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Comparison of two isometric handgrip protocols on sympathetic arousal in women

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HIGHLIGHTS

- Isometric handgrip is used in stress research because it increases arousal.
- We tested if two different handgrip tasks elicited similar arousal changes.
- Pupil dilation increased significantly to handgrip only in the 18-s protocol.
- The 18-s paradigm should induce arousal, regardless of experiment parameters.

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ABSTRACT

Isometric handgrip is commonly used in stress research because the task reliably increases sympathetic arousal. Various handgrip protocols have been used; they vary in handgrip strength, duration of grip, and the number of cycles of handgrip and rest. However, most protocols require the calibration of a maximum voluntary contraction (MVC) prior to the handgrip task, which is not always convenient (i.e., in a functional magnetic resonance imaging study). Here, we wanted to test whether two handgrip protocols with different strength, duration and cycle protocols would reliably elicit sympathetic arousal in the absence of calibrating an MVC. Sixty-two healthy naturally cycling women and women on hormonal contraception participated in one of the two isometric handgrip protocols using a hand therapy ball of medium resistance. Women completed one of the following handgrip protocols: 1) 30% of a perceived maximum voluntary contraction for 3 min or 2) 3 cycles of maximum voluntary contraction for 18 s with a one minute rest in between. All handgrip blocks were counterbalanced with a control condition. Sympathetic arousal was measured throughout the session via pupil diameter changes and salivary alpha-amylase. Results indicate that in the absence of calibrating an MVC, the handgrip tasks elicited different changes in sympathetic arousal. Pupil dilation responses increased significantly in the handgrip versus control blocks only in participants in the 18-s protocol. Additionally, more participants exhibited a salivary alpha-amylase response to the handgrip block in the 18-s condition compared to the 3-min condition. Thus, these results suggest that neuroimaging and behavioral studies with isometric handgrip should be able to successfully induce sympathetic nervous activity with the 18-s paradigm, regardless of the handgrip device and the ability to calibrate an MVC.

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1. Introduction

For decades, researchers have used isometric handgrip, a form of static exercise, to investigate the cardiovascular response to stress [1]. Isometric handgrip acts as pressor stimulus to the cardiovascular system through efferent sympathetic pathways [2], and it tends to increase arterial blood pressure and heart rate [3]. Researchers typically employ isometric handgrip to test cardiovascular responses to stress; however, it can also be used to investigate the underlying mechanisms of sympathetic nervous activity [3].

In studies of sympathetic nervous activity, the most common handgrip paradigm is a 3-min task [4] that implements the use of a dynamometer, a special device that quantitatively assesses hand muscle strength. In the 3-min paradigm, participants are first asked to maximally squeeze a hand-held dynamometer several times to determine their average maximum voluntary contraction (MVC). After the MVC is calibrated, participants maintain handgrip pressure on the dynamometer at 30–40% of their MVC for 3 min [4] or until they are too fatigued to continue [5]. Throughout the task, they receive constant feedback about the strength of their sustained handgrip. Although the duration of the

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task is short, studies have shown task-related increases in sympathetic nervous activity [5–10].

More recent studies with handgrip, namely those with neuroimaging, have modified the widely used 3-min 30% of MVC task. Instead, participants are asked to squeeze maximally for short periods of time [11–13]; squeeze periods are followed by rest periods. Topolovec and colleagues (2004) showed that not only was the 18 s maximum squeeze/60 s rest paradigm successful at inducing sympathetic nervous activity, but neuroimaging data from the study suggests that the underlying neural mechanism involves the nucleus of the solitary tract, which innervates the locus coeruleus, a major noradrenergic nucleus [14]. Thus, even though these maximal squeeze paradigms are relatively new, they seem to induce sympathetic arousal as well. Additionally, maximal squeeze paradigms can be run without the use of a dynamometer. For example, studies have used a cylinder [11–13] or a pressure transducer [13] for their hand grip task. In any case, it would seem that maximal squeezes can be done without the feedback component of the dynamometer.

Although both the 18-s and 3-min paradigms have been used in previous research studies, no studies have assessed how sympathetic responses might differ between them or whether all isometric handgrip tasks effectively increase sympathetic nervous activity, even in the absence of calibrating an MVC. Given that the effort involved in maximal intermittent static exercise is greater than the effort involved in sustained static exercise at 30% of maximal exertion, one might expect that the 18-s task induces greater sympathetic arousal.

Isometric handgrip reliably increases plasma norepinephrine [8, 15–19], but no studies with the 18-s or 3-min paradigms have examined pupil dilation responses or salivary alpha-amylase as indices of arousal, despite the fact that these are widely used non-invasive measures of noradrenergic activity [20,21]. The only study to use pupil dilation as an index of arousal used a paradigm with 2-min of isometric handgrip at 30% of maximal exertion; results showed increased pupil dilation to the handgrip task [9].

Thus, the present study was designed to address several unexplored aspects of isometric handgrip. First, we wanted to assess whether different isometric hand grip paradigms would successfully increase sympathetic nervous activity in the absence of calibrating an MVC. In theory, any of the isometric hand grip tasks should be able to increase sympathetic nervous activity even without calibrating a maximum voluntary contraction; sympathetic arousal during either the 3-min perceived 30% of MVC (3-min) or the shorter 18-s MVC (18-s) should still be possible.

Therefore, we hypothesized that the 3-min and the 18-s paradigms would both induce increases on different measures of sympathetic arousal; however, we also predicted that the 18-s paradigm would induce greater arousal than the 3-min paradigm. For the current study, we assessed changes on a previously used measure (pupil dilation) and a new measure, salivary alpha-amylase. Salivary alpha-amylase (sAA) is a known biomarker for norepinephrine, as changes in sAA correlate with changes in plasma norepinephrine [21]. It has been used in previous studies of sympathetic arousal [22–26], and seemed well-suited to measure sympathetic arousal during isometric handgrip.

