers – particularly between norepinephrine and salivary IL-1β –suggesting distinct regulatory pathways for stress-induced cytokine responses. These findings underscore the potential of salivary cytokine measurements as a valid, sensitive, and non-invasive tool for capturing acute inflammatory responses to psychosocial stress. Future research should extend this work to women, older populations and clinical contexts to better understand the role of stress-induced triggering of inflammation in health and disease.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author(s) used ChatGPT in order to improve readability and language. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

CRediT authorship contribution statement

Marvin Fischer: Writing – review & editing, Writing – original draft, Visualization, Methodology, Formal analysis, Data curation. **Lisa-Marie Walther:** Writing – review & editing, Investigation, Formal analysis, Data curation. **Angelina Gideon:** Writing – review & editing, Investigation. **Christine Sauter:** Writing – review & editing, Investigation, Formal analysis. **Christiane Waller:** Writing – review & editing. **Ivano Amelio:** Writing – review & editing. **Petra H. Wirtz:** Writing – review & editing, Writing – original draft, Supervision, Resources, Project

Table A1
Regression analysis between individual measurement timepoints of salivary Il-6 and IL-1β

Salivary IL-1β	Salivary IL-6	*β*	*p*
MZP2 (−1 min)	MZP2 (−1 min)	0.63	**<.001**
	MZP3 (+1 min)	0.49	**<.001**
	MZP4 (+10 min)	0.39	**.007**
	MZP6 (+30 min)	0.39	**.007**
MZP3 (+1 min)	MZP2 (−1 min)	0.56	**<.001**
	MZP3 (+1 min)	0.57	**<.001**
	MZP4 (+10 min)	0.28	.054
	MZP6 (+30 min)	0.34	**.018**
MZP4 (+10 min)	MZP2 (−1 min)	0.42	**.003**
	MZP3 (+1 min)	0.39	**.007**
	MZP4 (+10 min)	0.27	.068
	MZP6 (+30 min)	0.25	.092
MZP6 (+30 min)	MZP2 (−1 min)	0.56	**<.001**
	MZP3 (+1 min)	0.48	**<.001**
	MZP4 (+10 min)	0.40	**.005**
	MZP6 (+30 min)	0.48	**<.001**

Table A2
Regression analysis between individual measurement timepoints of salivary and plasma Il-6.

Salivary IL-6	Salivary IL-6	*β*	*p*
MZP2 (−1 min)	MZP2 (−1 min)	0.33	**0.022**
	MZP3 (+1 min)	0.27	0.069
	MZP4 (+10 min)	0.23	0.12
	MZP6 (+30 min)	0.30	**0.040**
MZP3 (+1 min)	MZP2 (−1 min)	0.38	**0.008**
	MZP3 (+1 min)	0.30	**0.041**
	MZP4 (+10 min)	0.32	**0.027**
	MZP6 (+30 min)	0.36	**0.011**
MZP4 (+10 min)	MZP2 (−1 min)	0.32	**0.031**
	MZP3 (+1 min)	0.34	**0.018**
	MZP4 (+10 min)	0.36	**0.011**
	MZP6 (+30 min)	0.35	**0.016**
MZP6 (+30 min)	MZP2 (−1 min)	0.30	**0.043**
	MZP3 (+1 min)	0.34	**0.019**
	MZP4 (+10 min)	0.38	**0.008**
	MZP6 (+30 min)	0.40	**0.006**
MZP7 (+45 min)	MZP2 (−1 min)	0.25	0.091
	MZP3 (+1 min)	0.28	0.056
	MZP4 (+10 min)	0.33	**0.025**
	MZP6 (+30 min)	0.36	**0.013**
MZP9 (+90 min)	MZP2 (−1 min)	0.09	0.54
	MZP3 (+1 min)	0.20	0.19
	MZP4 (+10 min)	0.12	0.40
	MZP6 (+30 min)	0.09	0.094

administration, Funding acquisition, Conceptualization.

Funding

This research was funded by the German Research Foundation (Deutsche Forschungsgemeinschaft, DFG): DFG Centre of Excellence "Centre for the Advanced Study of Collective Behaviour" (EXC 2117-422037984), the Swiss National Science Foundation (PP00P1_128565/1), the German Scholars Organization (GSO/CZS 2) (all to PHW).

