

Physiology of Maximum Clench Force

INTRODUCTION

Previous studies have shown that women can sustain longer times to muscle fatigue than men for various isometric contractions. This differential result has been repeatedly shown in various muscle groups including the adductor pollicis, elbow flexors, extrinsic finger flexors, and knee extensors (for review see Hicks, Kent-Braun, & Ditor, 2001). West et al. also found that in hand grip strength exercises of varying grip intensities, women consistently displayed longer times to fatigue compared to men (1995). While a consistent physiological explanation has not been validated, one common explanation has been that men can produce greater absolute forces when the test is based on strength (Drury et al., 2004; Staszkiwicz et al., 2003). A study by Hunter et al. suggested that the increased strength of men results in greater absolute forces which are associated with “increased intramuscular pressures, occlusion of blood flow, accumulation of metabolites, heightened metaboreflex responses, and impairment of oxygen delivery to the muscle” all resulting in a decreased resistance to fatigue in males (2001).

Based on the dimorphic expectation for maximum grip strength and maximum time to fatigue to vary with men having consistently greater grip strengths and women longer fatigue times, maximum clench force and time to fatigue data was collected in Physiology 335 labs at the University of Wisconsin Madison between 2004 and 2008. Participants were informed that the individual with the highest maximum clench force and the longest time to fatigue would be given a prize. The data collected from cohorts between these years showed that the average maximum clench force was 33.1 kg for men and 20.2 kg for women (Supplementary Figure 1). Additionally, the average time to fatigue for men was 53.8 seconds and 62.4 seconds for women. The preliminary data gathered by Professor Andrew Lokuta from the Physiology 335 cohorts revealed that men performed better than women on a maximum clench force test but,

based on the researchers personal observations, men did not seem to provide a maximum effort when performing a time to fatigue test when compared to the effort expended on their maximum clench force test. This observation was consistent over the 4 years of the study and based on visible signs of effort such as respiratory rate, muscle shaking, and sweat accumulation that seemed inconsistent between male participants suggesting that some participants were not giving maximum effort in the fatigue studies. It was hypothesized by Professor Andrew Lokuta that men applied lower maximum grip strengths during their time to fatigue tests in order to win the time to fatigue test and receive the prize. However, attempting to prove that a participant is not giving a maximum effort can be problematic. A direct physiological indicator may provide the best evidence to show if a participant is giving a maximum effort or not.

Examination of literature on how EDA and heart rate relate to exertion reveal that these variables are key biomarkers of work output. A study conducted by Tartz et al. affirms that greater grip forces were positively correlated with skin conductance if grip force reached above 0.2 kg, when measured in 24 subjects (2014). Additionally, other grip strength studies have shown that heart rate consistently increases in both men and women which is a common expectation of physical exertion studies (Petrofsky et al., 1975; Hunter et al., 2001). The results of the studies done by Tartz et al., Petrofsky et al., and Hunter et al. supported our hypothesis that EDA and heart rate would positively correlate with the amount of work exerted on a time to fatigue test. The confirmation of a correlation between these two biomarkers and grip strength on a time to fatigue test yield a possible physiological means for detecting if a participant is giving a true maximum effort. The goal of this study was to investigate if electrodermal activity (EDA) and heart rate covary with maximum clench force effort during a time to fatigue test. To accomplish this goal, grip strength of our participants was measured with a hand dynamometer, and electrodermal activity (EDA) and heart rate were recorded simultaneously to determine if a participant is giving a true maximum effort during a time to fatigue test. We examined the

hypothesis that there is an observable physiological biomarker which demonstrates whether a participant is exerting maximum effort or not, and we wanted to look at how this biomarker changed when maximum or assumed 50% of maximum clench force was applied in a time to fatigue study. With the assumption that participants gave their true maximum strength in the initial maximum grip strength test, changes in skin conductance and heart rate have the potential to serve as a proxy for detecting changes in a participant's level of effort. It is noted however that assuming maximum effort is given in the initial maximum grip strength test could be inaccurate for some participants.

The novel results of this study could bolster the methodologies employed in further studies where a participant's level of effort is of central importance. For example, in examining the physiological differences between effort exerted by men and women in their time to fatigue tests, ensuring a maximum effort from both parties would be central to discovering the physiological reasons for women's longer time to fatigue scores. Additionally, time to fatigue grip strengths are frequently employed as biomarkers of frailty, a condition of degrading skeletal muscle associated with aging, and identifying maximum effort is central in making this diagnosis (Bautmans et al., 2007; Hunter et al. 1998). By monitoring biomarkers like EDA and heart rate, which this study suggests are covariants, experimenters will be able to rule out psychological confounds which would encourage certain participants to purposely exert less force than their maximum in order to achieve a longer time to fatigue score. The difference in percentage of variance between baseline and maximum or attempted 50% sustained force could provide a reference point to assess whether or not someone is giving maximum effort. On the other hand, if future researchers are interested in how other variables psychologically correlate with participants purposely exerting less than maximum effort, covariants such as EDA and heart rate could be used as physiological markers with grip strength data.

MATERIALS

The three variables tested were clench force, skin conductivity (ElectroDermal Activity), and heart rate. Clench force was tested using a hand dynamometer (Model SS25LA, SN: 1506004319, Biopac Systems, Inc. Goleta, CA) and used to measure maximum clench force and time to reach half maximum clench force. Skin conductance was tested via the BSL EDA Finger Electrode Lead Set Xdcr (Model: SS3LA, SN: 13013875, Biopac Systems, Inc. Goleta, CA) that utilizes two BSL EDA gelled finger electrodes (Model: SS3LA) with gel 101. Heart rate data was collected via two electrodes from an EKG/ECHO electrode lead set (Model: EL503, Lot Number: 236819, Biopac Systems, Inc. Goleta, CA). Data was collected and analyzed using the Biopac Student Lab System (BSL 4 software, MP36). The Biopac Systems, Inc. Student Manual (Biopac Systems, Inc. ISO 9001:2008, Goleta, CA) was used for assistance with set-up and utilization of the Biopac equipment to ensure that all materials would function correctly.

METHODS

Procedure

Day 1

Participants in this experiment were students at the University of Wisconsin-Madison enrolled in Physiology 435 course during the Spring 2018 semester. Upon arrival, participants gave informed written consent and had an opportunity to ask questions in order to ensure that participants understood the procedure and movements they were being asked to perform. Participants were also asked in a survey to identify their dominant-hand, self-identifying gender, and sex during the consent process. Each participant was tested alone. Following consent of participation, the experimenter asked participants to sit while connecting equipment to measure the physiological data. The experimenter placed the dynamometer in the participants' dominant hand, and the transducer was connected to the acquisition unit. Next, a small spot of EDA gel was placed on both the middle and index finger of their non-dominant hand. The EDA finger

pads were then attached to the middle and index fingers of the non-dominant hand, and the lead was connected to the acquisition device. The ECG electrodes were attached to participants' right forearm and the left ankle. The leads were then attached to the Biopac acquisition device.

After all equipment was set up and attached to participants, participants were told they would be completing a time to fatigue test. Participants were instructed that they should squeeze the dynamometer with their maximum clench force while the experimenter measured the length of time before they reached 50% of the initial clench force. The experimenter explained that they would give verbal cues of "squeeze" to begin clenching and "relax" once participants had reached 50% of their initial force value (Figure 1). Participants were then asked if they had questions to ensure clarity of the experimental design before data collection.

Physiological data was recorded by the Biopac Systems, Inc. for the duration of the participants time to fatigue. Baseline data was collected for 30 seconds. One experimenter gave identical phrases of encouragement to all participants (Figure 1). After subjects were below 50% initial force for three seconds, the experimenter told them to "relax."

Participants remained seated with all equipment in place and were told they would be completing a maximum clench force study (Figure 1). The experimenter explained that they would be collecting three 2-second long maximum clench forces with their dominant hand with rest periods in between and that the experimenter would give them the verbal cues of "squeeze" and "relax" between trials. Participants were asked if they had any questions to ensure clarity of the experimental design before data collection.

Subjects were shown the Biopac Systems, Inc. acquisition software with only the data acquisition graph of clench force visible. Sixty seconds of baseline physiological data (EDA and heart rate) was recorded by the Biopac Systems, Inc. With the Biopac Systems, Inc. screen and clench force scale viewable, Subjects were instructed to "squeeze" for two seconds and then instructed to "relax". Subjects were then told their max clench force value and encouraged to

achieve a max clench force double their initial trial for the following two clench forces (Figure 1). After briefing, subjects were instructed to “squeeze” for two seconds and then instructed to “relax” for 10 seconds, and again told to “squeeze” for two seconds and then relax (Figure 1). Subjects were allowed to view the screen to encourage achievement of higher maximum clench forces for the second and third trials. EDA, ECG, and heart rate data were collected continuously through this time frame. Experimenters thanked the participants (Figure 1) and told them they would be compensated with a snack after they return next week for another time to fatigue trial.