Secondly, we wanted to assess the relationship between pupil dilation and sAA responses to isometric handgrip. Since previous work with isometric handgrip has shown that changes on different sympathetic measures are of similar magnitude and are in the same direction, we predicted that an increase in the pupil dilation response during handgrip would predict an increase in sAA. We also predicted that sAA responders (see Section 2) would exhibit greater pupil dilation responses to handgrip.

Lastly, we wanted to assess whether hormonal contraception use altered sympathetic responses to isometric handgrip. To date, no studies of isometric handgrip have explored the potential effects of hormonal contraception use on sympathetic nervous activity. Previous studies of sympathetic arousal have shown that women on hormonal contraception exhibit blunted noradrenergic responses to exercise [27] and arousing images [25] compared with naturally cycling women. Thus, insofar as the two groups of women in our study exhibited significantly different sex steroid hormone profiles, we predicted that women on hormonal contraception would show blunted noradrenergic responses to both hand grip tasks compared to naturally cycling women.

2. Materials and methods

2.1. Participants

Sixty-two female undergraduates from the University of Southern California between the ages of 18–34 participated in this study, which was approved by the university's Institutional Review Board. The participants received course credit or payment for their participation. Participants were asked to refrain from alcohol, caffeine, and cardiovascular exercise for twenty-four hours prior to each experimental session to control for outside influences that could affect baseline salivary alpha-amylase levels. To avoid contamination of salivary samples, participants were asked to fast 1 h prior to each experimental session as well as refrain from brushing teeth and chewing gum within the hour before their appointment. Their compliance with these criteria was confirmed with them upon their arrival.

Of the participants included in the final analyses, 42 were naturally cycling (NC women) and 20 were currently taking hormonal contraception (HC women). The NC women were recruited in the "follicular" phase (1–15 days from the start of menstruation) and the "luteal" phase of the menstrual cycle (15–30 days from the start of menstruation [28–31]). We used a forward day count from the first day of menstruation to determine menstrual cycle position. Of the HC women, all participants were on combined contraceptive formulations that had both ethinyl estradiol and a synthetic progestin; 3 HC women reported using triphasic formulations, 16 used monophasic formulations, and one participant's contraceptive phasicity was not reported.

2.2. Procedures

All experimental sessions were conducted between the hours of 12:00 and 18:00 to control for the effects of circadian rhythm on stress hormone levels. Upon arrival, participants rinsed their mouth by drinking an 8 oz. bottle of water; they also completed a demographic information packet. Approximately 10 min after their arrival, participants provided a 1-mL saliva sample using the "passive drool" collection method. Following the baseline saliva sample, participants completed a 5-pt. calibration on the iView X RED eye-tracking system (SensoMotoric Instruments). After successful calibration, participants were randomly assigned to one of five conditions for their first experiment block: 1) 3-min handgrip, 2) 18-s handgrip, 3) 3-min water bottle fingertip rest (control), 4) 18-s control, or 5) 3-min control. The five conditions were created to counterbalance the order of handgrip and control tasks across participants. Isometric handgrip tasks were completed with a hand therapy exercise ball of medium resistance (Gaiam), and the water bottle fingertip rest task was performed using an 8 oz. empty water bottle. All participants we asked to use their right hand for the isometric handgrip and control tasks. This was done to maintain consistency across the participants and to generate a paradigm that would easily translate into a neuroimaging study; fMRI studies with isometric handgrip have followed this hand protocol to aid in interpretation of neuroimaging data [12,32].

In each condition, participants were presented with a grayscale screen, and in the center of the screen was either a yellow or a blue circle (normed for luminance). During the yellow circle "rest" periods, participants were asked to rest and relax while maintaining their gaze on the screen. During the blue circle "squeeze" periods, participants were asked to either squeeze the hand therapy exercise ball at 30–40% of their perceived maximum grip for 3-min, maximally for three 18 s squeeze/60 s rest cycles, or they were asked to gently rest their fingertips on an empty water bottle. All conditions started with a 10-s yellow

circle display to determine initial baseline pupil diameter before the first squeeze block. For the 3-min conditions, participants saw the blue circle for 3 min and were asked to maintain the 30–40% of their perceived maximal handgrip or water bottle fingertip rest for the full 3 min. For the 18-s conditions, there were three cycles of the 18 s squeeze (blue) and 60 s rest (yellow), and participants were asked to maintain the maximum squeeze or water bottle fingertip rest for the full 18 s [12]. It should be noted that we decreased the number of cycles in the 18 s task from five [12] to three cycles; this was done to ensure that both the 18-s and 3-min tasks were of equivalent duration.

Immediately following the assigned task, participants provided a 1-mL saliva sample. Participants then recovered from the handgrip or the control tasks for 15-min [5]. During this recovery period, they also provided 1-mL samples at 7- and 15-min post-task. After the 15-min post-task sample, participants completed a second 5-pt. calibration on the iView X RED eye-tracking system (SensoMotoric Instruments), and underwent the same experiment format as in the first block (e.g., if the participant completed the 3-min handgrip, they now completed the 3-min control or if the participant completed the 18-s control, they now completed the 18-s handgrip task). Thus, in the second experiment block, each participant who completed the handgrip task in the first block now performed the control task and vice versa. The one exception was the fifth condition in which participants performed the control task for a second block. All control–control participants completed two 3-min control tasks. This control–control condition was used to ensure that there were no novelty effects driving arousal responses.

Immediately after the second task, participants provided a fifth 1-mL saliva sample; subsequent samples were provided at 7-min and 15-min post-task. During this time period, participants were also shown a slide show of mixed positive, negative, and neutral images. However, this slide show and the subsequent short-term memory test were part of a separate study and will not be discussed here. After completing the final saliva sample and the brief memory test, participants were debriefed and compensated with course credit or payment.