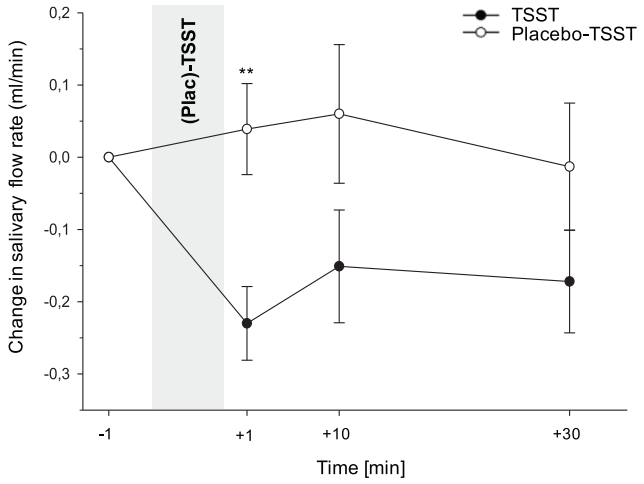

Fig. 4. Absolute changes in salivary flow rate. Changes in salivary flow rate between TSST and PlacTSST groups in ml/min. Asterisks denote significant differences between the TSST and PlacTSST groups ($p < 0.10$; *$p < 0.05$; **$p < 0.001$). Age and BMI were included as covariates. Data are presented as mean ± SEM.

Institutional review board statement

The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of the University of Konstanz, Germany.

Informed consent statement

Informed consent was obtained from all subjects involved in the study.

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.

Acknowledgment

We thank all students who helped in participant enrollment, study conduction, and data acquisition.

Appendix A

Additional results

Salivary flow rate reactivity to TSST and PlacTSST

When comparing changes in salivary flow from baseline to + 30 min between groups, we observed marginally significant differences in salivary flow between TSST and Plac-TSST groups (interaction effect group-by-time: $F(3.0, 135.67) = 2.31$, $p = 0.080$, $\eta_p^2 = 0.05$, $f = 0.22$). Post-hoc analysis revealed significantly lower salivary flow rate in the TSST group at + 1 min post-stress ($p = 0.002$) but no other timepoint (p's≥.097). Further post-hoc testing within groups separately revealed a significant time effect within the TSST group $F(2.66,77.11) = 4.58$, $p = 0.007$, $\eta_p^2 = 0.14$, $f = 0.40$) but not the Plac-TSST group ($p = 0.75$). In

TSST participants, salivary flow rate was (marginally) significantly decreased at all post-TSST measurement timepoints (+1, +10 and + 30 min) compared to baseline (p's$\leq$.069).

Appendix B

Additional discussion

Differences in stress reactivity kinetics between salivary and plasma IL-6

Salivary IL-6 is likely released in response to sympathetic activation of the salivary glands, which directly stimulates IL-6 secretion from salivary gland epithelial cells (Holmberg and Hoffman, 2014; Mathison et al., 1994; Proctor and Carpenter, 2007; Tanda et al., 1998). This might result in the observed immediate but short-lived increase in salivary IL-6 post-stress. In contrast, plasma IL-6 levels increase more gradually, likely due to catecholamine-driven mobilization of immune cells from different body compartments into the blood and heightened biosynthesis of IL-6 by peripheral immune and endothelial cells (Dhabhar, 2018; Zhang et al., 2021). The latter process requires immune cell activation and cytokine release into the circulation, which might lead to a delayed but sustained IL-6 response in plasma (Marsland et al., 2017). These mechanistic differences might account for the earlier peak and faster recovery observed in salivary IL-6 compared to plasma IL-6 following acute stress.