Day 2

Participants returned and were tested alone. They gave informed written consent and had an opportunity to ask questions in order to ensure that participants understood the procedure and movements they were being asked to perform. After all equipment was attached to participants, they were told they would be completing a time to fatigue test. Participants were instructed that they should squeeze the dynamometer with what they thought was 50% of their maximum clench force for as long as possible while the experimenter would measure the length of time. The experimenter explained that they would give verbal cues of “squeeze” to begin clenching and the participant with the longest half-max time to fatigue would receive a prize (Figure 2). Unlike day 1, in order to encourage participants to clench for as long as possible, participants were not told that they would be stopped at 50% of their initial half-max clench force. Physiological data was recorded by the Biopac Systems, Inc. for the duration of the participants’ time to fatigue. Baseline data was collected for 30 seconds. Participants then clenched at their half maximum clench force when instructed to “squeeze” and held this clench. One experimenter gave identical phrases of encouragement to all participants (Figure 2). After subjects were below 50% initial sustained clench force for three seconds, the experimenter told them to “relax”. Ten additional seconds of physiological data was recorded. The participants were thanked for their participation.

Data Analysis

Baseline data was calculated by averaging heart (rate beats per minute), EDA (microSiemens), and a resting value of the hand dynamometer (kg) over the 30 second baseline collection time for both day 1 (H1, E1, and F1 on Figure 6 respectively) and day 2 (H3, E3, and F4 on Figure 7 respectively).

Maximum effort data was calculated by averaging 3 trials of 2 second clench forces during maximum clench force tests, providing six seconds total of data collection per participant (F3 on Figure 6). Discarded outlier trials from average clench force trials were defined by:

$< \text{Quartile 1} - (1.5 \cdot \text{IQR})$ and $> \text{Quartile 3} + (1.5 \cdot \text{IQR})$ (Hoaglin et al., 1986).

Day 1 maximum time to fatigue physiological data was calculated by averaging heart rate and calculating maximum EDA throughout the full time to fatigue data collection period. Baseline average heart rate and EDA were subtracted from average time to fatigue heart rate and maximum time to fatigue EDA, respectively, to obtain change in average heart rate and change in EDA during a time to fatigue test. Day 2 half-maximum time to fatigue data was calculated the same way.

Average clench force of the initial two seconds of the day 1 time to fatigue tests were subtracted from calculated average maximum clench force to identify participants that were deviating from the experimental instructions to reach maximum clench force on the time to fatigue test. Average clench force, Average EDA and average heart rate from each participant's day one time to fatigue test were compared with the average EDA and average heart rate from the participant's day two time to fatigue test. Average half-maximum clench force in the day 2 time to fatigue test was compared to both the day 1 maximum clench force average and the time to fatigue initial maximum to determine how accurately subjects were achieving a half-maximum clench force on the day 2 trial. Absolute change in EDA and change in average heart rate from baseline to time to fatigue were compared between day 1 and day 2 to additionally account for influence of varied baselines between testing days.

In classifying this experimental data, there are two key assumptions. First, because this experiment gauged the difference in the amount of force applied on days 1 and 2, the amount of force applied on day 1 during a maximum effort test was arbitrarily assumed to be the participants true maximum clench force in kg (F3 on Figure 6). Deviation refers to the percentage change that a participant displayed from their true maximum clench force (F3 on Figure 6) to their clench force applied on time to fatigue tests on either day 1 or day 2 (as represented by F2 and F5 on Figures 6 and 7 respectively). Secondly, during data collection of day 2 data we assumed all participants would first overshoot their expected 50% effort before leveling out to a more appropriate grip strength level which they could sustain throughout the time to fatigue test (see Figure 7). Accordingly, when determining the grip strength applied for the day 2 time to fatigue test, researchers chose a point after the initial overshoot (see F5 on Figure 7).

Regression curves were created for male and female participants by plotting day 1 % change in EDA as the dependent variable of the max-force time to fatigue to determine an appropriate curve to plot future participants onto, in order to identify if they significantly deviated from expected physiological markers. This regression was repeated for % increase in heart rate vs. time to fatigue max-effort. Regressions were also developed from the day 2 data with the same plot structure. Coefficient of determination analysis was applied to all graphs to determine strength of the proposed regression.

Positive Control

The change in skin conductance and heart rate due to a stimulus could be measured with Biopac Systems, Inc devices as shown by Figure 4. Figure 3 shows baseline measurements of all physiological measurements. Each group member performed a Valsalva maneuver to cause a physiological change heart rate and EDA from the resting state. The change in heart rate is shown in Row 1 of Figure 4, where peaks larger than the baseline are observed. The change in ECG is shown in Row 2 of Figure 4 and a peak of 1.5mV is observed,

while the baseline is just under 1mV. The change in EDA is shown in Row 3 of Figure 4, where peaks between 12.0 and 12.3 microSiemens are observed compared to the baseline of 10 microSiemens. This positive control data showed that heart rate and EDA measurements pertaining to the experiment could be measured after the stimulus of a Valsalva maneuver. Positive control data was collected for 5 individuals to ensure physiological changes could be consistently detected using the Biopac Systems, Inc. devices (*Figure will be shown in final paper).

Negative Control

Before collecting clench force data, baseline physiological data of EDA and heart rate was collected for 30 seconds when group members were sitting down in a resting position with their eyes closed.

RESULTS

Two-tailed t-tests were used to analyze the physiological comparisons for all measures unless specified as a logarithmic regression analysis.

Average percent deviation between maximum clench force (F3 of Figure 6) and initial maximum clench during the time to fatigue test on day 1 (F2 of Figure 6) was significantly less than the average percent deviation between maximum clench force (F3) and initial maximum clench during the time to fatigue test on day 2 (F5 of Figure 7), $p = 4.60566E-11$ (Figure 8).

Average percent change from baseline EDA (E1 in Figure 6) to maximum EDA (E2 of Figure 6) during the time to fatigue test with maximum effort exerted on day 1 was significantly higher than the percent change from baseline EDA (E3 of Figure 7) to maximum EDA (E3 in Figure 7) during the time to fatigue test with 50% effort on day 2, $p = 0.0295$ (Figure 9).

Similarly, percent change from baseline (H1 of Figure 6) to maximum heart rate (H2 of Figure 6) during the time to fatigue test with maximum effort on day 1 was significantly higher

than the percent change from baseline (H3 of Figure 7) to maximum heart rate (H4 of Figure 7) during the time to fatigue test with 50% effort exerted on day 2, $p = 0.0024$ (Figure 10).

A logarithmic regression analysis comparing the percent change from maximum clench force (F3) to initial clench of the time to fatigue test (F2 and F5) with the percent change between baseline EDA (E1 and E3) to maximum EDA (E2 and E4) as well as percent change between baseline heart rate (H1 and H3) to average heart rate (H2 and H4) showed a positive, though insignificant, trend between clench force and both EDA and heart rate with $R^2 = 0.07566$ and $R^2 = 0.25125$, respectively (Figure 11).

Similarly, a logarithmic regression analysis comparing percent of maximum clench force achieved at day 1 initial clench force at time to fatigue test (F2) and day 2 initial clench force at time to fatigue test (F5) with change in EDA from baseline (E1 and E3) to maximum EDA (E2 and E4) did not show a significant relationship between the variables, $R^2 = 0.00478$ (Figure 12A). Logarithmic regression analysis comparing percent of maximum clench force achieved at day 1 initial clench force at time to fatigue test (F2) and day 2 initial clench force at time to fatigue test (F5) with change in heart rate from baseline (H1 and H3) to maximum heart rate (H2 and H4) showed a moderately significant relationship between the variables, $R^2 = 0.68883$ (Figure 12B).

A two-tailed t-test was performed to analyze EDA data that suggested feedforward activation during the 5 seconds before the time to fatigue trials. Average percentage change in EDA accounted for by feedforward activation showed a significant difference between blinded and unblinded participants, $p = .028495$, (Figure 13). EDA data from both day 1 and day 2 time to fatigue tests was pooled, and any subjects data that showed percentage change $<0.001\%$ were considered to be a 0% change.