2.3. Eye movements and pupil dilation

Pupil dilation was measured using the iView X RED eye-tracking software at a sampling rate of 120 Hz. We selected this measure for analysis because pupil dilation is considered a reliable measure of sympathetic arousal [33,34]. Also, a recent study with isometric handgrip showed pupil diameter increased in response to a 2-min isometric handgrip task [9]. Fixation events and pupil dilation data for each participant were exported using the eye-tracking analysis software program BeGaze 2 (SensoMotoric Instruments). To calculate the pupil diameter change in response to the handgrip and control tasks, we examined pupil diameter values across the entire task and we adopted methods from Hayashi and Someya [9]. These methods were implemented to assess the change in pupil diameter across the task duration from the baseline. First, for a more general assessment of pupil responses to handgrip versus a control task, we assessed pupil diameter changes across the entire task. To do this, we averaged the pupil values across a handgrip task or control block; to normalize data between participants, we divided these task block averages by the baseline pupil diameter in the 10 s preceding the corresponding task block, as follows:

$$\frac{\text{Average pupil diameter across entire handgrip or control task}}{\text{Average pupil diameter across 10 s baseline preceding handgrip or control}} \times 100\% = \text{whole task pupil response}.$$

For a handgrip-specific pupil analysis, we also assessed the pupil data using established methods adopted from Hayashi and Someya [9]. We determined the 20 fixations with the greatest pupil diameter during each 1-min interval in the 3-min task and during each 18-s interval in the 18-s task to obtain the top-20 pupil diameter values. Therefore, each task had three experimental intervals. These top-20 were averaged for each interval. In order to normalize the data between participants, we calculated a percent of the baseline pupil diameter for each handgrip and control interval in each task, as follows:

$$\frac{\text{Average of top}-20\text{ pupil diameter across one handgrip or control interval}}{\text{Average pupil diameter across 10 s baseline preceding handgrip or control}} \times 100\% = \text{top}-20\text{ pupil response per interval}.$$

These average top-20 pupil responses were then averaged across the three intervals for handgrip and control blocks during the 3-min and 18-s tasks, and this value was the top-20 pupil response used for subsequent analyses.

2.4. Saliva samples

Saliva samples were immediately frozen for a minimum of 24 h to allow mucins to precipitate. Prior to the assays, they were thawed and centrifuged at 3000 $\times g$ for 15 min to extract particulates from saliva. Clear supernatant was decanted into microtubes.

2.5. Salivary alpha-amylase measurement

Alpha-amylase levels were measured using Salimetrics, LLC (State College, PA) enzyme kinetic assay kits and measured optically using Molecular Devices, LLC SpectraMax M3 Multi-mode Microplate Reader (Sunnyvale, CA).

Previous studies have shown the greatest sAA response to arousing stimuli to be between baselines and immediately post-task [22,25]; therefore, we calculated the values for the sAA response to handgrip and the control task by subtracting the amount of sAA in the sample immediately before the task from the amount in the sample taken immediately after the end of the task. Participants with an increase of 3 U/mL or more in their level of sAA were classified as "sAA responders" (Salimetrics, LLC). All other participants were considered "sAA non-responders" [22].

2.6. Salivary measurement of sex steroid hormones

Salivary levels of 17β-estradiol and progesterone were measured using Salimetrics, LLC (State College, PA) ELISA kits and measured optically using Molecular Devices, LLC SpectraMax M3 Multi-mode Microplate Reader (Sunnyvale, CA). We assayed two saliva samples for 17β-estradiol and progesterone; from these samples, we determined the average levels of these hormones. We used the assays to determine overall group differences between NC and HC women, and mean ± SEM values of 17β-estradiol and progesterone were within the expected ranges for women (Salimetrics, LLC, State College, PA). The observed ranges from the assay of 17β-estradiol (2.3 ± 0.77 pg/mL) and progesterone (72.58 ± 44.55 pg/mL) in NC women were also similar to the expected ranges.

2.7. Statistical analysis

We used one-way ANOVAs to assess differences in sex steroid hormone levels, health and stress ratings, and baseline salivary alpha-amylase (sAA) levels. We also used a series of repeated-measures ANOVAs to analyze differences in pupil diameter and salivary alpha amylase responses to handgrip v. control tasks in handgrip (HG) participants as well as to each of the handgrip paradigms.

3. Results

3.1. Participants

Participants' mean age was 20.48 ± 0.32 years (mean ± SEM), and NC and HC women did not differ significantly in age ($F_{(1, 60)} = 1.10$, $p > 0.1$). These two groups of women also did not differ significantly in their baseline levels of sAA ($F_{(1, 60)} = 2.24$, $p > 0.1$), health ratings ($F_{(1, 60)} = 0.97$, $p > 0.1$), stress ratings ($F_{(1, 60)} = 2.82$, $p = 0.098$), and their ratings of stress compared to usual ($F_{(1, 60)} = 0.62$, $p > 0.1$). Baseline characteristics of the entire female cohort, NC women and HC women are outlined in Table 1.

3.2. Sex hormones and menstrual cycle position

Menstrual cycle position in NC women was determined by self-report and verified using salivary assays. Follicular ($N = 23$) and luteal ($N = 19$) women did not differ significantly in their levels of progesterone ($F_{(1, 40)} = 0.29$, $p > 0.1$) or 17β-estradiol ($F_{(1, 40)} = 0.80$, $p > 0.1$), so we collapsed these women into one group of naturally cycling women (NC women) for all subsequent analyses. When comparing NC ($N = 42$) and HC ($N = 20$) women, however, we also found no significant differences in their levels of progesterone ($F_{(1, 60)} = 0.32$, $p > 0.1$) or 17β-estradiol ($F_{(1, 60)} = 3.22$, $p = .078$).