Interrelations between salivary cytokines

With respect to interrelations between stress-reactive salivary cytokines, we found that higher *total* aggregated IL-6 levels were significantly associated with higher *total* aggregated IL-1β levels within the first 30 min post-stress. However, no association was observed when examining *changes* in aggregated salivary IL-6 and IL-1β levels over time in response to stress ($AUC_{i\ \text{baseline}\ -30\ \text{min}}$). Our observation that individuals who produce higher total amounts of salivary IL-6 also tend to produce higher amounts of IL-1β is in line with previous research in children reporting positive intercorrelations between levels of salivary cytokines, including IL-6 and IL-1β (Riis et al., 2015). To the best of our knowledge this is the first study to investigate interrelations between changes in salivary cytokines in response to acute stress induced via TSST in healthy adults. Therefore, the observation that the degree to which IL-6 increases from baseline in response to stress does not predict the degree to which IL-1β increases, represents a novel finding. The lack of association in stress-induced aggregated changes (i.e., AUC_i scores) of salivary cytokines suggests that the dynamic responses of IL-6 and IL-1β to acute stress may be regulated by independent biological mechanisms or that the cytokines exhibit different sensitivities to stress-related pathways. These findings highlight the importance of distinguishing between total cytokine output and acute stress-induced changes when assessing salivary cytokine stress responses.

Data availability

Data will be made available on request.

References

Allen, A.P., Kennedy, P.J., Cryan, J.F., Dinan, T.G., Clarke, G., 2014. Biological and psychological markers of stress in humans: focus on the trier social stress test. Neurosci. Biobehav. Rev. 38, 94–124. https://doi.org/10.1016/j.neubiorev.2013.11.005.

Allen, A.P., Kennedy, P.J., Dockray, S., Cryan, J.F., Dinan, T.G., Clarke, G., 2017. The trier social stress test: principles and practice. Neurobiol. Stress 6, 113–126. https://doi.org/10.1016/j.ynstr.2016.11.001.

Angstwurm, M.W., Gärtner, R., Ziegler-Heitbrock, H.W., 1997. Cyclic plasma IL-6 levels during normal menstrual cycle. Cytokine 9 (5), 370–374. https://doi.org/10.1006/cyto.1996.0178.

Auer, A., Walther, L.-M., Jendryczko, D., Auer, L., & Wirtz, P. H. (2024, 2024/04/01/). Is your stress my stress? A standardized, randomized-controlled paradigm to study physiological stress contagion based on direct stress observation. *Psychoneuroendocrinology,* 162, 106964. https://doi.org/10.1016/j.psyneuen.2024.106964.

Beis, D., von Känel, R., Heimgartner, N., Zuccarella-Hackl, C., Bürkle, A., Ehlert, U., Wirtz, P.H., 2018. The role of norepinephrine and α-adrenergic receptors in acute stress-induced changes in granulocytes and monocytes. Biopsychosoc. Sci. Med. 80 (7), 649–658. https://doi.org/10.1097/psy.0000000000000620.

Belstrøm, D., Damgaard, C., Könönen, E., Gürsoy, M., Holmstrup, P., Gürsoy, U.K., 2017. Salivary cytokine levels in early gingival inflammation. J. Oral Microbiol. 9 (1), 1364101. https://doi.org/10.1080/20002297.2017.1364101.

Bianchi, V.E., 2019. The Anti-Inflammatory Effects of Testosterone. J. Endocr. Soc. 3 (1), 91–107. https://doi.org/10.1210/js.2018-00186.

Blanca, M.J., Alarcón, R., Arnau, J., Bono, R., Bendayan, R., 2017. Non-normal data: is ANOVA still a valid option? Psicothema 29 (4), 552–557. https://doi.org/10.7334/psicothema2016.383.

Blanca, M.J., Arnau, J., García-Castro, F.J., Alarcón, R., Bono, R., 2023. Non-normal data in repeated measures ANOVA: impact on type i error and power. Psicothema 35 (1), 21–29. https://doi.org/10.7334/psicothema2022.292.

Cohen, J., 1988. Statistical power analysis for the behavioural sciences. Erlbaum.

Dhabhar, F.S., 2018. The short-term stress response – Mother nature's mechanism for enhancing protection and performance under conditions of threat, challenge, and opportunity. Front. Neuroendocrinol. 49, 175–192. https://doi.org/10.1016/j.yfrne.2018.03.004.

Dill, D.B., Costill, D.L., 1974. Calculation of percentage changes in volumes of blood, plasma, and red cells in dehydration. J. Appl. Physiol. 37 (2), 247–248. https://doi.org/10.1152/jappl.1974.37.2.247.