DISCUSSION

This research project examines covariance between two physiological variables, electrodermal activity (EDA), heart rate, and clench force. Collected data confirmed our original hypothesis that EDA and heart rate outputs would positively correlate with the percentage effort exerted during a time to fatigue test of endurance. After considering and normalizing to baseline data for each candidate, our data revealed exciting categorical associations between percentage of effort put forth on a clench force tests and percentage increases in EDA and heart rate. Additionally, an intriguing finding was revealed that blinded participants displayed increased feed-forward EDA activation on a time to fatigue endurance task as compared to their un-blinded counterparts. The results indicate promising trends which could be further investigated in future studies. Such future studies might even consider developing computational models which reliably predict the amount of effort being applied on strength and endurance tests based on physiological readouts such as EDA and heart rate.

In the day 2 time to fatigue test, our data suggests that participants were consistently providing less force than the 50% of their original maximum grip strength that they were instructed to provide. The participants' day one maximum clench force in the time to fatigue test was on average 98.596% of their day one maximum clench force, as shown in Figure 8. When participants were told to clench what they deemed to be 50% of their maximum grip strength, they gave a time to fatigue grip strength that was on average 38.624% of their day 1 max grip strength. An explanation for this could be that participants did not want to overshoot their true 50% of maximum grip strength in order to achieve the longest time to fatigue in the study, or simply were unsure of what 50% of their maximum grip strength felt like, as the maximum grip strength measurement was performed at least a week prior. The result provides support for the purpose of our study in that participants may cheat, or give less than maximum effort, during a time to fatigue test in order to achieve the longest time to fatigue. In a future study, this result could be used to determine whether or not a participant is cheating in a time to fatigue test.

Change in EDA and heart rate were both greater on day 1 when giving maximum clench force than on day 2. Average percentage change in EDA from baseline corresponding with attempted 50% effort in the day 2 time to fatigue trial, 15.259%, was significantly lower than the average percentage change in EDA during the maximum effort time to fatigue trial performed on day 1, 30.075% (Figure 9). Similarly, average percentage change in heart rate from baseline to attempted 50% effort in the day 2 time to fatigue trial, 77.085%, was significantly lower than the day 1 time to fatigue average percentage change in heart rate, 122.608% (Figure 10). These results suggest that effort given and percentage change in EDA covary, as well as effort given and percentage change in heart rate. As grip strength given in a time to fatigue test decreases (Figure 8), average percentage change in EDA and heart rate also decrease. This data is consistent with the hypothesis put forward by Andrew Lokuta and the results of his study with Physiology 335 classes at UW-Madison. Our results show that both EDA and heart rate can be used as biomarkers to determine whether or not someone is truly giving maximum effort in a time to fatigue test.

In addition to the correlations between increased grip strength and increased EDA and heart rate explained by Figures 8, 9, and 10, the physiological outputs from this experiment were mapped onto several logarithmic regression plots. We hypothesized that there would be a correlation between the entire population of participants and percentage change in EDA and clench force when giving maximum and 50% effort, enabling us to determine, in one day, if the participant was giving maximum effort (Figure 11). Modeling percentage change in EDA and heart rate in conjunction with percentage change in grip strength in the day two trial did not strongly support our hypothesis that heart rate and EDA can be examined in one trial to determine whether or not someone was cheating.

In order to further investigate the relationship between the two dependent variables of heart rate and EDA, logarithmic regression plots were also formulated using raw values for each physiological readout (Figures 12A and 12B). This method gave an individual approach to

determining maximum effort in that one can compare elevation of EDA and heart of an individual while giving maximum effort and later compare this change with elevation of EDA and heart rate during another task to determine the percentage of maximum effort given. The regression plot displaying percentage deviation and change in EDA raw values did not yield a significant line of regression, which could be explained by the minute scale of EDA changes seen in response to various clench force efforts on time to fatigue tests. Likely, in future studies, the percent change in EDA values as plotted on Figure 11 would be a more valuable statistical readout than the raw values which are plotted on Figure 12A. If more resources were available in the future, it would be interesting for researchers to conduct a similar study with higher numbers of participants in order to see if the regression plot displaying percentage changes would yield a significant regression line.

A final logarithmic regression displaying percentage deviation and change in raw heart rate values did yield a significant line of regression (Figure 12B). Based on previous literature, such as the Wyatt et al. study, heart-rate reaches a threshold during physiological exertion (2005). Therefore, the physiological output is best modeled by a logarithmic model. While keeping in mind the study's limited sample size and constraints, Figure 12B suggests that using the logarithmic formula $y = 60.253 \ln(x-234.85)$ could tentatively be used to predict a participants expected percentage effort of their maximum clench force, on a time to fatigue test, based on their change in heart rate. Such a logarithmic model could be useful in further gauging, based on a physiological readout of heart rate, whether student participants are truly exerting their maximum effort on a time to fatigue test.

Our data showed an interesting and significant increase in participants EDA values in the five seconds leading up to the time to fatigue trials on both Day 1 and Day 2 (Figure 14). During the trials, we counted each participant down from five seconds to when they were to begin their time to fatigue trials. During these five seconds, we noticed EDA increasing even though the participants had not began clenching yet (Figure S4). We compared the percentage

change in EDA of our participants in the five seconds leading up to the beginning of their time to fatigue trials with our own percentage change in EDA in the five seconds before time to fatigue trials, considering the participants blinded and ourselves not blinded. The blinded participants had a significantly greater average percentage change in EDA than we did. An explanation of this trend could be a possible mechanism of feed forward activation which corroborates findings from Gordon et al. and Shibasaki et al. showing that sweating precedes static exercise. The data suggests that because we, as both moderators and participants of the study, knew the entirety and purpose of the study, we did not get nervous or need to physiologically prepare for the time to fatigue test. On the other hand, the blinded participants were unaware of how it would feel to participate in the study and were in competition with the rest of the class causing them physiologically prepare for the test and have a larger percentage change in EDA in the five seconds before their time to fatigue trials. This data can be examined as an immediate example of feedforward activation, however further studies with greater numbers and variety of participants should be done to test this mechanism and solidify its claim.

We assumed that a true maximum clench force is the result of the average of three trials (F3). Because participants initially over estimated 50% of their maximum clench force given on day one, but determined the correct force shortly after, we collected clench force data for 2 seconds (Figure 7; F5) shortly after the initial peak to collect a consistent initial maximum clench for participants. The assumption is that every participant would (1) over-estimate 50% of their initial clench force and (2) would re-adjust their grip to what they believed to be their true 50% maximum. Additionally, we noticed that the heart rate of participants would level out towards the end of the time to fatigue test (Figure 7). Whereas during the day one trials (Figure 6), heart rate remained more consistent over the course of the time to fatigue test. A possible explanation is that as participants effort over the day two time to fatigue began to drop because they did not achieve their true 50% maximum.

The data from our study shows that males achieved a greater average maximum clench force compared to their female counterparts (Figure S2). This validates the common explanation, set forth by Drury et al and Staszkiwicz et al, that men tend to produce greater absolute forces on a hand dynamometer strength test than females (2004). Although men produce greater absolute forces, they performed relatively poorly compared to women on time to fatigue tests (Figure S3). Although a physiological explanation has not been established, our findings are consistent with studies conducted by West et al (1995). The data summarized in both Figures S2 and S3 are consistent with the data collected by Dr. Lokuta in his study with the Physiology 335 class. One difference, however, is that average time to fatigue for both men and women were larger in Dr. Lokuta's data when than the times achieved by participants in our study (Figure S1). One possible explanation to explain this discrepancy is that we did not provide an incentive (other than verbal encouragement) to produce the longest time to fatigue possible. Studies have shown incentives improve performance and increase motivation for study participants (Richter, 2014). This may explain why both men and women have longer fatigue times compared to the study we conducted. Despite this lack of incentive, our results follow the same trend.

After finding several of the measured physiological outputs to show significant trends and statistical differences, it was concluded that the null hypothesis could be rejected, but only with the added consideration of several constraints. The above study's intriguing results suggest that both EDA and heart rate positively correlate with the amount of effort put forth in a hand dynamometer time to fatigue test. However, the experimental findings of this study cannot be extrapolated to all age groups because this study was performed on a fairly homogenous population of university students between the ages of 19 and 25. Additionally, due to availability of study participants, there were uneven numbers of male (n=5) and female (n=18) participants. In considering that males and females display significantly different maximum grip strengths and time to fatigue test times (Figure S2 and S3), it is possible that the physiological co-variants for

males and females could also differ. In using a logarithmic regression curve, there was a slight concern that variance could have been increased by not consistently separating male and female data points. However, further experimentation with a larger sample size and more even distribution, is necessary to determine if differences exist between male and female grip strength co-variants. No extensive variability in data quality was noted, which suggested that the equipment and measures used in this experiment were valid and could be reliably used for future studies.