3.3. sAA levels throughout the experimental session

Throughout the experimental session, we collected saliva samples at seven different time points (see Section 2). We used a repeated measured ANOVA with experimental condition (3-min HG in Block1 v. 3-min HG in Block2 v. 18-s HG in Block1 v. 18-s HG in Block2 v. Control–Control) as the between-subjects factor and saliva collection time point as the within-subjects factor to assess potential differences in sAA levels across the experimental session. The repeated measures ANOVA revealed a significant main effect of time point ($F_{(6, 342)} = 3.33$, $p < 0.01$), but no interaction between experimental condition and time point ($F_{(24, 342)} = 0.50$, $p > 0.1$; Fig. 1) and no significant between-subjects effect of experimental condition on sAA levels ($F_{(4, 57)} = 1.5$, $p > 0.1$).

3.4. Control–control manipulation check — pupil and sAA response

The control–control group ($N = 13$) was implemented to be a manipulation check. We used two repeated-measures ANOVAs to test whether there were differences between the first and second control block in the participants' whole task and top-20 pupil response. For the whole task pupil response, we found no effect of control block order ($F_{(1, 12)} = 0.006$, $p > 0.1$); there was also no effect of control block order on the top-20 pupil response ($F_{(1, 12)} = 0.009$, $p > 0.1$).

We also ran a repeated-measures ANOVA to assess the effect of control block order on the sAA response. There was no significant effect of control block order on the sAA response to the control task ($F_{(1, 12)} = 0.616$, $p > 0.1$). Thus, the control–control manipulation check showed that control block order did not affect the response to the control task.

3.5. Handgrip versus control task — pupil diameter response

First, we assessed how the pupil diameter changed across each block in HG participants ($N = 49$).

3.5.1. Whole task pupil analysis

For this analysis, we used the whole task pupil response, and we tested whether this response differed between the handgrip and the control block in HG participants. In a repeated-measures ANOVA, there were no significant differences in the whole task pupil response to the handgrip and the control block ($F_{(1, 48)} = .01$, $p > 0.1$).

3.5.2. Top-20 pupil diameter values analysis

We also assessed the data using the handgrip-specific top-20 pupil diameter value method [9]. In a repeated measures ANOVA, there were no significant differences in the top-20 pupil response to the handgrip and the control block ($F_{(1, 48)} = .33$, $p > 0.1$).

3.5.3. Pupil response by NC/HC women

We next assessed whether contraceptive status (NC women v. HC women) modulated the whole task and top-20 pupil response to the handgrip and the control block in HG participants. Separate repeated-measures ANOVAs revealed no significant effect of contraceptive status on either the whole task pupil responses ($F_{(1, 47)} = .005$, $p > 0.1$) or the top-20 pupil response ($F_{(1, 47)} = 0.1$, $p > 0.1$) to the handgrip and control block.

3.5.4. Summary

For the whole task and the top-20 pupil response analyses, HG participants did not show enhanced pupil dilation to the handgrip compared to the control task. Additionally, contraceptive status did not modulate the whole task or the top-20 pupil response to handgrip. Since the HG group was comprised of two different handgrip paradigms, our next set of pupil dilation analyses examined pupil dilation responses to the handgrip and control tasks in the 18-s compared to the 3-min paradigm. Prior to those analyses, however, we assessed sAA responses in HG participants.

3.6. Handgrip versus control task — sAA response

To examine sAA responses (i.e., change in sAA, percent change of sAA from baseline) to handgrip and control tasks in HG participants ($N = 49$), we conducted several analyses. With two repeated-measures ANOVAs, we found no significant differences between the handgrip and the control block in sAA change ($F_{(1, 48)} = 0.68$, $p > 0.1$) or the percent change in sAA ($F_{(1, 48)} = 0.75$, $p > 0.1$). We also identified that 28.6% of all HG participants were sAA responders to the handgrip task, regardless of paradigm.

Table 1
Baseline characteristics of the female cohort, NC women, and HC women. There were no significant differences between NC and HC women in any of the baseline characteristics.

	All women	NC women	HC women
Age (years)	20.48 (*SD* = 2.5)	20.71 (*SD* = 2.8)	20.0 (*SD* = 1.6)
Years of education	14.36 (*SD* = 1.3)	14.5 (*SD* = 1.4)	14.12 (*SD* = 1.0)
Health rating	7.36 (*SD* = 0.89)	7.29 (*SD* = 0.91)	7.53 (*SD* = 0.85)
Stress rating	4.8 (*SD* = 1.96)	5.11 (*SD* = 1.8)	4.22 (*SD* = 2.1)
Stress compared to usual	4.7 (*SD* = 1.8)	4.83 (*SD* = 1.7)	4.45 (*SD* = 1.9)
Baseline sAA before block 1 (U/mL)	102.75 (*SD* = 79.6)	113.07 (*SD* = 87.7)	81.07 (*SD* = 54.93)
Estradiol (pg/mL)	2.18 (*SD* = 0.80)	2.3 (*SD* = 0.77)	1.92 (*SD* = 0.82)
Progesterone (pg/mL)	70.39 (*SD* = 45.1)	72.58 (*SD* = 44.55)	65.62 (*SD* = 47.1)