Ehrenreich, H., Schuck, J., Stender, N., Pilz, J., Gefeller, O., Schilling, L., Poser, W., Kaw, S., 1997. Oct). Endocrine and hemodynamic effects of stress versus systemic CRF in alcoholics during early and medium term abstinence. Alcohol. Clin. Exp. Res. 21 (7), 1285–1293.

Engeland, C.G., Bosch, J.A., Rohleder, N., 2019. Salivary biomarkers in psychoneuroimmunology. Curr. Opin. Behav. Sci. 28, 58–65. https://doi.org/10.1016/j.cobeha.2019.01.007.

Gervasio, J., Zheng, S., Skrotzki, C., & Pachete, A. (2022, 2022/02/01/). The effect of oral contraceptive use on cortisol reactivity to the Trier Social Stress Test: A meta-analysis. *Psychoneuroendocrinology,* 136, 105626. https://doi.org/https://doi.org/10.1016/j.psyneuen.2021.105626.

Gideon, A., Sauter, C., Fieres, J., Berger, T., Renner, B., Wirtz, P.H., 2020. Kinetics and interrelations of the renin aldosterone response to acute psychosocial stress: a neglected stress system. J. Clin. Endocrinol. Metabol. 105 (3), dgz190. https://doi.org/10.1210/clinem/dgz190.

Gideon, A., Sauter, C., Pruessner, J.C., Farine, D.R., Wirtz, P.H., 2022. Determinants and mechanisms of the renin-aldosterone stress response. Psychosom. Med. 84 (1), 50–63. https://doi.org/10.1097/psy.0000000000001018.

Grisanti, L.A., Perez, D.M., Porter, J.E., 2011a. Modulation of immune cell function by α(1)-adrenergic receptor activation. Curr. Top. Membr. 67, 113–138. https://doi.org/10.1016/b978-0-12-384921-2.00006-9.

Grisanti, L. A., Woster, A. P., Dahlman, J., Sauter, E. R., Combs, C. K., & Porter, J. E. (2011, Aug). α1-adrenergic receptors positively regulate Toll-like receptor cytokine production from human monocytes and macrophages. *J Pharmacol Exp Ther,* 338 (2), 648-657. https://doi.org/10.1124/jpet.110.178012.

Het, S., Rohleder, N., Schoofs, D., Kirschbaum, C., Wolf, O.T., 2009. Neuroendocrine and psychometric evaluation of a placebo version of the 'Trier Social stress Test'. Psychoneuroendocrinology 34 (7), 1075–1086. https://doi.org/10.1016/j.psyneuen.2009.02.008.

Het, S., Schoofs, D., Rohleder, N., Wolf, O.T., 2012. Stress-induced cortisol level elevations are associated with reduced negative affect after stress: indications for a mood-buffering cortisol effect. Psychosom. Med. 74 (1), 23–32. https://doi.org/10.1097/PSY.0b013e31823a4a25.

Hinterdobler, J., Schott, S., Jin, H., Meesmann, A., Steinsiek, A.L., Zimmermann, A.S., Wobst, J., Müller, P., Mauersberger, C., Vilne, B., Baecklund, A., Chen, C.S., Moggio, A., Braster, Q., Molitor, M., Krane, M., Kempf, W.E., Ladwig, K.H., Hristov, M., Hulsmans, M., Hilgendorf, I., Weber, C., Wenzel, P., Scheiermann, C., Maegdefessel, L., Soehnlein, O., Libby, P., Nahrendorf, M., Schunkert, H., Kessler, T., Sager, H.B., 2021. Oct 14). Acute mental stress drives vascular inflammation and promotes plaque destabilization in mouse atherosclerosis. Eur. Heart J. 42 (39), 4077–4088. https://doi.org/10.1093/eurheartj/ehab371.

Holmberg, K. V., & Hoffman, M. P. (2014). Anatomy, Biogenesis and Regeneration of Salivary Glands. In A. J. M. Ligtenberg & E. C. I. Veerman (Eds.), *Saliva: Secretion and Functions* (Vol. 24, pp. 0). S.Karger AG. https://doi.org/10.1159/000358776.