CONCLUSION

The results of this study allow us to confidently reject the null hypothesis and validate that skin conductance (EDA) and heart rate covary with initial maximum clench force, a measurement of isometric effort, during a time to fatigue test. After the data was analyzed, it was found that EDA and heart rate are observable physiological biomarkers that demonstrate if a participant is giving a true maximum effort (see discussion on true max effort). Interestingly, the data collected in this study provides a useful logarithmic regression curve comparing changes in effort level and the associated difference in heart rate (Figure 13). Therefore, within the constraints of our study, we could predict a participant's change in effort given their maximum clench force and heart rate. This regression could prove to be useful in future studies that seek to measure a participant's level of effort when utilizing these physiological variables.

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APPENDIX

Time (duration)	Dialogue <i>Name of dialogue section: specific script</i>
Start	<i>Time to fatigue briefing:</i> You will be completing a time to fatigue study. You will clench the dynamometer as hard as you can and keep that clench for as long as you can. Once your force reaches 50% of your initial maximum clenching we will end the trial. We will give you periodic updates on how close you are to 50%. Once you consistently are at 50% of your max clench force we will tell you to “relax”. Again, please try to give your maximum effort. Do you have any questions?
Baseline (0-30 seconds)	<i>Baseline briefing:</i> We are going to collect some baseline data for 30 seconds and then we will begin the time to fatigue trial. I will instruct you when to squeeze after the 30 second baseline period is up. Please keep the dynamometer flat on your palm during the first 30 seconds. When I count down “5-4-3-2-1 squeeze,” please squeeze the dynamometer as hard as you can for as long as you can.
Time to Fatigue trial (~10-90 sec)	<i>Time to fatigue initiation:</i> 5-4-3-2-1 squeeze
<ul style="list-style-type: none"> ● After clench strength has fallen to about 60% of initial 	<i>Time to fatigue encouragement 1:</i> You’re doing great!
<ul style="list-style-type: none"> ● After clench strength has fallen to about 30% of initial 	<i>Time to fatigue encouragement 2:</i> Don’t give up! You can do this!
<ul style="list-style-type: none"> ● At 3 seconds of less than 50% of max clench force 	<i>Time to fatigue completion:</i> Relax

1 minute recovery period	<i>Max clench for briefing:</i> You will now be completing a maximum clench force study. You will perform 3 maximum clench force trials, each trial will last only 2 seconds. You will then rest for 10 seconds between each trial. When I count down “5-4-3-2-1 squeeze,” please squeeze as hard as you can for two seconds and then I will tell you to rest. We will show you a graph of your clench force measured in kilograms. We will repeat this 3 times total. Do you have any questions?
Max Clench Trial 1 (0-2 sec)	<i>Max clench force initiation 1:</i> 5-4-3-2-1 squeeze <i>Max clench force completion 1:</i> Rest
10 second rest period	<i>Max clench force encouragement 1:</i> Your maximum clench force was approximately _____. This time try and reach _____ (2 times value of trial 1).
Max Clench Trial 2 (0-2 sec)	<i>Max clench force initiation 2:</i> Squeeze <i>Max clench force completion 2:</i> Rest
10 Second Rest Period	<i>Max clench force encouragement 2:</i> Nice job, lets try to hit that number again.
Max Clench Trial 3 (0-2 sec)	<i>Max clench force initiation 3:</i> Squeeze <i>Max clench force completion 3:</i> Rest
End	<i>End of study briefing:</i> Thanks for your participation.

Figure 1: This table outlines the verbal script of the researchers and the timeframe of all verbal cues for the Day 1 procedure when maximum clench time to fatigue and maximum clench force are recorded.

Time (duration)	Dialogue <i>Name of dialogue section: specific script</i>
Start	<i>Time to fatigue briefing:</i> You will be completing a time to fatigue study. This time, you will clench the dynamometer at what you believe is half of your maximum clench force and keep that clench for as long as you can. We will record how long you can clench for. The person who has the longest clench time wins a prize. Do you have any questions?
Baseline (0-30 seconds)	<i>Baseline briefing:</i> We are going to collect some baseline data for 30 seconds and then we will begin the time to fatigue trial. I will instruct you when to squeeze after the 30 second baseline period is up. Please keep the dynamometer flat on your palm during the first 30 seconds. When I count down “5-4-3-2-1 squeeze,” please squeeze the dynamometer at what you believe is half of your maximum clench force for as long as you can.
Time to Fatigue trial (~10-90 sec)	<i>Time to fatigue initiation:</i> 5-4-3-2-1 squeeze

<ul style="list-style-type: none"> After clench strength has fallen to about 60% of initial 	<i>Time to fatigue encouragement 1: You're doing great!</i>
<ul style="list-style-type: none"> After clench strength has fallen to about 30% of initial 	<i>Time to fatigue encouragement 2: Don't give up! You can do this!</i>
<ul style="list-style-type: none"> At 3 seconds of less than 50% of half-max sustained clench force 	<i>Time to fatigue completion: Relax</i>
End	<i>End of study briefing: Thank you for your participation.</i>

Figure 2: This table outlines the verbal script of the researchers and the timeframe of all verbal cues for the Day 2 procedure when data for subjective half clench force time to fatigue was collected.

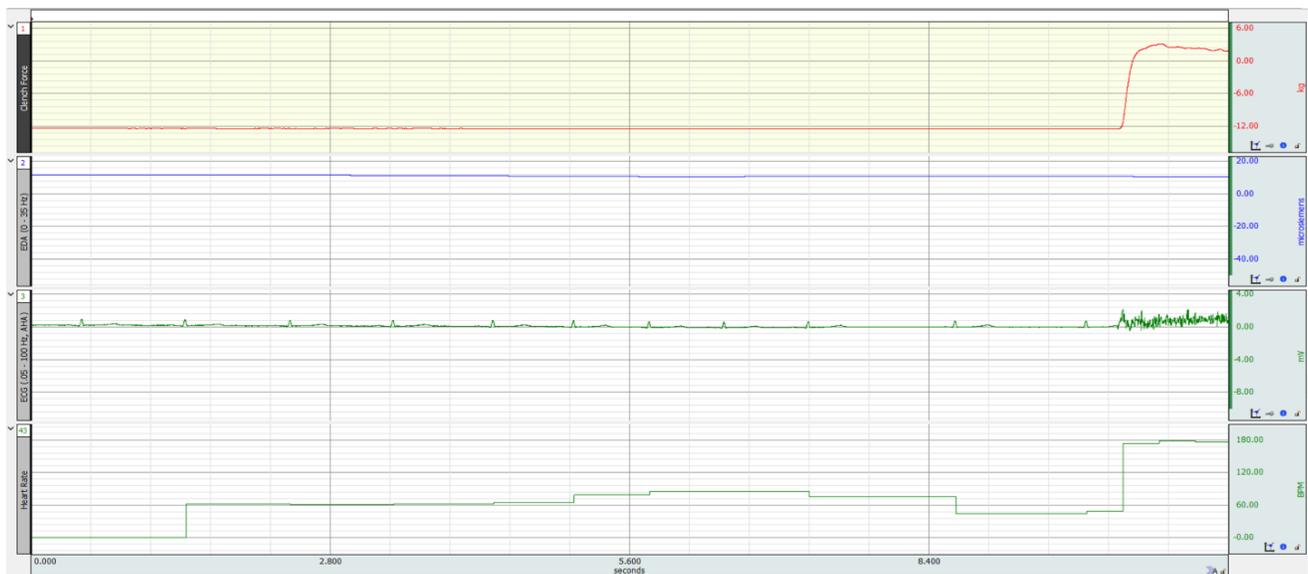


Figure 3: A prediction of baseline measurements taken 30 seconds prior to max clench force test. In ascending order: baseline measurements for clench force (kg), skin conductance (uSiemens), and pulse (mV and BPM). The last second of the graph (far right side) shows the initiation of a maximum clench force thus indicating that the negative control data collected was different than the data that will be collected after the initiation of the stimuli (clench force).

