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Evaluation of the socially evaluated cold-pressor group test (SECPT-G) in the general population

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Background. In stress research, economic instruments for introducing acute stress responses are needed. In this study, we investigated whether the socially evaluated cold-pressor group test (SECPT-G) induces salivary alpha-amylase and/or cortisol responses in the general population and whether it is associated with anthropometric, experimental, and lifestyle factors.

Methods. A total of 91 participants was recruited. Salivary cortisol and alpha-amylase levels were assessed prior (t_0), immediately after (t_1), and ten minutes after the SECPT-G (t_2).

Results. A strong cortisol increase was found immediately after the SECPT-G, which further increased between t_1 and t2. This was independent of most of the control variables. However, men showed stronger cortisol increases than women. No sAA responses were found at all.

Conclusions. We conclude that the SECPT-G is a good means of an acute stress test when cortisol – but not necessarily sAA – responses are intended.

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Abstract

Background. In stress research, economic instruments for introducing acute stress responses are needed. In this study, we investigated whether the socially evaluated cold-pressor group test (SECPT-G) induces salivary alpha-amylase and/or cortisol responses in the general population and whether it is associated with anthropometric, experimental, and lifestyle factors.
Methods. A total of 91 participants was recruited. Salivary cortisol alpha-amylase levels were assessed prior (t_0), immediately after (t_1), and ten minutes after the SECPT-G (t_2).
Results. A strong cortisol increase was found immediately after the SECPT-G, which further increased between t_1 and t2. This was independent of most of the control variables. However, men showed stronger cortisol increases than women. No sAA responses were found at all.
Conclusions. We conclude that the SECPT-G is a good means of an acute stress test when cortisol – but not necessarily sAA – responses are intended.

Introduction

Eliciting stress responses is an important tool in bio-behavioral health research, but public speaking paradigms which are usually used are labor intensive and, therefore, an impediment to recruiting larger samples. One standard procedure in stress research is the socially evaluated cold-pressor test (SECPT; Schwabe, Haddad, & Schachinger, 2008). The SECPT combines a physiological stressor (immersing one's hand in ice water; e.g., Lovallo, 1975) with socially-evaluative components (being watched by the experimenter and being videotaped by a camera). The SECPT is an economic alternative to other stress tests (e.g., the Trier Social Stress Test

(TSST); Kirschbaum, Pirke, & Hellhammer, 1993 or the Maastricht Acute Stress Test (MAST); Smeets et al., 2012) because it only takes a few minutes, and because it can be performed with only one experimenter.

In 2014, Minkley and colleagues showed that the SECPT can also be performed in groups (socially evaluated cold-pressor test for groups, SECPT-G) and that this is, thus, an even more economic variant of the original SECPT set-up (Minkley, Schröder, Wolf, & Kirchner, 2014). Minkley and colleagues evaluated the SECPT-G in a sample of 61 middle-aged, normal weight, non-smoking participants. They found strong cardiovascular (blood pressure and heart rate variability) and hypothalamic-pituitary-adrenal (HPA) axis (cortisol) responses. To the best of our knowledge, the SECPT-G has not been evaluated in the general population so far.

However, it has been shown previously that demographic, anthropometric, and lifestyle factors are associated with the stress response. Although there are divergent findings, a stronger HPA axis response has been found in young men than in young women (Kirschbaum, Wüst, & Hellhammer, 1992; Kudielka & Kirschbaum, 2005; Stephens, Mahon, McCaul, & Wand, 2016). Furthermore, a delayed post stress recovery has been found in women (Owen, Poulton, Hay, Mohamed-Ali, & Steptoe, 2003). Moreover, in women, stress response has been associated with the phase of the menstrual cycle and with the use of oral contraceptives (Kirschbaum, Kudielka, Gaab, Schommer, & Hellhammer, 1999). It has been found that age is negatively related with HPA axis response (i.e., cortisol secretion after an acute stressor is decreased in older adults; Kudielka, Buske-Kirschbaum, Hellhammer, & Kirschbaum, 2004). Furthermore, it has been found that people with higher BMI show decreased HPA axis responses to acute psycho-social stressors (Jones et al., 2012). However, in other studies, positive associations between BMI and cortisol response to an acute stressor were found (McInnis et al., 2014). Moreover, it has been found that people with a low socio-economic status and a low income show stronger HPA axis responses (Owen et al., 2003). Lifestyle factors can influence HPA axis reactivity as well. It has been found that smoking leads to chronically elevated cortisol levels and to a reduced response to acute stressors (Kirschbaum, Wüst, & Strasburger, 1992; Kudielka, Hellhammer, & Wüst, 2009; Rohleder & Kirschbaum, 2006). Furthermore, although previous findings are divergent, chronic stress can affect the acute stress response as well (e.g., Kudielka, Bellingrath, & Hellhammer, 2006). Besides, regular physical activity also affects HPA axis activity and, therefore, the response to acute stressors (Luger et al., 1987). From all these studies, it becomes clear that it is necessary that an evaluation of a stress paradigm should be performed in a broad population and that the effects of demographic, anthropometric, and lifestyle factors should be considered. Therefore, in our study, we expanded the sample to a more general population (i.e., including all age and weight groups, as well as smokers).