Horstmann, J.P., Marzi, I., Relja, B., 2016. Adrenergic stimulation alters the expression of inflammasome components and interleukins in primary human monocytes. Exp. Ther. Med. 11 (1), 297–302. https://doi.org/10.3892/etm.2015.2850.

Izawa, S., Sugaya, N., Kimura, K., Ogawa, N., Yamada, K.C., Shirotsuki, K., Mikami, I., Hirata, K., Nagano, Y., Nomura, S., 2013. Oct). an increase in salivary interleukin-6 level following acute psychosocial stress and its biological correlates in healthy young adults. Biol. Psychol. 94 (2), 249–254. https://doi.org/10.1016/j.biopsycho.2013.06.006.

Kirschbaum, C., Kudielka, B.M., Gaab, J., Schommer, N.C., Hellhammer, D.H., 1999. Impact of gender, menstrual cycle phase, and oral contraceptives on the activity of the hypothalamus-pituitary-adrenal axis. Biopsychosocial Science and Medicine 61 (2). https://journals.lww.com/bsam/fulltext/1999/03000/impact_of_gender,_menstrual_cycle_phase,_and_oral.6.aspx.

Kirschbaum, C., Pirke, K.M., Hellhammer, D.H., 1993. The 'Trier social stress Test'–a tool for investigating psychobiological stress responses in a laboratory setting. Neuropsychobiology 28 (1–2), 76–81. https://doi.org/10.1159/000119004.

Kirschbaum, C., Wüst, S., Hellhammer, D., 1992. Consistent sex differences in cortisol responses to psychological stress. Psychosom. Med. 54 (6), 648–657. https://doi.org/10.1097/00006842-199211000-00004.

Kosaka, T., Kokubo, Y., Ono, T., Sekine, S., Kida, M., Kikui, M., Yamamoto, M., Watanabe, M., Amano, A., Maeda, Y., Miyamoto, Y., 2014.. Salivary inflammatory cytokines may be novel markers of carotid atherosclerosis in a Japanese general population: the Suita study. Atherosclerosis 237 (1), 123–128. https://doi.org/10.1016/j.atherosclerosis.2014.08.046.

Kudielka, B.M., Kirschbaum, C., 2005. Apr). sex differences in HPA axis responses to stress: a review. Biol. Psychol. 69 (1), 113–132. https://doi.org/10.1016/j.biopsycho.2004.11.009.

Kuebler, U., Arpagaus, A., Meister, R.E., von Känel, R., Huber, S., Ehlert, U., Wirtz, P.H., 2016. Oct). Dark chocolate attenuates intracellular pro-inflammatory reactivity to acute psychosocial stress in men: a randomized controlled trial. Brain Behav. Immun. 57, 200–208. https://doi.org/10.1016/j.bbi.2016.04.006.

Kuebler, U., Zuccarella-Hackl, C., Arpagaus, A., Wolf, J.M., Farahmand, F., von Känel, R., Ehlert, U., Wirtz, P.H., 2015. May). Stress-induced modulation of NF-κB activation, inflammation-associated gene expression, and cytokine levels in blood of healthy men. Brain Behav. Immun. 46, 87–95. https://doi.org/10.1016/j.bbi.2014.12.024.

Kühnel, A., Hagenberg, J., Knauer-Arloth, J., Ködel, M., Czisch, M., Sämann, P.G., Brückl, T., Spoormaker, V.I., Erhardt, A., Grandi, N.C., Ziebula, J., Elbau, I.G., Lucae, S., Binder, E.B., Kroemer, N.B., Be, C.W.G., 2023. Stress-induced brain responses are associated with BMI in women. Commun. Biol. 6 (1), 1031. https://doi.org/10.1038/s42003-023-05396-8.

La Fratta, I., Tatangelo, R., Campagna, G., Rizzuto, A., Franceschelli, S., Ferrone, A., Patruno, A., Speranza, L., De Lutiis, M.A., Felaco, M., Grilli, A., Pesce, M., 2018. The plasmatic and salivary levels of IL-1β, IL-18 and IL-6 are associated to emotional difference during stress in young male. Sci. Rep. 8 (1), 3031. https://doi.org/10.1038/s41598-018-21474-y.