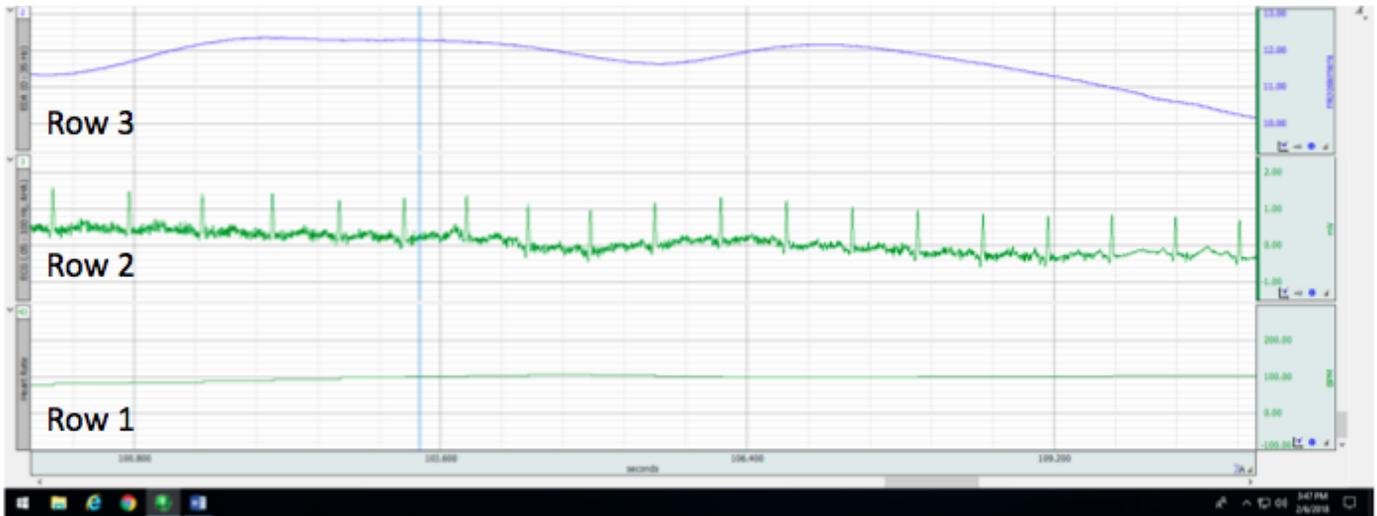


Figure 4: Graph showing changes in skin conductance (row 3), and heart rate (row 1,2) following a Valsalva Maneuver. Note: rows 1 and 2 both show data for heart rate. Row 1 is in BPM and row 2 is in mV. Note: heart rate in mV was used solely for the integration of BPM and not used in data analysis.



Figure 5: A prediction of a maximum clench force (left peak) followed by 50% maximum clench force (right peak) given in a positive control test.

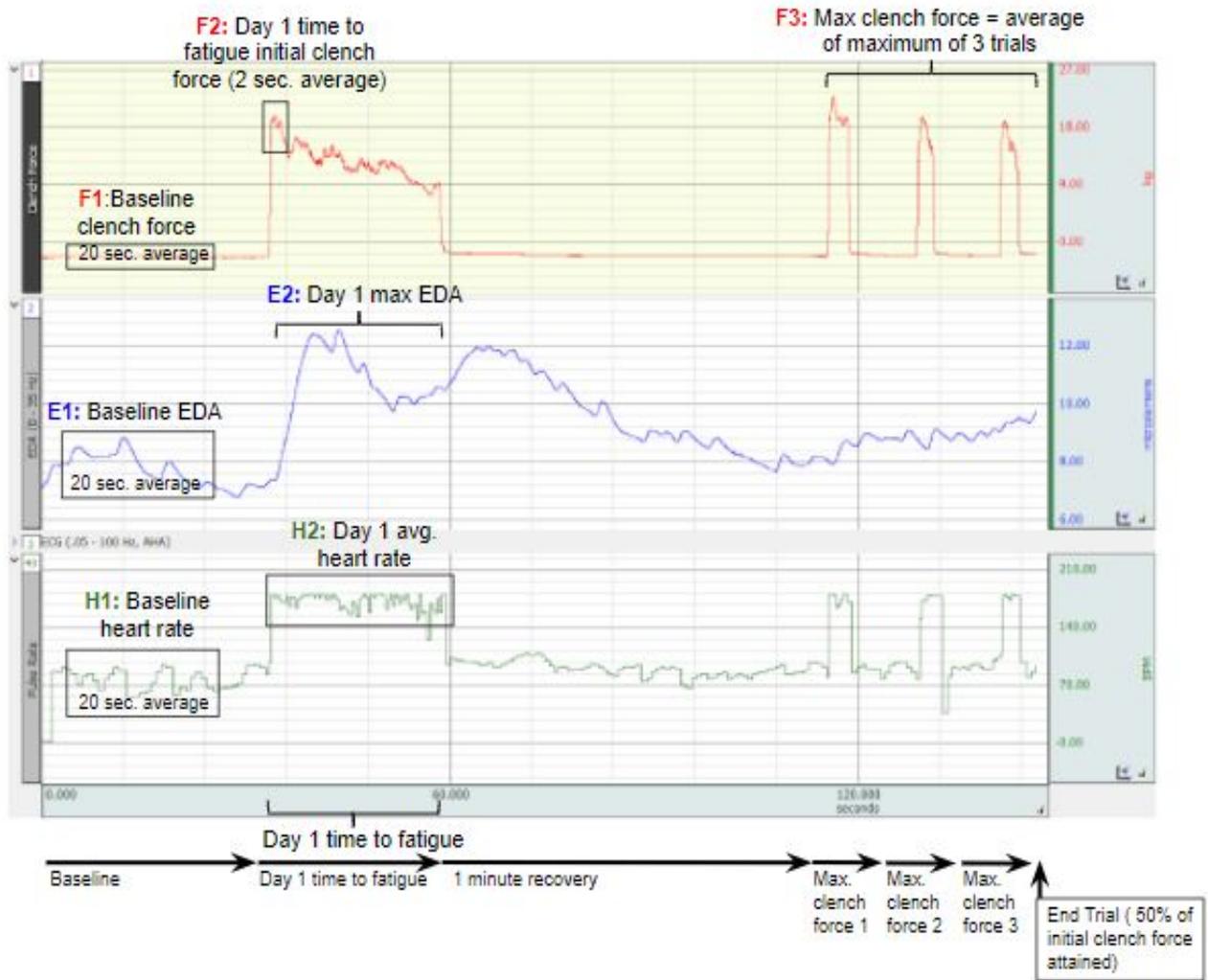


Figure 6. Representative screenshot of data taken for a typical participant during the Day 1 Procedure for the time to fatigue and maximum clench force trials. Baseline clench force, initial clench force in the time to fatigue test, and average maximum clench (kg) were averaged across the intervals indicated. Percent change from baseline to maximum EDA (microSiemens) and heart rate (BPM) were calculated from the time intervals indicated.

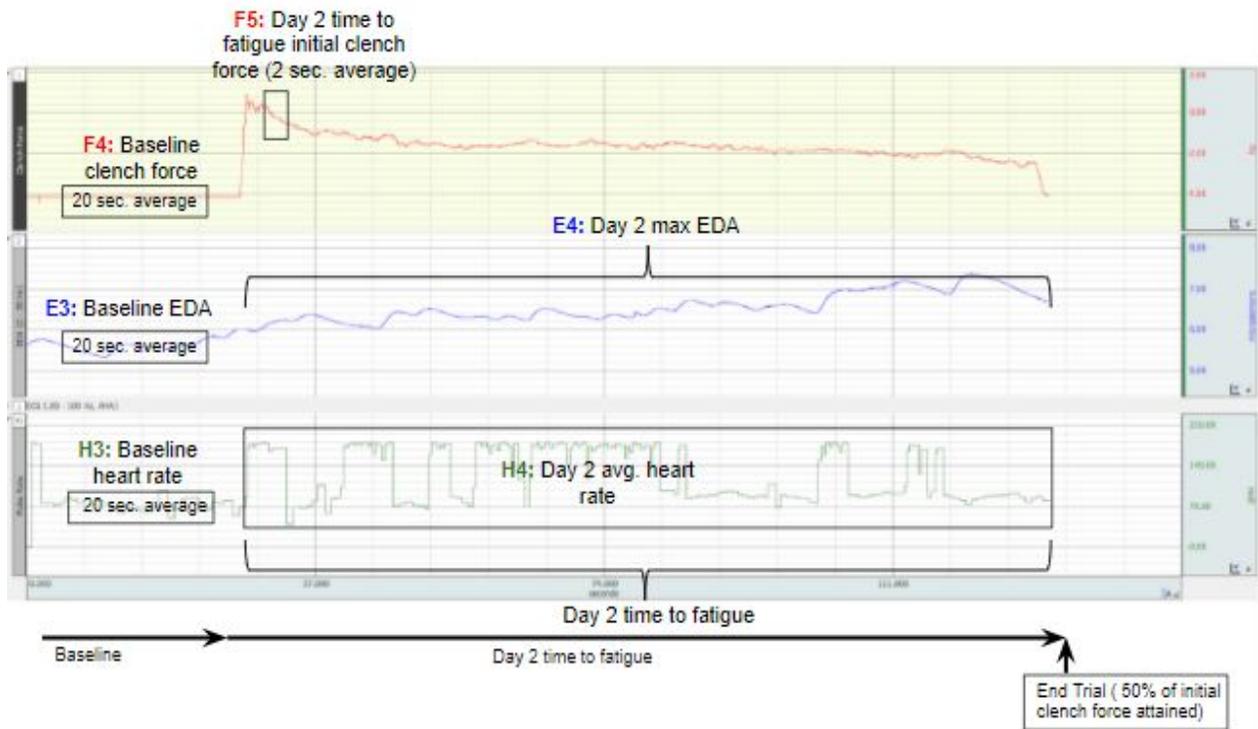


Figure 7. Representative screenshot of a typical participant during Day 2 time to fatigue trial. Baseline clench force and initial clench force (kg) were averaged across the intervals indicated. Percent change from baseline to maximum EDA (microSiemens) and heart rate (BPM) were calculated from the intervals indicated.