Furthermore, in none of the previous studies it has been investigated whether the SECPT or the SECPT-G also leads to an increase in salivary alpha-amylase (sAA) secretion. Some authors suggest – although there are some valid concerns that need to be taken into account (e.g., Bosch, Veerman, Geus, & Proctor, 2011) – that sAA can be used as a marker for sympathetic nervous system activity (e.g., Nater, Rohleder, Schlotz, Ehlert, & Kirschbaum, 2007; Rohleder & Nater,

2009) and, therefore, it should absolutely be investigated in stress studies as well. Hence, in our study, we investigated whether a sAA response could be elicited by means of the SECPT-G. Our approach was threefold. First, we investigated whether the SECPT-G introduces an HPA axis response (i.e., a cortisol increase) in the general population. Second, we examined whether the SECPT-G also induces an SNS response (i.e., an increase in sAA). Third, we explored whether anthropometric, and lifestyle factors (e.g., age, BMI, sex, use of oral contraceptives, physical activity, smoking, chronic stress) as well as experimental (time of day and immersion time) are associated with the physiological stress responses (HPA axis and SNS) to the SECPT-G.

Materials & Methods

Participants

The SECPT-G was evaluated in a sample of $N = 96$. The participants came to our laboratory in the context of a public event (open day of the university) and were then asked whether they would like to participate in a stress experiment. Because of missing data, five participants had to be excluded from statistical analysis. The remaining $N = 91$ participants had a mean age of 36.8 ± 14.3 years (min: 18 years, max: 73 years) and a BMI of 24.1 ± 3.7 kg/m^2 (min: 16.1, max: 35.4). Forty-three (47.3%) of the participants were male, eleven (12.1%) were smokers, and 22 (24.2%) had already consumed alcoholic beverages on the experimental day, but no one had consumed more than the equivalent of two drinks and no one had consumed alcoholic beverages within two hours before the experiment. Mean activity levels were 4921 ± 5169 metabolic minutes per week (min: 0, max: 27810). Most of the participants were German ($N = 80$, 87.9%). The others were Greek ($N = 4$, 2.2%), Austrian ($N = 3$, 3.3%), Russian ($N = 3$, 3.3%), or Italian ($N = 1$, 1.1%). The nationality of one participant was not specified. As graduation, one of the participants reported (1.1%) certificate of secondary education (‘Hauptschulabschluss’), 19 (29.2%) secondary school level (‘mittlere Reife’), 5 (5.5%) graduation (‘Ausbildung’), 10 (11%) vocational diploma (‘Fachabitur’), 23 (25.3%) general qualification for university entrance (‘Abitur’), 9 (9.9%) Bachelor degree, 17 (18.7%) diploma or master degree, and 4 (4.4%) PhD. Three (3.3 %) of the participants did not report their graduation. As current occupational position, 35 (38.5%) of the participants reported full-time employee, 17 (18.7%) student, 14 (15.4%) part-time employee, 9 (9.9%) unemployed, 8 (8.8%) self-employed, 4 (4.4%) PhD student, and 3 (3.3%) retired. Seven (14.6%) of the female participants reported usage of an oral contraceptive. All participants reported that they had not eaten or consumed beverages at least one hour before the start of the experiment. All participants gave their written and informed consent. The study was carried out in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki) and was approved by the local ethics committee of the Friedrich-Alexander University Erlangen-Nuremberg (# 6_18 B).

Procedure

General Procedure. The experiment was performed twelve times in groups of eight participants on one evening between 18:30 h and 00:30 h. Each session lasted about 25 minutes. Participants were informed that they would take part in a stress experiment. After they gave their consent for participation, they waited in a room that was not the experimental room, where they disinfected their hands, and rinsed their mouth with water. This lasted about five minutes. After this, they were brought as a group to the experimental room where they were made familiar with the saliva collection procedure. Saliva was collected by means of salivettes (Sarstedt, Nümbrecht, Germany). During saliva collection, subjective stress perception was rated on a ten-point Likert scale with the anchors "not stressed at all" and "extremely stressed". Subsequently after instruction, the first saliva sample (t_0) was collected. After this, the SECPT-G (see below for further specifications) was explained and then started immediately. The second saliva sample (t_1) was collected immediately after the SECPT-G. To fill the gap between the third saliva sample (t_2) which was collected ten minutes after the SECPT-G, participants filled out some questionnaires (see below).