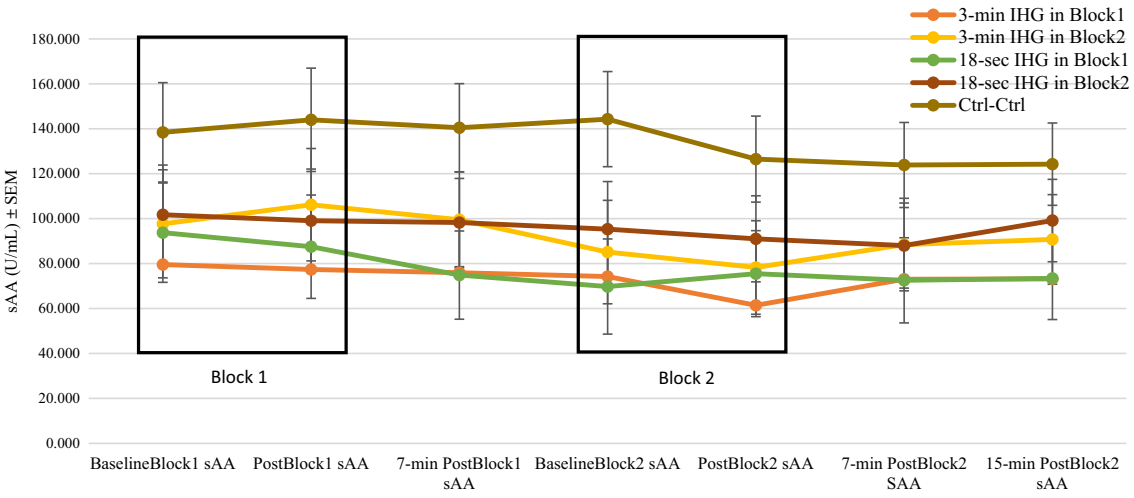

Fig. 1. sAA levels throughout the experimental sessions. The mean sAA levels ± SEM at each of the seven times points for the 3-min IHG in Block1 ($n = 12$), 3-min IHG in Block2 ($n = 11$), 18-sec IHG in Block1 ($n = 13$), 18-sec IHG in Block2 ($n = 13$), and Ctrl–Ctrl ($n = 13$) conditions are presented above.

3.6.1. sAA response by NC/HC women

We next assessed whether contraceptive status modulated the sAA response to the handgrip and the control block in HG participants. Separate repeated-measures ANOVAs revealed no significant effects of contraceptive status on the sAA change ($F_{(1, 47)} = 0.71$, $p > 0.1$) or percent change in sAA ($F_{(1, 47)} = 0.10$, $p > 0.1$) in response to the handgrip and control block. Interestingly, when we examined the percentage of sAA responders to handgrip in NC and HC women, we found that 33% of NC women responded to the grip task while only 18.8% of HC women responded.

3.6.2. Summary

Though we anticipated there to be substantially more sAA responders to handgrip in the HG participants, we did not see that pattern. Since the HG participants were comprised of those in the 18-s and the 3-min tasks, this analysis did not account for the possibility that there may be more sAA responders to one of the handgrip tasks than the other. In subsequent analyses below, we compared sAA responses to the 18-s versus 3-min tasks.

3.7. 18-s versus 3-min task — pupil diameter response[1]

Next, we tested the whole task pupil response across each experiment block in 18-s participants ($N = 26$) and 3-min participants ($N = 23$).

3.7.1. Whole task pupil analysis

Since we were comparing the response between handgrip paradigms, we did not include the CTRL participants in these analyses. We first tested whether the whole task pupil response differed in the handgrip and the control blocks in 18-s and 3-min participants. Using a repeated-measures ANOVA, with experiment block (handgrip versus control) as a within-subjects factor and handgrip paradigm (18-s versus 3-min) as a between-subjects factor, we found a significant interaction between experiment block and handgrip paradigm ($F_{(1, 47)} = 6.48$, $p < 0.05$) for the whole task pupil response. We also found a significant between-subjects effect for the pupil response in the handgrip paradigm ($F_{(1, 47)} = 15.1$, $p < .001$). These data indicate that the whole task pupil response to handgrip is significantly greater in the 18-s paradigm compared to the 3-min paradigm (see Fig. 2a).

3.7.2. Top-20 pupil diameter values analysis

Since we observed a significant interaction between the experiment block and handgrip paradigm in the whole task pupil analysis, we also tested whether the top-20 pupil response differed between the two paradigms. Using a repeated-measures ANOVA with experiment block (handgrip v. control) as a within-subjects factor and handgrip paradigm (18-s v. 3-min) as a between-subjects factor, we also found a significant interaction between experiment block and handgrip paradigm ($F_{(1, 47)} = 4.66$, $p < 0.05$) for the top-20 pupil response. However, unlike with the whole task pupil analysis, we did not find a significant main effect. These results indicate a significantly greater top-20 pupil response to handgrip in the 18-s compared to the 3-min paradigm. Though this analysis should favor the 3-min task, since there are more pupil values collected during its 1-min handgrip blocks than during the 18-s blocks, we still observe greater sympathetic arousal to handgrip in the 18 s condition (Fig. 2b).

We also assessed whether the top-20 pupil response differed by trial in the 18-s and 3-min tasks. Using a repeated measures ANOVA with experiment interval (1 v. 2 v. 3) as a within-subjects factor and handgrip paradigm as a between-subjects factor, we found a significant main effect of experiment interval ($F_{(2, 94)} = 7.7$, $p < 0.01$), but no significant interaction between experiment interval and handgrip paradigm ($p > 0.1$). However, it should be noted that numerically, the top-20 pupil response in the 3-min condition decreased across each interval; this step-wise decrease was not observed in the 18-s condition (Fig. 3).

3.7.3. Summary

Analyses with the whole task and top-20 pupil dilation responses indicate that sympathetic arousal responses to handgrip are greater in the 18-s condition than in the 3-min condition. The pupil dilation analyses support the notion that isometric handgrip during the 18-s paradigm induced greater sympathetic arousal than the control task, while handgrip during the 3-min paradigm did not.

3.8. 18-s versus 3-min task — sAA response

We also assessed sAA responses to the 18-s versus 3-min paradigms. Two repeated-measures ANOVAs with experiment block (handgrip versus control) as a within-subjects factor and handgrip paradigm (18-s versus 3-min) as a between-subjects factor were used to assess potential differences in the sAA response. The analyses revealed no significant main effects or interactions between the factors for change in sAA and percent change in sAA from the baseline.