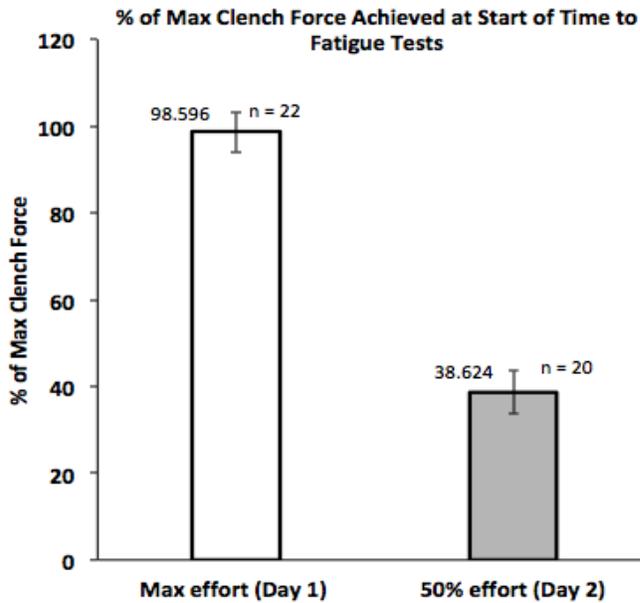


Figure 8. Average percent deviation from maximum clench force (kg, F3) in initial maximum clench given in the Day 1 and Day 2 time to fatigue tests (F2 for Day 1 and F5 for Day 2 with $n=22$ and $n=20$, respectively), $p = 4.606E-11$.

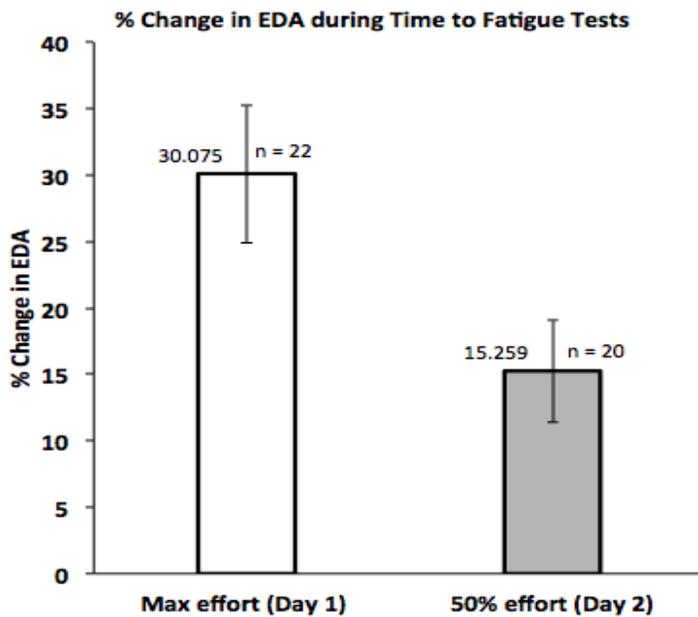


Figure 9. Average percent change from baseline EDA (microSiemens, E1 on Day 1 and E3 on Day 2) to maximum EDA during the time to fatigue test with maximum effort on Day 1 (E2) and maximum EDA during the time to fatigue test with subjective 50% effort on Day 2 (E4), $p = 0.0295$.

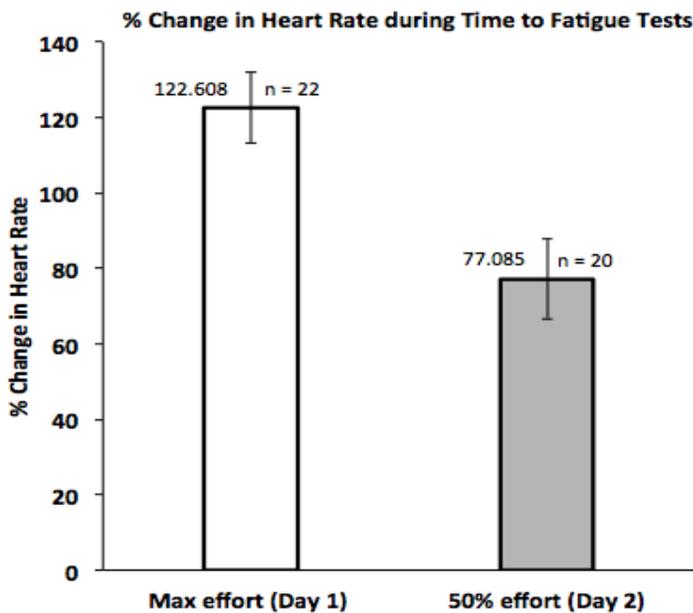


Figure 10. Average percent change from baseline (H1) to maximum heart rate (BPM, H2) during the time to fatigue test with maximum effort on Day 1 compared with average percent change from baseline (H3) to maximum heart rate (H4) during the time to fatigue test with subjective 50% effort on Day 2, $p = 0.0024$.

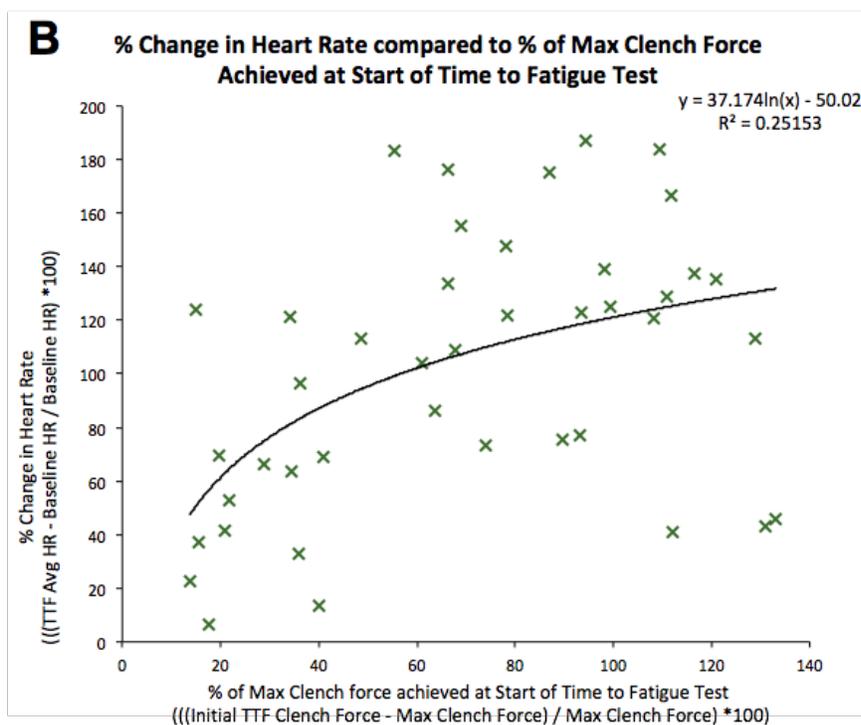
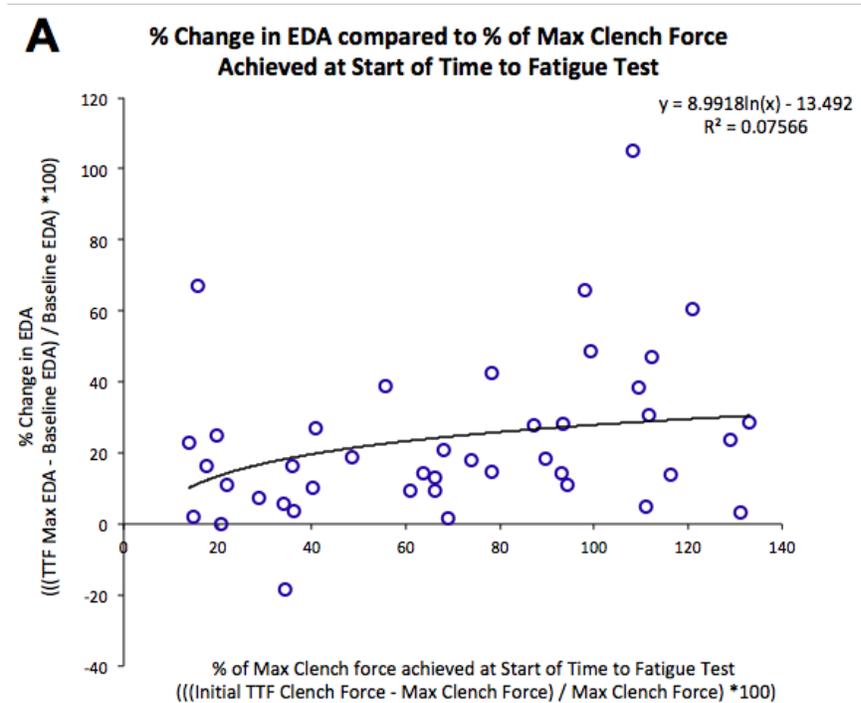