Stress induction. In the experimental room, all participants were asked to stand around a large table with transparent boxes filled with ice water in front of them. They were instructed to immerse their hands in the ice water as long as possible for up to three minutes. Mean immersion time was 2:30 ± 0:55 min (max: 3:00 min., min: 0:39 min.). The hand of each participant was directly opposite of the hand of another person with the aim to introduce a competitive situation. Remaining time was displayed on a large-display digital clock that was visible for all of the participants. An auditory countdown announced the last five seconds. Therefore, our protocol slightly differed from that reported by Schwabe and colleagues (2008) and Minkley and colleagues (2014) because in those previous studies no countdown was used. Another difference was that we did not use a camera. Two experimenters were present during the SECPT-G. They wore medical uniforms and were instructed to behave distanced and have a neutral mimic.

Assessment of demographic variables and lifestyle factors. Between t_1 and t_2, participants filled out questionnaires which assessed demographic variables (e.g., age, sex, weight, height, graduation, profession, and whether they were smokers). Furthermore, the screening scale of the Trier Inventory of Chronic Stress (TICS-SSCS; Schulz & Schlotz, 1995) and the short form of the International Physical Activity Questionnaire (IPAQ; Craig et al., 2003) were filled-out by the participants.

Sample processing

Saliva samples were stored at -30 °C after collection for later analyses. Before cortisol and sAA measurement, two freeze-thaw cycles were performed. Immediately before measurement, samples were centrifuged at 2000 g and 20 °C for ten minutes. Salivary alpha-amylase was measured with an in-house enzyme kinetic assay using reagents from Roche Diagnostics (Mannheim, Germany), as previously described (Bosch, Geus, Veerman, Hoogstraten, & Amerongen, 2003; Rohleder & Nater, 2009). In brief, saliva was diluted at 1:625 with ultrapure

water, and diluted saliva was incubated with substrate reagent (α-amylase EPS Sys; Roche Diagnostics) at 37° C for three minutes before a first absorbance reading was taken at 405 nm with a Tecan Infinite 200 PRO reader (Tecan, Crailsheim, Germany). A second reading was taken after five minutes incubation at 37 °C and increase in absorbance was transformed to sAA concentration (U/ml), using a standard curve prepared using "Calibrator f.a.s." solution (Roche Diagnostics). Salivary cortisol concentrations were determined in duplicate using chemiluminescence immunoassay (CLIA, IBL, Hamburg, Germany). Intra- and inter-assay coefficients of variation were below 10% for both sAA and cortisol.

Statistical analysis

Analyses of variance for repeated measurements (rmANOVAs) with the within-subject factor time (t_0, t_1, t_2) were calculated. As post-hoc tests, *t*-tests with adjusted alpha levels according to the Bonferroni correction were calculated. Partial eta-squares (η_p^2) for ANOVAs and Cohen's *d* for t-tests were considered as measures of effect sizes If necessary, Cohen's *d* was corrected according to the method that was proposed by Morris (2008). To investigate whether one of the control variables (age, sex, BMI, graduation, profession, use of oral contraceptive, amount of physical activity, chronic stress, smoking, time of day, and immersion time) was responsible for the main effect of the factor time, these variables were entered as covariates into further rmANOVAs. For further analysis of significant effects of the covariates, bivariate Pearson correlations r were calculated. For these analyses, an adjusted alpha level of $\alpha = .05/11 = .005$ was used because 11 control variables were investigated. Because of positive skewness, sAA and cortisol levels were transformed by means of the natural logarithm prior to statistical analysis.

Results

Subjective stress perception

Subjective stress perception did not significantly differ between the three time points ($F(2, 180) = 2.75$, $p = .067$, $\eta_p^2 = .03$; t_0: 2.6 ± 1.3, t_1: 3.0 ± 1.8, t_2: 3.1 ± 1.7). However, there was a trend towards higher ratings after the SECPT-G (t_0 and t_1: $t(90) = -1.89$, $p = .062$, $d = 0.14$, Fig1a).