Lehmann, A.P., Nijakowski, K., Swora-Cwynar, E., Łuczak, J., Czepulis, N., Surdacka, A., 2020. Characteristics of salivary inflammation in obesity. Pol. Arch. Int. Med. 130 (4), 297–303. https://doi.org/10.20452/pamw.15186.

Lehmann-Kalata, A., Miechowicz, I., Korybalska, K., Swora-Cwynar, E., Czepulis, N., Łuczak, J., Orzechowska, Z., Grzymisławski, M., Surdacka, A., Witowski, J., 2018. Salivary fingerprint of simple obesity. Cytokine 110, 174–180. https://doi.org/10.1016/j.cyto.2018.05.006.

Lockwood, K.G., Marsland, A.L., Cohen, S., Gianaros, P.J., 2016. Nov). sex differences in the association between stressor-evoked interleukin-6 reactivity and C-reactive protein. Brain Behav. Immun. 58, 173–180. https://doi.org/10.1016/j.bbi.2016.07.001.

Lydersen, S., & Giskeødegård, G. F. (2022, 2022/09/19). Measurements below the detection limit. *Tidsskrift for Den norske legeforening*. https://tidsskriftet.no/en/2022/09/medicine-and-numbers/measurements-below-detection-limit.

Marsland, A.L., Walsh, C., Lockwood, K., John-Henderson, N.A., 2017. The effects of acute psychological stress on circulating and stimulated inflammatory markers: a systematic review and meta-analysis. Brain Behav. Immun. 64, 208–219. https://doi.org/10.1016/j.bbi.2017.01.011.

Mathison, R., Davison, J.S., Dean Befus, A., 1994. Neuroendocrine regulation of inflammation and tissue repair by submandibular gland factors. Immunol. Today 15 (11), 527–532. https://doi.org/10.1016/0167-5699(94)90209-7.

Matomäki, P., Kainulainen, H., Kyröläinen, H., 2018. Corrected whole blood biomarkers – the equation of Dill and Costill revisited. Physiol. Rep. 6 (12), e13749. https://doi.org/10.14814/phy2.13749.

Moghbeli, M., Khedmatgozar, H., Yadegari, M., Avan, A., Ferns, G.A., Ghayour Mobarhan, M., 2021. Chapter five - Cytokines and the immune response in obesity-related disorders. In: Makowski, G.S. (Ed.), Advances in Clinical Chemistry, Vol. 101. Elsevier, pp. 135–168.

Mohamad, N.V., Wong, S.K., Wan Hasan, W.N., Jolly, J.J., Nur-Farhana, M.F., Ima-Nirwana, S., Chin, K.Y., 2019. Jun). the relationship between circulating testosterone and inflammatory cytokines in men. Aging Male 22 (2), 129–140. https://doi.org/10.1080/13685538.2018.1482487.

Nater, U.M., Rohleder, N., Gaab, J., Berger, S., Jud, A., Kirschbaum, C., Ehlert, U., 2005. Human salivary alpha-amylase reactivity in a psychosocial stress paradigm. Int. J. Psychophysiol. 55 (3), 333–342. https://doi.org/10.1016/j.ijpsycho.2004.09.009.

Newton, T.L., Fernandez-Botran, R., Lyle, K.B., Szabo, Y.Z., Miller, J.J., Warnecke, A.J., 2017. Salivary cytokine response in the aftermath of stress: an emotion regulation perspective. Emotion 17 (6), 1007–1020. https://doi.org/10.1037/emo0000156.

O'Brien, S.M., Fitzgerald, P., Scully, P., Landers, A., Scott, L.V., Dinan, T.G., 2007. Impact of gender and menstrual cycle phase on plasma cytokine concentrations. Neuroimmunomodulation 14 (2), 84–90. https://doi.org/10.1159/000107423.

Ostrowska, L., Smarkusz-Zarzecka, J., Gornowicz, A., Lendzion, K., Zyśk, B., Pogodziński, D., 2023. Analysis of selected salivary adipokines and cytokines in patients with obesity-a pilot study. Int. J. Mol. Sci. 24 (4). https://doi.org/10.3390/ijms24044145.