Figure 11. Logarithmic regression plot comparing the percent change from maximum clench force (F3) to initial clench of the time to fatigue test (F2 and F5) with the percent change between baseline EDA (E1 and E3) to maximum EDA (E2 and E4) as well as percent change between baseline heart rate (H1 and H3) to maximum heart rate (H2 and H4).

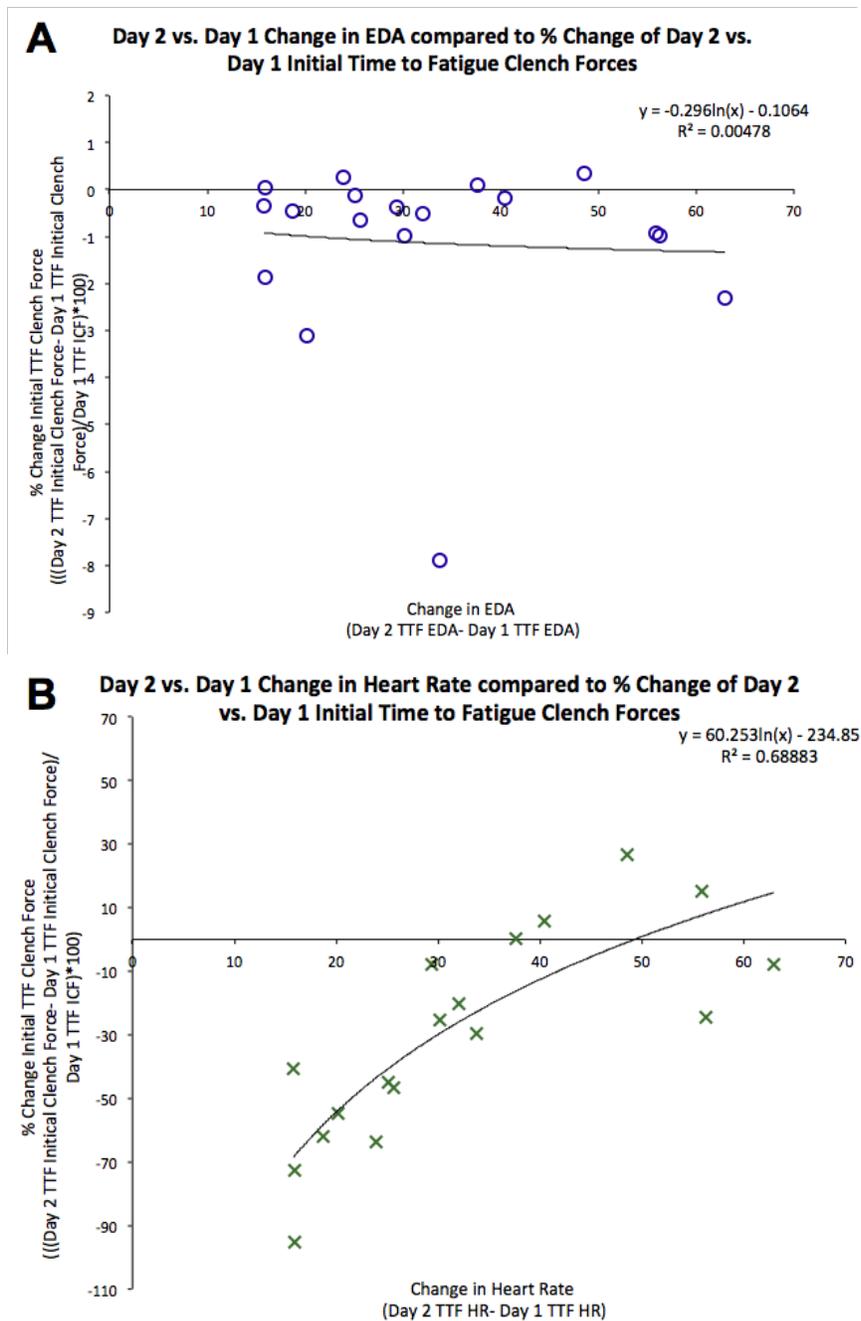


Figure 12. (A) Logarithmic regression plot with percent deviance between Day 1 maximum clench force during the time to fatigue test (F2) and Day 2 maximum clench force during the time to fatigue test (F5) on the y-axis and change in EDA from baseline EDA (E1 and E3) to maximum EDA (E2 and E4) on the x-axis. (B) Logarithmic regression plot with percent deviance between Day 1 maximum clench force during the time to fatigue test (F2) and Day 2 maximum clench force during the time to fatigue test (F5) on the y-axis and change in heart rate from baseline heart rate (H1 and H3) to maximum heart rate (H2 and H4) on the x-axis.

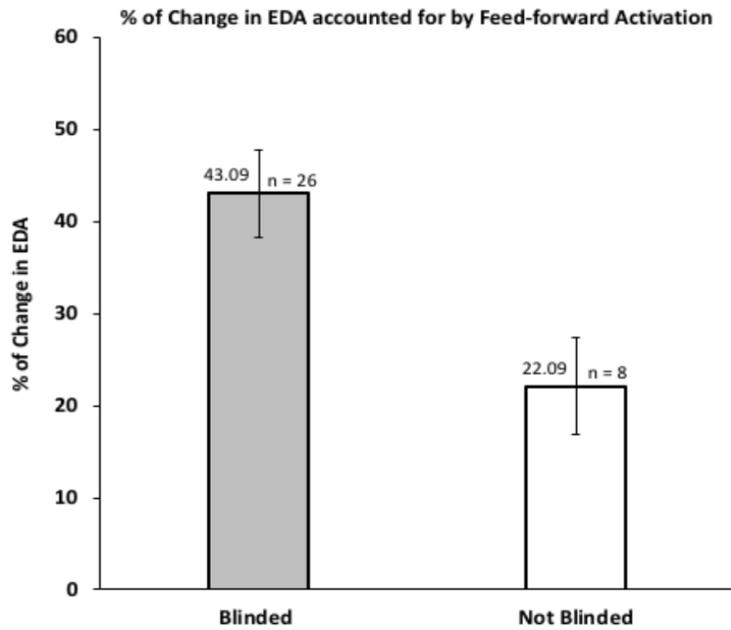
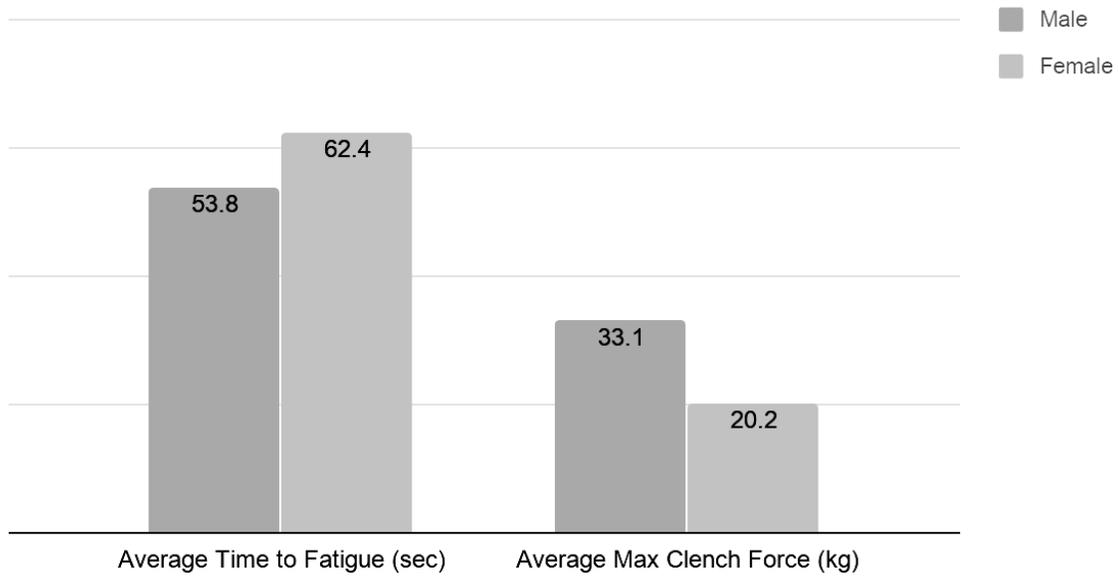