<Figure 1 about here>

HPA axis response

Cortisol levels significantly increased after the SECPT-G ($F(1.50, 134.68) = 26.2$, $p < .001$, $\eta_p^2 = .23$; t_0: 1.9 ± 1.7, t_1: 2.6 ± 3.1, t_2: 4.3 ± 4.7 nmol/l; Fig. 1b). This effect was significant between all of the time points (t_0 and t_1: $t(90) = -2.93$, $p = .004$, $d = 0.55$; t_1 and t_2: $t(90) = -4.62$, $p < .001$, $d = 0.44$; t_0 and t_2: $t(90) = -6.00$, $p < .001$, $d = 0.82$).

Alpha-amylase response

Mean sAA levels did not differ between the three time points ($F(2, 180) = 0.22$, $p = .801$, $\eta_p^2 = .002$; t_0: 113.7 ± 103.2, t_1: 112.1 ± 102.2, t_2: 114.8 ± 103.5 U/ml, Fig. 1c).

Effects of anthropometric measures, experimental, and lifestyle factors

Overall results. To investigate whether any of the control variables were associated with the stress response, these variables were included as covariates in further rmANOVAs. The only significant main effects of covariates were found for time of day and for immersion time when entering them into the analysis for sAA (time of day: $F(1, 88) = 11.07$, $p = .001$, $\eta_p^2 = .11$; immersion time: $F(1, 89) = 13.812$, $p < .001$, $\eta_p^2 = .14$). However, no interactions between time of day or immersion time with sAA response as well as no main effects of sAA response were found. For t_0, t_1, as well as for t_2, sAA levels were lower at later times of the day (t_0: $r(90) = -.28$, $p = .009$; t_1: $r(90) = -.33$, $p = .001$; t_2: $r(90) = -.35$, $p = .001$). Participants with longer immersion times showed higher sAA levels at all three time points (t_0: $r(90) = .34$, $p = .001$; t_1: $r(90) = .33$, $p = .001$; t_2: $r(90) = .39$, $p < .001$).

For none of the other control variables, significant main effects were found, neither for sAA, nor for cortisol (Tab. 1). However, for cortisol, the main effect of the factor time disappeared when including age, BMI, smoking and immersion time into the analysis. In these cases, marginally significant interactions between the control variables age and smoking and the factor time were found (age * time: $F(1.59, 141.6) = 5.957$, $p = .006$, $\eta_p^2 = .06$; smoking * time: $F(1.59, 141.52) = 4.54$, $p = .018$, $\eta_p^2 = .05$). Furthermore, for cortisol, beside a main effect of time, a significant sex * time interaction was found ($F(1.59, 141.5) = 6.15$, $p = .005$, $\eta_p^2 = .07$). This was further analyzed.

<Table 1 about here>

Associations between sex and the cortisol response. In men, at t_2, cortisol levels were marginally higher than in women ($t(74.5) = 1.70$, $p = .093$), but both groups showed the typical cortisol time effects (an increase between t_0 and t_1 as well as between t_1 and t_2, Fig. 2).

<Figure 2 about here>

Associations between age and the cortisol response. Because we found a marginally significant interaction between the factors age and time ($p = .006$, adjusted α-level = .005) and because it is known that age can influence the stress response for other stress induction paradigms (Kudielka et al., 2004), this was further analyzed. Age was marginally associated with the cortisol levels prior to the SECPT-G and immediately after (t_0: $r(91) = -.20$, $p = .057$; t_1: $r(91) = -.20$, $p = .060$). At these time points, cortisol levels were lower in older participants. At t_2, cortisol levels were independent of age ($p = .760$).

For a further exploratory analysis, age was grouped into quartiles (Q_1: 18-24, Q_2: 24-33, Q_3: 33-50, and Q_4: 50-75 years, $N_{Q1} = 25$, $N_{Q2} = 23$, $N_{Q3} = 24$, $N_{Q4} = 19$). The youngest participants (Q_1)

only showed a cortisol increase between t_0 and t_2 ($t(24) = -2.77$, $p = .011$), but no increase between t_0 and t_1 ($p = .189$) or t_1 and t_2 ($p = .239$). For older middle-aged (Q_3), and older (Q_4) participants, only a cortisol increase between t_1 and t_2 and between t_0 and t_2 (all $p < .001$), but not between t_0 and t_1 was found (Q_3: $p = .054$, Q_4: $p = .294$). Younger middle-aged adults (Q_2) showed a cortisol increase between t_0 and t_1 ($p = .047$) and between t_1 and t_2 ($p = .002$) as well as between t_0 and t_2 ($p = .001$).

Discussion

Our study confirms that the SECPT-G is a well-suited experimental procedure for introducing an HPA-axis stress response. It, therefore, offers a very economical alternative to less economic stress induction set-ups like the TSST. However, in our study, no sAA response was found. Thus, when an sAA response is required, other set-ups (e.g., the TSST or the MAST) might be better alternatives.