Parkin, G.M., Kim, S., Mikhail, A., Malhas, R., McMillan, L., Hollearn, M., Granger, D.A., Mapstone, M., Yassa, M.A., Thomas, E.A., 2023. Jan). Associations between saliva and plasma cytokines in cognitively normal, older adults. Aging Clin. Exp. Res. 35 (1), 117–126. https://doi.org/10.1007/s40520-022-02292-9.

Proctor, G.B., Carpenter, G.H., 2007. Regulation of salivary gland function by autonomic nerves. Auton. Neurosci. 133 (1), 3–18. https://doi.org/10.1016/j.autneu.2006.10.006.

Pruessner, J.C., Kirschbaum, C., Meinlschmid, G., Hellhammer, D.H., 2003. Oct). two formulas for computation of the area under the curve represent measures of total hormone concentration versus time-dependent change. Psychoneuroendocrinology 28 (7), 916–931. https://doi.org/10.1016/s0306-4530(02)00108-7.

Quinn, M. E., Stanton, C. H., Slavich, G. M., & Joormann, J. (2020, Jan). Executive Control, Cytokine Reactivity to Social Stress, and Depressive Symptoms: Testing the Social Signal Transduction Theory of Depression. *Stress,* 23 (1), 60-68. https://doi.org/10.1080/10253890.2019.1641079.

Riis, J.L., Granger, D.A., DiPietro, J.A., Bandeen-Roche, K., Johnson, S.B., 2015. Salivary cytokines as a minimally-invasive measure of immune functioning in young children: correlates of individual differences and sensitivity to laboratory stress. Dev. Psychobiol. 57 (2), 153–167. https://doi.org/10.1002/dev.21271.

Rodríguez Medina, D., leija alva, G., Dominguez, B., Hernandez-Pozo, M. d. R., Cruz-Albarran, I. A., Morales-Hernández, L., & Marmolejo-Ramos, F. (2019). Effects of the Trier Social Stress Test on the distributions of IL-6 and MAP levels. *Heliyon,* 5, 1-9. https://doi.org/10.1016/j.heliyon.2019.e01580.

Rohleder, N., Schommer, N.C., Hellhammer, D.H., Engel, R., Kirschbaum, C., 2001. Sex differences in glucocorticoid sensitivity of proinflammatory cytokine production after psychosocial stress. Psychosom. Med. 63 (6), 966–972. https://doi.org/10.1097/00006842-200111000-00016.

Saban, K.L., Mathews, H.L., Bryant, F.B., Tell, D., Joyce, C., DeVon, H.A., Witek Janusek, L., 2018. Oct). Perceived discrimination is associated with the inflammatory response to acute laboratory stress in women at risk for cardiovascular disease. Brain Behav. Immun. 73, 625–632. https://doi.org/10.1016/j.bbi.2018.07.010.

Schmidt, A.F., Finan, C., 2018. Linear regression and the normality assumption. J. Clin. Epidemiol. 98, 146–151. https://doi.org/10.1016/j.jclinepi.2017.12.006.

Schneiderman, N., Ironson, G., Siegel, S.D., 2005. Stress and health: psychological, behavioral, and biological determinants. Annu. Rev. Clin. Psychol. 1, 607–628. https://doi.org/10.1146/annurev.clinpsy.1.102803.144141.

Segerstrom, S.C., Miller, G.E., 2004. Psychological stress and the human immune system: a meta-analytic study of 30 years of inquiry. Psychol. Bull. 130 (4), 601–630. https://doi.org/10.1037/0033-2909.130.4.601.

Slavish, D.C., Graham-Engeland, J.E., Smyth, J.M., Engeland, C.G., 2015. Salivary markers of inflammation in response to acute stress. Brain Behav. Immun. 44, 253–269. https://doi.org/10.1016/j.bbi.2014.08.008.

Stamou, M.I., Colling, C., Dichtel, L.E., 2023. Adrenal aging and its effects on the stress response and immunosenescence. Maturitas 168, 13–19. https://doi.org/10.1016/j.maturitas.2022.10.006.