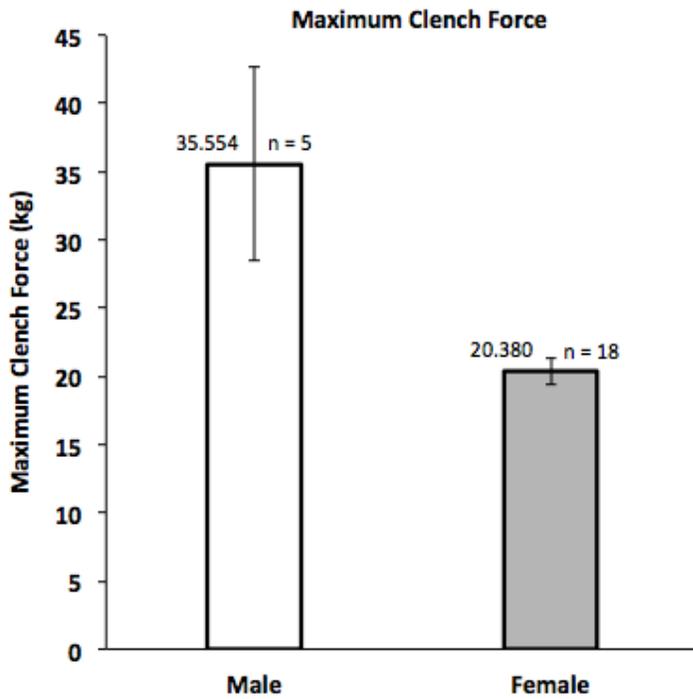
Figure 13. Average percentage change in EDA during the 5 seconds leading up to time to fatigue trials for blinded versus unblinded participants. Pooled data of both Day 1 and Day 2 and males and females, $p = 0.028495$.

SUPPLEMENTAL FIGURES

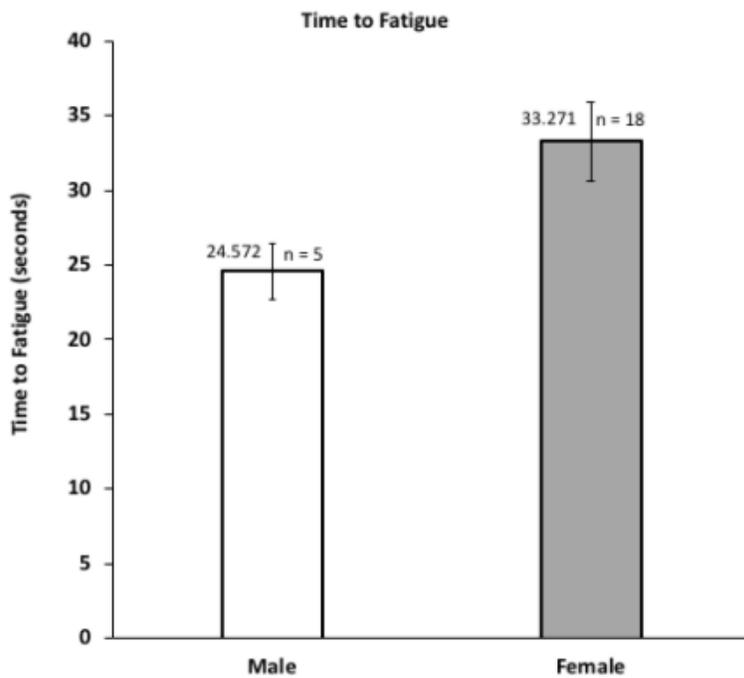
Preliminary Discrepancies in Male and Female Time to Fatigue and Maximum Clench Force



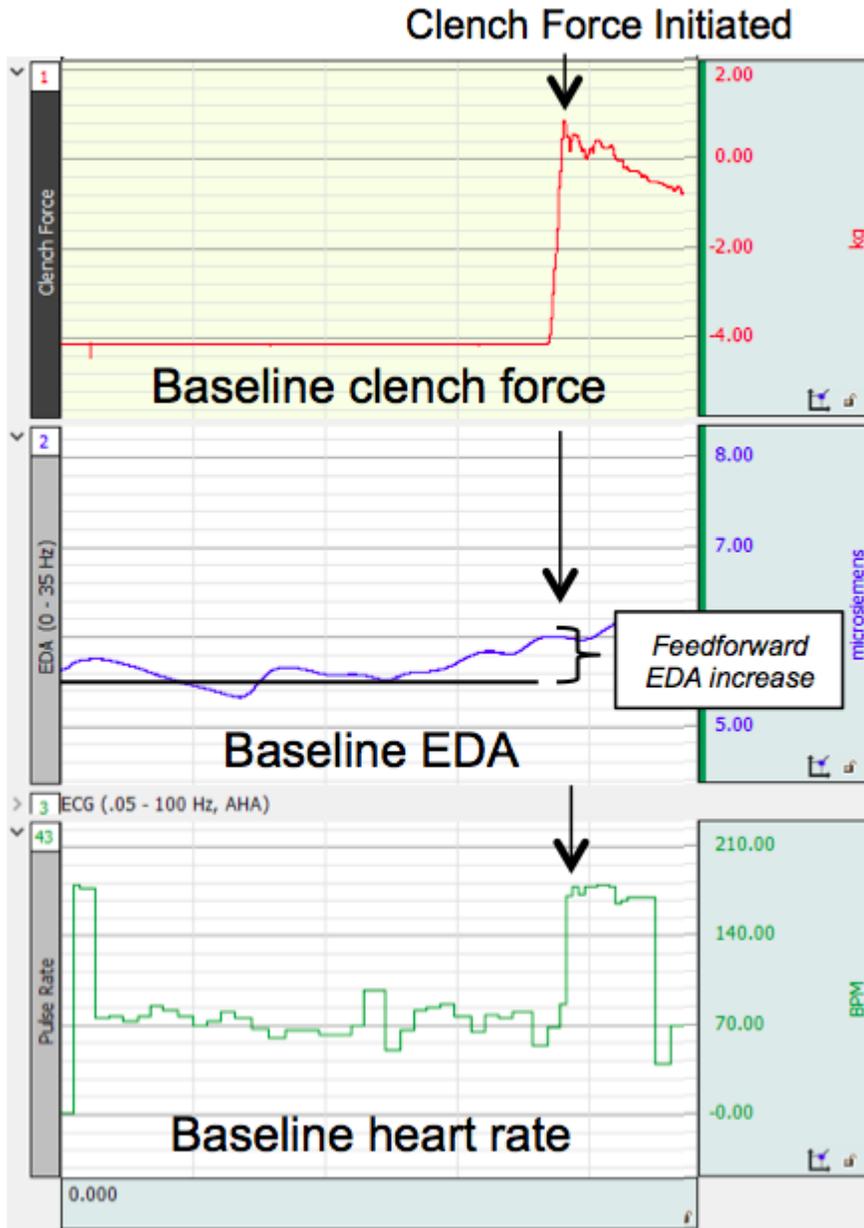
Supplemental Figure 1. Dr. Andrew Lokuta's data of average time to fatigue and maximum clench force tests results compiled from Physiology 335 courses between 2004 and 2008. Researchers conducting these trials suspected that male participants were not clenching at a true maximum initial force in the time to fatigue study and thus their average time to fatigue (53.8s) is artificially high.



Supplemental Figure 2. Average maximum clench force of Males and Females (F3 for Day 1), $p = 0.001256491$.



Supplemental Figure 3. Average Day 1 time to fatigue for Males and Females, $p=0.102417291$.



Supplemental Figure 4. EDA Feed forward activation recorded in the Biopac Systems Inc. before initiating a maximum clenching time to fatigue test. A feedforward response is not observed for heart rate.