The lack of sAA response in our study is unexpected, because a number of previous studies that investigated the effects of a cold-pressor test (CPT) without a socially-evaluative component did find sAA increases. In these studies, an sAA increase was found immediately after the CPT (Skoluda et al., 2015; van Stegeren, Wolf, & Kindt, 2008). A potential reason for our failure to find an sAA response might be that the study was performed in the late evening when a naturally decay in sAA levels takes place (Nater et al., 2007). This was also confirmed by the main effect of the factor time of day in our study. Furthermore, sAA levels are usually high (although they slightly decay) in the evening (e.g., Nater et al., 2007) and might have, therefore, masked or prevented an effect of our treatment. Future studies will, therefore, have to explore whether it is possible to induce sAA responses by means of the SECPT-G as performed in our experiment at other times of the day. Furthermore, it should be investigated whether the classical SECPT (not performed in groups) introduces an sAA response at different times of the day.

The cortisol response was independent of many anthropometric and lifestyle factors as well as of experimental factors, e.g., time of day and immersion time. However, interestingly, some groups showed a marginal different time course of the stress response. Both the older middle-aged and the older participants only showed a cortisol increase between t_1 and t_2, but not between t_0 and t_1. Thus, the HPA axis response started later in older people. Furthermore, basal cortisol levels (t_0 and t_1) were significantly lower in older participants which is in line with previous findings (Kudielka et al., 2004). Furthermore, the youngest participants only showed an increase between t_0 and $_{t2}$, but not between t_0 and t_1 or between t_1 and t_2. Thus, the HPA axis response was also slower and weaker in these participants. This contradicts previous findings which found stronger cortisol responses in younger participants than in older for the TSST (Kudielka et al., 2004). Furthermore, we found a stronger cortisol response in men than in women which has been found for other stress induction set-ups as well (e.g., Kirschbaum, Wüst, & Hellhammer, 1992). We conclude that the SECPT-G introduces an HPA axis responses that is associated with known

anthropometric and lifestyle factors in a similar – but not the same – way as with other stress induction set-ups and is, therefore, a good alternative.

Beside the late time of the day, our study is subject to some further limitations. One is that we did not use a control group which immersed their hands in warm water. Previous studies have shown – though with a slightly different procedure and with other samples – that this does not introduce a stress response. Because our main goal was to show that the SECPT-G is a suitable application for studies in the general population and not that a warm water test introduces no response, this does not affect our conclusions much. However, there is a residual uncertainty that the stress response was not introduced by the SECPT-G itself, but by other situational factors (e.g., during test preparation) in our study.

Another limitation is that – although our sample is not the typical healthy student population at the age of early 20 – it can be assumed that the people that came to our laboratory were interested in science and were, thus, still a specific population. Furthermore, the time point of the collection of the third saliva sample was quite early, in comparison to other studies that found the cortisol peak approximately 20 minutes after onset of the stressor (Minkley et al., 2014; Schwabe et al., 2008). Therefore, it is very likely that cortisol levels would have increased further. However, since our study was conducted during a public event, it was not possible to investigate longer recovery periods. This will have to be done in future research. Moreover, our study design should be supplemented by collection of other stress markers (e.g., blood pressure, heart rate variability, inflammatory markers) in future research.

Conclusions

Our study confirms that the SECPT-G is a stress induction tool which elicits a strong HPA axis response, which is mostly independent of many anthropometric, experimental, and lifestyle factors, and which can, therefore, be used for research in the general population. However, for investigation of older participants it should be noted that this group shows a marginal different time course of the cortisol response which – partially – supports previous findings for other stress induction set-ups. We conclude that the SECPT-G is particularly useful for studying the general population regardless of common exclusion factors which makes it a good means for clinical applications. In future research, age-related differences in the HPA axis response to the SECPT-G should be examined further. Furthermore, it should be investigated in future research whether an sAA response can be introduced at earlier times of the day.

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References

Bosch, J. A., Geus, E. J. C. de, Veerman, E. C. I., Hoogstraten, J., & Amerongen, A. V. N. (2003). Innate secretory immunity in response to laboratory stressors that evoke distinct patterns of cardiac autonomic activity. Psychosomatic Medicine, 65(2), 245–258.

Bosch, J. A., Veerman, E. C. I., Geus, E. J. de, & Proctor, G. B. (2011). α-Amylase as a reliable and convenient measure of sympathetic activity: Don't start salivating just yet! Psychoneuroendocrinology, 36(4), 449–453.