Stephens, M.A.C., Mahon, P.B., McCaul, M.E., Wand, G.S., 2016. Hypothalamic–pituitary–adrenal axis response to acute psychosocial stress: Effects of biological sex and circulating sex hormones. Psychoneuroendocrinology 66, 47–55. https://doi.org/10.1016/j.psyneuen.2015.12.021.

Steptoe, A., Hamer, M., Chida, Y., 2007. The effects of acute psychological stress on circulating inflammatory factors in humans: a review and meta-analysis. Brain Behav. Immun. 21 (7), 901–912. https://doi.org/10.1016/j.bbi.2007.03.011.

Szabo, Y., Newton, T., Miller, J., Lyle, K., Fernández-Botrán, R., 2016. Acute stress induces increases in salivary IL-10 Levels. Stress 19, 1–28. https://doi.org/10.1080/10253890.2016.1206885.

Szabo, Y.Z., Fernandez-Botran, R., Newton, T.L., 2019. Cumulative trauma, emotion reactivity and salivary cytokine levels following acute stress in healthy women. Anxiety Stress Coping 32 (1), 82–94. https://doi.org/10.1080/10615806.2018.1524377.

Szabo, Y.Z., Slavish, D.C., Graham-Engeland, J.E., 2020. The effect of acute stress on salivary markers of inflammation: a systematic review and meta-analysis. Brain Behav. Immun. 88, 887–900. https://doi.org/10.1016/j.bbi.2020.04.078.

Tanda, N., Ohyama, H., Yamakawa, M., Ericsson, M., Tsuji, T., McBride, J., Elovic, A., Wong, D.T.W., Login, G.R., 1998. IL-1β and IL-6 in mouse parotid acinar cells: characterization of synthesis, storage, and release. Am. J. Physiol. Gastrointestinal Liver Physiol. 274 (1), G147–G156. https://doi.org/10.1152/ajpgi.1998.274.1.G147.

Tell, D., Mathews, H.L., Burr, R.L., Witek Janusek, L., 2018. During stress, heart rate variability moderates the impact of childhood adversity in women with breast cancer. Stress 21 (2), 179–187. https://doi.org/10.1080/10253890.2018.1424132.

Turner, A. I., Smyth, N., Hall, S. J., Torres, S. J., Hussein, M., Jayasinghe, S. U., Ball, K., & Clow, A. J. (2020, Apr). Psychological stress reactivity and future health and disease outcomes: A systematic review of prospective evidence. *Psychoneuroendocrinology,* 114, 104599. https://doi.org/10.1016/j.psyneuen.2020.104599.

Uh, H.W., Hartgers, F.C., Yazdanbakhsh, M., Houwing-Duistermaat, J.J., 2008. Evaluation of regression methods when immunological measurements are constrained by detection limits. BMC Immunol. 9, 59. https://doi.org/10.1186/1471-2172-9-59.

Walther, L.-M., Gideon, A., Sauter, C., Leist, M., Wirtz, P.H., 2024. Peripheral blood leukocyte subpopulation changes in reaction to an acute psychosocial stressor as compared to an active placebo-stressor in healthy young males: mediating effects of major stress-reactive endocrine parameters. Cells 13 (23), 1941. https://www.mdpi.com/2073-4409/13/23/1941.

Wang, T., He, C., 2018. Pro-inflammatory cytokines: the link between obesity and osteoarthritis. Cytokine Growth Factor Rev. 44, 38–50. https://doi.org/10.1016/j.cytogfr.2018.10.002.

Wirtz, P.H., Ehlert, U., Emini, L., Suter, T., 2008. Higher body mass index (BMI) is associated with reduced glucocorticoid inhibition of inflammatory cytokine production following acute psychosocial stress in men. Psychoneuroendocrinology 33 (8), 1102–1110. https://doi.org/10.1016/j.psyneuen.2008.05.002.

Zhang, R. G., Niu, Y., Pan, K. W., Pang, H., Chen, C. L., Yip, C. Y., & Ko, W. H. (2021, Dec). β(2)-Adrenoceptor Activation Stimulates IL-6 Production via PKA, ERK1/2, Src, and Beta-Arrestin2 Signaling Pathways in Human Bronchial Epithelia. *Lung,* 199 (6), 619-627. https://doi.org/10.1007/s00408-021-00484-0.