[1] Since we did not identify any significant differences between NC and HC women when we assessed their pupil and sAA responses to handgrip in the HG versus CTRL analyses, we did not test for differences in these groups in the different handgrip paradigms.

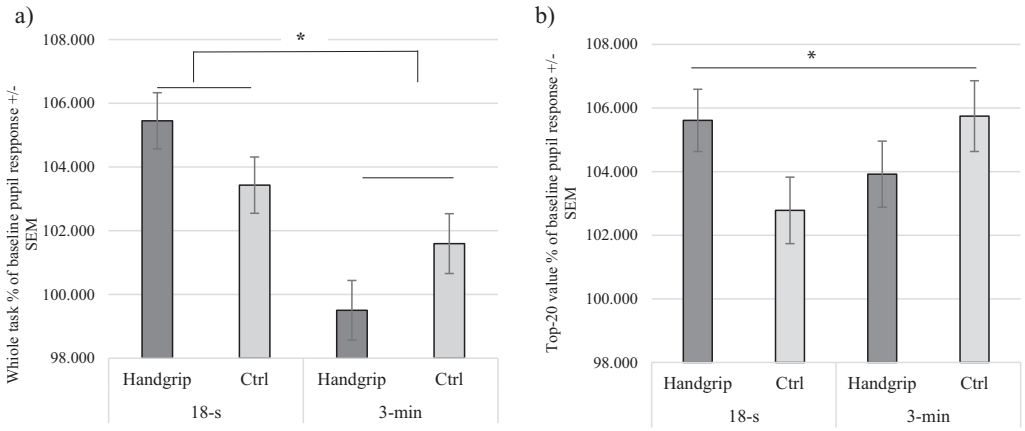

Fig. 2. Pupil responses differ to handgrip versus control in 18-s and 3-min tasks. a) In the whole task pupil response to handgrip analysis, there is a significant interaction between experiment block and handgrip paradigm. b) In the top-20 pupil value response to handgrip there is a significant interaction between experiment block and handgrip paradigm, $^{*}p < .05$.

The results from the repeated-measures ANOVAs show that the sAA response does *not* differ by handgrip paradigm; additionally, the sAA response also does not appear to increase for handgrip compared to control. Initially, we found that 28.6% of all HG participants were sAA responders to handgrip. Here, we examined the percentage of sAA responders in each handgrip paradigm to determine if one of the paradigms elicited more sAA responders. Of the 18-s participants, 38.5% were sAA responders compared to only 17.4% in the 3-min condition. Thus, the number of sAA responders to the 18-s paradigm was nearly doubled that of the 3-min condition. A chi-square test of independence could not be run on account of the small cell size for 3-min sAA responders ($N = 4$).

The difference in the percentage of sAA responders was not observed in the control block comparison; 35% of participants in both the 18-s and 3-min conditions were sAA responders. These results suggest that future studies with sAA and handgrip should utilize the 18-s paradigm, especially in the absence of calibrating an MVC, and analyses using sAA responder and non-responder classifications may provide more information about sympathetic arousal than looking at mean changes in sAA levels given the short duration of the task [35].

3.9. Relationship between pupil diameter and sAA in handgrip tasks

Finally, we wanted to assess the relationship between pupil diameter and sAA during the handgrip block. Since our previous analyses showed that the 18-s paradigm was the only one to effectively induce sympathetic arousal in participants, we investigated the relationship between pupil diameter responses and sAA responses only in the 18-s condition. We used the handgrip-specific top-20 pupil response for these analyses.

3.9.1. Pupil response in sAA responders/non-responders in 18-s condition

A repeated-measures ANOVA for the top-20 pupil value response with experiment block (handgrip v. control) as a within-subjects factor and sAA response (responder v. non-responder) as a between-subjects factor revealed a significant within-subject effect of experiment block ($F_{(1, 24)} = 7.63$, $p < 0.05$). Participants in the handgrip condition exhibited larger pupil responses than those in the control condition. There was also a near significant between-subjects effect for sAA response ($F_{(1, 24)} = 4.02$, $p = 0.056$), suggesting that sAA responders had a

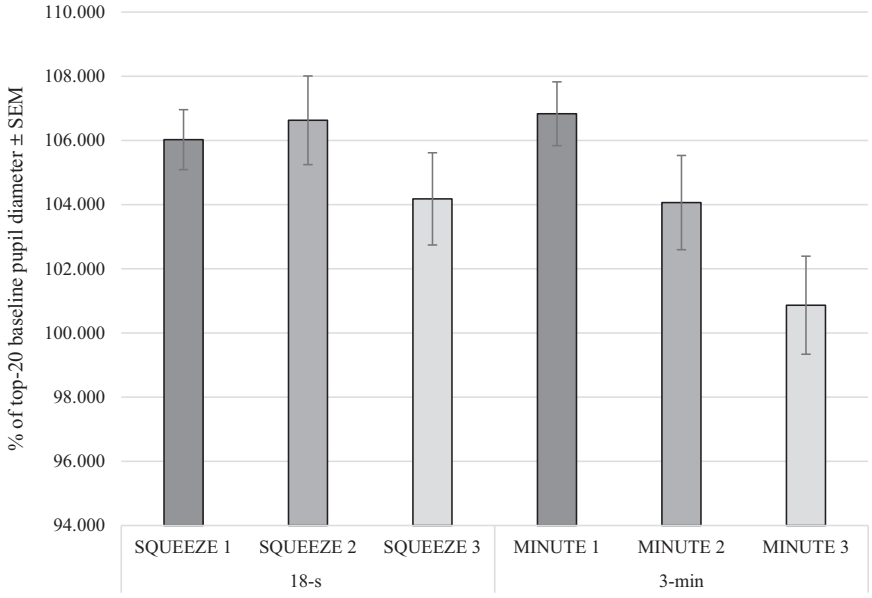

Fig. 3. Top-20 pupil response by squeeze interval in the 18-s and by minute in the 3-min paradigms. We observed a main effect of experiment trial ($p < .01$), but there was no significant interaction between experiment trial and handgrip paradigm.