Craig, C. L., Marshall, A. L., Sjorstrom, M., Bauman, A. E., Booth, M. L., Ainsworth, B. E., . . . Sallis, J. F. (2003). International physical activity questionnaire: 12-country reliability and validity. Medicine and Science in Sports and Exercise, 35(8), 1381–1395.

Jones, A., McMillan, M. R., Jones, R. W., Kowalik, G. T., Steeden, J. A., Deanfield, J. E., . . . Muthurangu, V. (2012). Adiposity is associated with blunted cardiovascular, neuroendocrine and cognitive responses to acute mental stress. PloS One, 7(6), e39143.

Kirschbaum, C., Wüst, S., & Strasburger, C. J. (1992). 'Normal'cigarette smoking increases free cortisol in habitual smokers. Life Sciences, 50(6), 435–442.

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. Psychosomatic Medicine, 61(2), 154–162.

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.

Kirschbaum, C., Wüst, S., & Hellhammer, D. (1992). Consistent sex differences in cortisol responses to psychological stress. Psychosomatic Medicine, 54(6), 648–657.

Kudielka, B. M., Bellingrath, S., & Hellhammer, D. H. (2006). Cortisol in burnout and vital exhaustion: An overview. G Ital Med Lav Ergon, 28(1 Suppl 1), 34–42.

Kudielka, B. M., Buske-Kirschbaum, A., Hellhammer, D. H., & Kirschbaum, C. (2004). HPA axis responses to laboratory psychosocial stress in healthy elderly adults, younger adults, and children: Impact of age and gender. Psychoneuroendocrinology, 29(1), 83–98.

Kudielka, B. M., Hellhammer, D. H., & Wüst, S. (2009). Why do we respond so differently? Reviewing determinants of human salivary cortisol responses to challenge. Psychoneuroendocrinology, 34(1), 2–18.

Kudielka, B. M., & Kirschbaum, C. (2005). Sex differences in HPA axis responses to stress: A review. Biological Psychology, 69(1), 113–132.

Lovallo, W. (1975). The cold pressor test and autonomic function: A review and integration. Psychophysiology, 12(3), 268–282.

Luger, A., Deuster, P. A., Kyle, S. B., Gallucci, W. T., Montgomery, L. C., Gold, P. W., . . . Chrousos, G. P. (1987). Acute hypothalamic–pituitary–adrenal responses to the stress of treadmill exercise. New England Journal of Medicine, 316(21), 1309–1315.

McInnis, C. M., Thoma, M. V., Gianferante, D., Hanlin, L., Chen, X., Breines, J. G., . . . Rohleder, N. (2014). Measures of adiposity predict interleukin-6 responses to repeated psychosocial stress. Brain, Behavior, and Immunity, 42, 33–40. https://doi.org/10.1016/j.bbi.2014.07.018

Minkley, N., Schröder, T. P., Wolf, O. T., & Kirchner, W. H. (2014). The socially evaluated cold-pressor test (SECPT) for groups: Effects of repeated administration of a combined physiological and psychological stressor. Psychoneuroendocrinology, 45, 119–127.

Morris, S. B. (2008). Estimating effect sizes from pretest-posttest-control group designs. Organizational Research Methods, 11(2), 364–386.

Nater, U. M., Rohleder, N., Schlotz, W., Ehlert, U., & Kirschbaum, C. (2007). Determinants of the diurnal course of salivary alpha-amylase. Psychoneuroendocrinology, 32(4), 392–401.

Owen, N., Poulton, T., Hay, F. C., Mohamed-Ali, V., & Steptoe, A. (2003). Socioeconomic status, C-reactive protein, immune factors, and responses to acute mental stress. Brain, Behavior, and Immunity, 17(4), 286–295.

Rohleder, N., & Kirschbaum, C. (2006). The hypothalamic-pituitary-adrenal (HPA) axis in habitual smokers. International Journal of Psychophysiology : Official Journal of the International Organization of Psychophysiology, 59(3), 236–243. https://doi.org/10.1016/j.ijpsycho.2005.10.012

Rohleder, N., & Nater, U. M. (2009). Determinants of salivary alpha-amylase in humans and methodological considerations. Psychoneuroendocrinology, 34(4), 469–485. https://doi.org/10.1016/j.psyneuen.2008.12.004

Schulz, P., & Schlotz, W. (1995). Trierer Inventar zur Erfassung von chronischem Stress (TICS): Skalenkonstruktion und teststatistische Überprüfung.

Schwabe, L., Haddad, L., & Schachinger, H. (2008). HPA axis activation by a socially evaluated cold-pressor test. Psychoneuroendocrinology, 33(6), 890–895.

Skoluda, N., Strahler, J., Schlotz, W., Niederberger, L., Marques, S., Fischer, S., . . . Nater, U. M. (2015). Intra-individual psychological and physiological responses to acute laboratory stressors of different intensity. Psychoneuroendocrinology, 51, 227–236.

Smeets, T., Cornelisse, S., Quaedflieg, C. W., Meyer, T., Jelicic, M., & Merckelbach, H. (2012). Introducing the Maastricht Acute Stress Test (MAST): A quick and non-invasive approach to elicit robust autonomic and glucocorticoid stress responses. Psychoneuroendocrinology, 37(12), 1998–2008.

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.

Van Stegeren, A., Rohleder, N., Everaerd, W., & Wolf, O. T. (2006). Salivary alpha amylase as marker for adrenergic activity during stress: Effect of betablockade. Psychoneuroendocrinology, 31(1), 137–141. https://doi.org/10.1016/j.psyneuen.2005.05.012

Van Stegeren, A. H., Wolf, O. T., & Kindt, M. (2008). Salivary alpha amylase and cortisol responses to different stress tasks: Impact of sex. International Journal of Psychophysiology : Official Journal of the International Organization of Psychophysiology, 69(1), 33–40.

Table 1(on next page)

Associations between cortisol and sAA increase after the SECPT-G and anthropometric variables, experimental, as well as lifestyle factors.

	Cortisol:						**Alpha-amylase:**					
	Main effect time		Interaction time * covariate		Main effect covariate		Main effect time		Interaction time * covariate		Main effect covariate	
Covariate	*p*	η_p^2	*p*	η_p^2	*p*	η_p^2	*p*	η_p^2	*p*	η_p^2	*p*	η_p^2
None	**< .001**	.37					n.s.	< .01				
Age	n.s.	.01	.006	.06	n.s.	.02	n.s.	< .01	n.s.	< .01	n.s.	.01
Sex	**< .001**	.36	**.005**	.07	n.s.	< .01	n.s.	< .01	n.s.	< .01	n.s.	< .01
BMI	n.s.	.01	n.s.	.04	n.s.	< .01	n.s.	< .01	n.s.	< .01	n.s.	< .01
Graduation	**< .001**	.38	n.s.	.01	n.s.	.01	n.s.	< .01	n.s.	.01	n.s.	.01
Profession	**< .001**	.38	n.s.	.01	n.s.	.01	n.s.	< .01	n.s.	.01	n.s.	.01
Use of oral contraceptives (women only)	**< .001**	.30	n.s.	.01	n.s.	.01	n.s.	.01	n.s.	.02	n.s.	.01
Physical activity	**< .001**	.11	n.s.	.01	n.s.	< .01	n.s.	.01	n.s.	.01	n.s.	< .01
Chronic stress	**< .001**	.16	n.s.	.02	n.s.	< .01	n.s.	< .01	n.s.	< .01	n..s	.03
Smoking	n.s	.01	.012	.05	n.s.	< .01	n.s.	.01	n.s.	.01	n.s.	< .01
Time of day	**< .001**	.07	n.s.	.03	n.s.	.03	n.s.	.01	n.s.	.01	**.001**	.11
Immersion time	.012	.05	n.s.	.001	n.s.	< .01	n.s.	.01	n.s.	.01	**< .001**	.13

1

Figure 1(on next page)

Subjective stress ratings (a), mean cortisol levels (b), and mean sAA levels (c) prior to the SECPT-G (t_0), immediately after (t_1), and ten minutes after it (t_2).