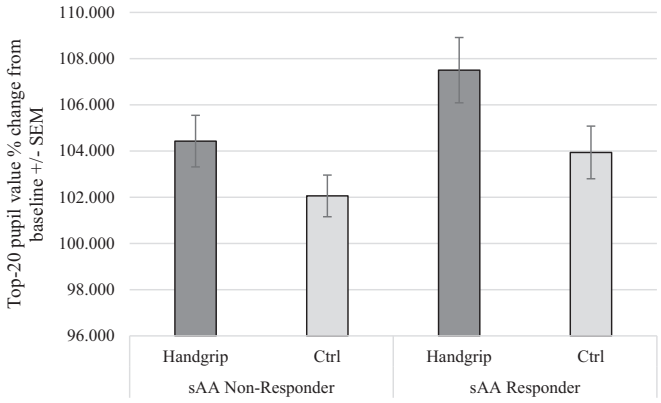

Fig. 4. Pupil responses in sAA responders and non-responders of the 18-s task. In the top-20 pupil value response to handgrip, sAA responders had near significantly greater pupil dilation compared to non-responders, $p = .056$.

near significantly greater pupil response compared to the sAA non-responders (Fig. 4).

3.9.2. Pupil response × percent change in sAA from the baseline

A correlation analysis showed a trend toward a significant positive relationship between the top-20 pupil value response and percent change in sAA from the baseline ($F_{(1, 24)} = 2.2$, $p = 0.15$). The 18-s condition was comprised of 26 participants, and it is possible that with more power, this relationship would reach significance.

4. Discussion

The present study was designed to test whether isometric handgrip induced sympathetic nervous activity in the absence of calibrating a maximum voluntary contraction. We also wanted to investigate the effects of hormonal contraception use on the sympathetic responses to handgrip. We assessed pupil diameter and salivary alpha-amylase responses to isometric handgrip in two different paradigms; each task had a handgrip block counterbalanced with a control block. We also included a control–control group as a manipulation check for the control task.

Surprisingly, in HG participants, we saw no significant increases in the whole task pupil response, the top 20 value pupil diameter response [9], or the salivary alpha-amylase in handgrip blocks compared to the control blocks. However, when we accounted for whether HG participants were in the 18-s or 3-min paradigm, we did observe increases in sympathetic nervous activity in response to handgrip. We found that the whole task and top-20 pupil dilation responses increased significantly in the handgrip compared to the control blocks for 18-s participants; this was not the case for participants in the 3-min condition. Our findings suggest that, in the absence of calibrating a maximum voluntary contraction, the 18-s paradigm will be more likely to successfully induce sympathetic arousal during isometric handgrip than the 3-min 30% of perceived maximal effort condition.

A potential explanation for this phenomenon might be that participants are able to self-calibrate a maximum voluntary contraction (MVC) to be sustained for a brief period of time, but not a certain percentage of a perceived MVC that needs to be sustained for a longer period of time. To identify and maintain a certain percentage of an MVC, feedback to the participant about the intensity of their isometric handgrip is important. With a special device like a dynamometer, participants receive initial feedback about their MVC, subsequent feedback about the percentage of the MVC to maintain for 3 min, and feedback on how well they maintain their percentage of the MVC. However, if participants are completing the task with a physical therapy squeeze ball, such as in this experiment, there is no feedback component. Thus, both the participant and the experimenter have no way of knowing how hard the participant was squeezing the physical therapy ball or how long they maintained the prescribed intensity of handgrip.

Also, based on the minute-by-minute top-20 pupil dilation responses, it seems likely that the 3-min participants in our experiment did not maintain their isometric handgrip at 30–40% of their perceived MVC for the full 3 min as their pupil responses decreased with each interval across the 3-min task. Failure to maintain isometric handgrip at 30% of their perceived MVC would prevent these participants from fatigue, which is important for inducing sympathetic arousal in a sustained handgrip task [36]. Thus, for the 3-min task, the feedback component might be essential for inducing sympathetic arousal. However, consistent with previous work [12], the feedback component does not appear to be as critical for inducing sympathetic nervous activity in the 18-s paradigm. Based on our measures of sympathetic arousal, participants were capable of successfully completing an estimated MVC for a brief period of time. The 18-s participants showed increased pupil responses to the handgrip compared to the control blocks, and there were more sAA responders to the 18-s handgrip block compared to the 3-min handgrip block. Thus, if future studies want to utilize an isometric handgrip task without calibrating an MVC, the 18-s paradigm [12] should be used instead of the 3-min one.

Alternatively, the lack of sympathetic arousal in the 3-min condition might not be related to the absence of a feedback component. The whole-task pupil dilation results for the 3-min task suggest that there may be parasympathetic activation as these participants showed pupil constriction from the baseline across the handgrip task. Some studies have shown increased parasympathetic activity in response to sustained isometric exercise. For example, Nishiyasu and colleagues found evidence of parasympathetic cardiac tone enhancement during a sustained isometric handgrip test (50% of MVC for 30-s or 60-s); this parasympathetic activity balanced out cardiac sympathetic activity induced by the isometric handgrip, and there were no observable increases in sympathetic arousal [37]. Additionally, there is evidence that elevated sympathetic tone can be overridden by intense vagus nerve discharge in a response known as "accentuated antagonism" [38]. Therefore, a small increase in sympathetic arousal in the 3-min handgrip paradigm may have been overridden by parasympathetic tone. However, this potential explanation needs to be further explored in studies collecting measures that directly assess parasympathetic activity [37].

A secondary aim of this study was to relate the magnitude and the direction of the pupil dilation and sAA response to isometric handgrip. Other studies in the isometric handgrip literature examined multiple measures of sympathetic arousal and related the direction and magnitude of the changes (e.g. [4,7,39]). The current isometric handgrip experiment also examined the relationship between measures of sympathetic nervous activity. To date, no study has directly related pupil dilation and sAA responses to arousing tasks; this experiment was designed to be the first. Since the 3-min paradigm was not successful at inducing sympathetic nervous activity during handgrip, we only examined this relationship in 18-s participants. Participants in the 18-s paradigm showed a trend toward a significant positive relationship between their pupil dilation and their sAA responses to isometric handgrip; sAA responders had significantly greater pupil dilation than sAA non-responders.

Identifying this relationship is important not only for the isometric handgrip literature, but also for general research in stress and arousal. Changes in pupil diameter during emotionally arousing tasks have been positively correlated with changes in skin conductance [40] and blood pressure [41,42]. These data are the first to our knowledge to show that pupil dilation responses may positively relate to changes in sAA. Future studies with isometric handgrip or other stressors should consider using these indices of sympathetic activity; they should also explore the potentially positive, significant relationship between them.

Although the present study produced several interesting and novel findings, it is not without caveats. First, we originally designed the experiment to assess differences in sympathetic nervous activity in

women on and off hormonal contraception. Previous research has shown that hormonal contraceptive users exhibit blunted sympathetic arousal responses compared to naturally cycling women [25,27]. In the present study, we recruited women reported to be in hormonally distinct phases (e.g. follicular, luteal, hormonal contraceptive users). However, we did not find significant differences between NC and HC women in their levels of salivary 17β-estradiol or progesterone. Some research has shown that hormone status is not significantly associated with changes in salivary alpha-amylase [43]. Alternatively, the lack of differences in hormone levels may have been the reason we found no differences in the pupil diameter or sAA responses to the handgrip task between NC and HC women.

A possible explanation for the equivalent sex hormone levels in these groups of women is that a proportion of the NC women in this study may have had anovulatory cycles; therefore, their hormone profiles may have been irregular [44]. Although the women in this study reported a regular menstrual cycle, anovulatory cycles are not uncommon in the college-aged females and they often go undetected. For example, in a recent study of college aged women, 26.9% of the female undergraduates had anovulatory cycles [44]; this proportion is similar to an earlier study that employed different methods to identify the ovulation point [45].

Additionally, of the 42 NC women included in this study, 10 had previously used hormonal contraception. Prior use of hormonal contraceptives may alter sex steroid hormone levels in the natural menstrual cycles of women. Future work should expand upon these current findings to assess the influence of sex steroid hormones on handgrip related-sympathetic nervous activity in women of different menstrual phases and contraceptive status. In this particular study, it is likely that the lack of hormone differences between NC and HC women contributed to there being no differences in their sympathetic arousal response to handgrip.

A second limitation to this study is that we did not collect simultaneous pressor and tachycardic assessments in response to the different isometric paradigms. While sAA [21] and pupil dilation [40] have been recognized as effective indices of sympathetic arousal, they have not been utilized much in isometric handgrip paradigms. Pressor and tachycardic measurements have been used in a number of isometric handgrip studies [3]; including blood pressure and heart rate changes in response to the two handgrip paradigms would have strengthened the findings from the present study.

Another caveat to this study is that the significant interaction between experiment block and handgrip paradigm in our top-20 pupil value analysis was driven in part by the large pupil dilation response to the 3-min control block. One possible explanation for this increase is that the condition where 3-min participants completed the control block second elicited a greater pupil response. However, a repeated-measures ANOVA for 3-min participants with experiment block as a within-subjects factors and block order as a between-subjects factor revealed no effect of block order ($p > 0.1$).

We also considered the possibility that 3-min participants had lower baseline levels of sAA, leaving them greater opportunity to respond sympathetically to either the handgrip or control task. However, an ANOVA revealed that baseline levels of sAA did not differ between 18-s and 3-min participants ($p > 0.1$). It is also possible that during the control blocks, 3-min participants were exhibiting greater anticipatory stress. Given the relatively mundane nature of the control task, the 3-min duration may have led the participants to anticipate the appearance of other stimuli. While this possibility remains purely speculative, the increased pupil dilation response to the 3-min control block should be explored in future studies to determine the underlying reason for this unexpected sympathetic nervous activity.

Additionally, we did not have a comparison condition using a device to calibrate MVC. Although we are able compare our findings to numerous studies in the literature that used this type of the device, our conclusions would be strengthened if we were able to assess how the use of different handgrip devices in the 18-s and 3-min paradigms affects sympathetic nervous activity. Thus, future studies interested in this particular comparison should include different handgrip devices to assess the influence of feedback on sympathetic arousal.

Finally, this study was designed to assess sympathetic arousal responses to isometric handgrip in women on and off hormonal contraception. However, to have a more complete picture of sympathetic arousal responses to these different isometric handgrip paradigms, males would need to be tested in a comparable protocol. Future studies of sympathetic arousal and isometric handgrip should include both men and women.

4.1. Conclusions

In spite of these limitations, this study has important implications for future studies with isometric handgrip. The handgrip blocks of the 18-s paradigm [12] increased sympathetic nervous activity, even in the absence of calibrating an MVC; whereas the 3-min handgrip blocks showed no significant indication of increasing sympathetic nervous activity [4]. These results suggest that neuroimaging and behavioral studies with isometric handgrip should be able to successfully induce sympathetic nervous activity with the 18-s paradigm. To further understand the underlying mechanisms of the observed phenomena in young women, future neuroimaging work should further investigate the neural mechanisms underlying sympathetic arousal during the 18-s paradigm in women of varying sex hormone statuses. These future studies would not only contribute to our knowledge of the effects of sex steroid hormones on the sympathetic arousal response; they would also facilitate a better understanding of the neural mechanisms underlying sympathetic nervous activity in women.

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