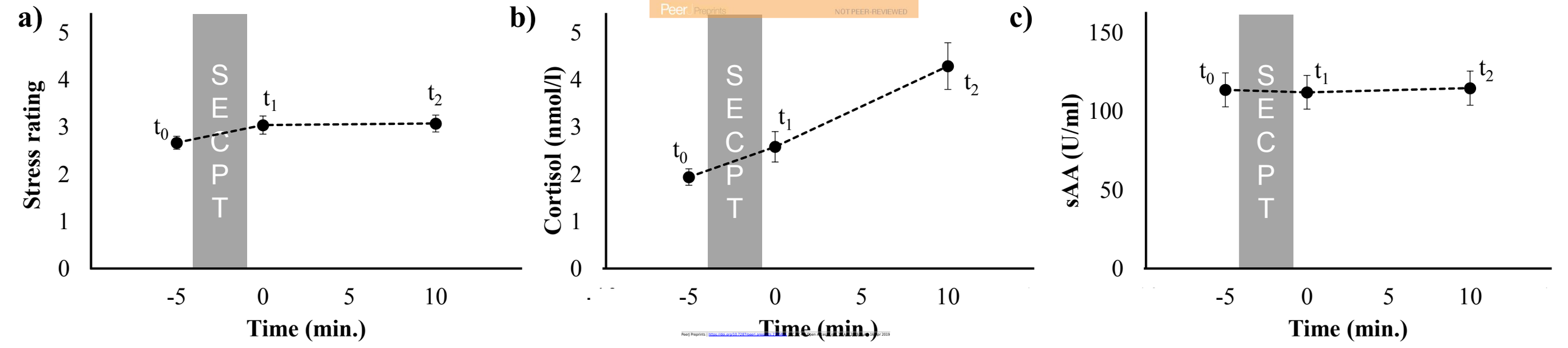
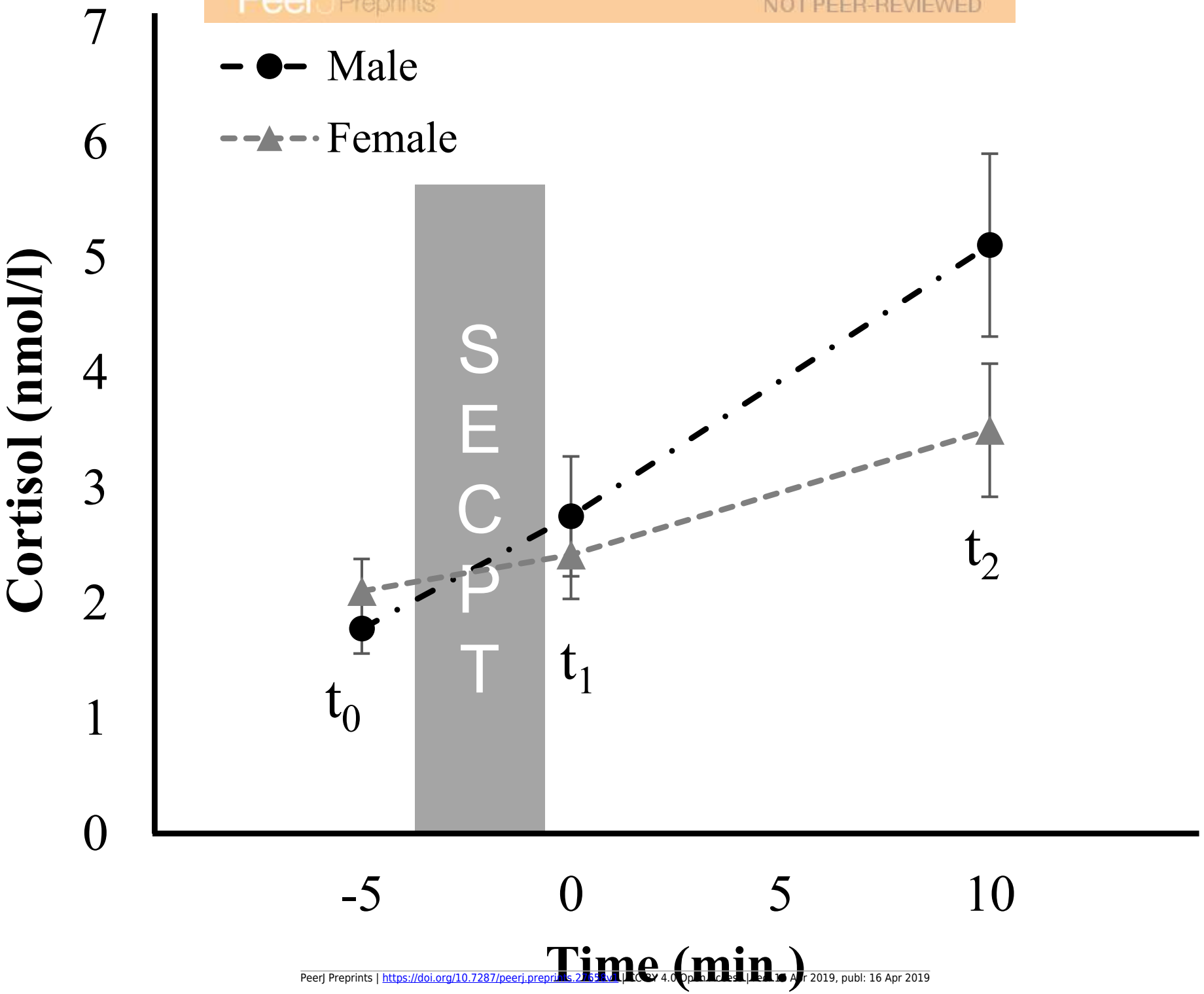


Figure 2(on next page)

Associations between sex and the cortisol response.



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