

Mouthpiece use During Heavy Resistance Exercise Affects Serum Cortisol and Lactate

W. D. Dudgeon, L. A. Buchanan, A. E. Strickland, T. P. Scheett, D. P. Garner

Accepted Manuscript Version

This is the unedited version of the article as it appeared upon acceptance by the journal. A final edited version of the article in the journal format will be made available soon.

As a service to authors and researchers we publish this version of the accepted manuscript (AM) as soon as possible after acceptance. Copyediting, typesetting, and review of the resulting proof will be undertaken on this manuscript before final publication of the Version of Record (VoR). Please note that during production and pre-press, errors may be discovered which could affect the content.

© 2017 The Author(s). This open access article is distributed under a Creative Commons Attribution (CC-BY) 4.0 license.

Publisher: Cogent OA

Journal: *Cogent Medicine*

DOI: <http://doi.org/10.1080/2331205X.2017.1403728>

Mouthpiece use During Heavy Resistance Exercise Affects Serum Cortisol and Lactate

W. D. Dudgeon, L. A. Buchanan, A. E. Strickland, T. P. Scheett, D. P. Garner

W. D. Dudgeon¹, L. A. Buchanan¹, A. E. Strickland², T. P. Scheett, D. P. Garner^{2*}

¹Department of Health & Human Performance, College of Charleston

²Department of Health, Exercise & Sport Science, The Citadel

*D. P. Garner, Department of Health, Exercise & Sport Science, The Citadel, Charleston, SC 29409

garnerd1@citadel.edu

Phone: 843-953-6323

Fax: 843-953-6798

***Corresponding Author**

Wesley Dudgeon received a Ph.D. in exercise science with a focus on applied physiology at the University of South Carolina in 2006. He is currently a tenured, associate professor at the College of Charleston and the head of department of health and human performance. His research interests include mouthpiece use during exercise and sport nutrition.

ABSTRACT

Recent reports suggest the use of mouthpieces may be beneficial at improving aerobic and anaerobic exercise performance. However, the mechanisms of these reported improvements have yet to be elucidated. The purpose of this study was to explore the possible mechanisms of improved performance using the ArmourBite® mouthpiece. Using a within subject randomized treatment design, 15 experienced resistance trained males (19-26 years of age) performed 6 sets of 10 repetitions of free weight back squats at 80% of 1RM with and without a mouthpiece. Blood samples were collected before exercise, after 3 sets (Mid), immediately post (Post), 30 minutes post (Post-30), 60 minutes post (Post-60) and 120 minutes post (Post-120) exercise. Samples were analyzed for lactate and ELISA was used to determine cortisol. Mouthpiece use resulted in more repetitions completed without assistance (54.36±0.61 vs. 53.27±0.79, p=0.046 and fewer assisted repetitions (6.73 ± 0.79 vs. 5.64 ± 0.61 repetitions, p=0.046 compared to the control group. Lactate concentrations were lower in the treatment versus control group at the Post (11.54±2.23 vs.13.07±2.96 mmol/L, p=0.023 Post-30 (4.45±1.94 vs.5.41±1.90 mmol/L, p=0.021, and Post-60 (2.07±0.94 vs.2.55±0.96 mmol/L, p=0.048) sampling periods. Mouthpiece use lowered cortisol levels at Mid and Post-30 (19.39±6.90 vs. 27.84±14.56 µg/dL, p=0.02 22.91±8.47 vs. 31.81±10.79µg/dL, p=0.04. Cortisol AUC values showed significant differences within the AUC Pre-Post control and treatment (55.16±23.84 vs. 41.95±2.65 µg/dL, p=0.02 groups. These data suggest that mouthpiece use may increase performance and decrease stress when used during intense resistance exercise.

Keywords: performance mouth guard, jaw clenching, anaerobic exercise, physiological parameters

1 Background

Mouthguard use during sport has been utilized as a method to prevent oral-facial injury, with a review of dental trauma literature citing participation in sport as being the greatest cause of dental injury (Glendor, 2009; Hughston, 1980). However, the use of such appliances has also been cited to improve athletic performance. Early research in this area focused on the use of MORA devices (mandibular orthopedic repositioning appliance) stated to improve performance along with the protection of teeth (Fonder, 1976). The review of this research occurring in the late seventies and early eighties, was varied and provided no solid evidence that these devices improved performance based on the methodology used in the studies. Smith, Stenger, and Grunwaldt found improvements in strength with MORA devices in professional football players, with Grunwaldt finding an 8-11% improvement in Cybex muscle testing in these athletes with the oral appliance (Smith, 1982; Stenger, 1977). Yet in testing college athletes, researchers were unable to detect differences in strength with the use of an oral appliance (Welch, Edington, & Ritter, 1986; Yates, Koen, Semenick, & et al., 1984). However, problems existed for each of these studies, ranging from small sample sizes to varied fitness levels of athletes to lack of uniformity of devices used between studies. Thus, research in the area of mouthpiece use during exercise, and measurement of these parameters, remained stagnant until the early 2000s when interest in this topic renewed partly due to the subjective feedback provided by athletes wearing mouthpieces designed by Shock Doctor, Bite Tech and Makkar Athletic; mouthguard companies that marketed the effectiveness of mouthguard use during exercise for performance enhancement.

Recently, a review of the anaerobic parameters during exercise showed that when using a mouthpiece improvements include increased torque, bench throw power and force, vertical jump,

and the Wingate anaerobic test (Arent, McKenna, & Golem, 2010; Busca, Morales, Solana-Tramunt, & et al., 2016; Dunn-Lewis, Luk, Comstock, & et al., 2012; Durante-Pereira, del Rey-Santamaria, Javierre-Garces, & et al., 2008; Ebben, Leigh, & Geiser, 2008; Morales, Busca, Solana-Tramunt, & et al., 2015). Dunn-Lewis and colleagues cited significant increases in bench throw power and force, increased rate of power production in the vertical jump for the Pure Balance mouthguard versus no mouthguard and an over the counter mouthguard, citing improvements in both men and women in the area of upper body power exercises (Dunn-Lewis et al., 2012). Busca and colleagues and Durante-Pereira and colleagues in testing countermovement vertical jumps cited significant improvements in mean power and height in the mouthpiece versus no mouthpiece condition (Busca et al., 2016; Durante-Pereira et al., 2008). Other studies found significant improvements, 4% peak power and 1% mean power, during the Wingate test using an oral appliance (Arent et al., 2010; Morales et al., 2015). Morales and colleagues also measured lactate during anaerobic testing and cited a significant 8% decrease in lactate measures with mouthpiece use (Morales et al., 2015). Although this research appears to support the use of a mouthpiece during anaerobic exercise, more research in the area of objective measures is needed to quantify a mouthpiece effect. To do this, Garner and colleagues have assessed lactate and cortisol during both aerobic and anaerobic exercise, finding significant improvements in both lactate and cortisol with mouthpiece use (Garner & McDivitt, 2009b; Garner, Dudgeon, & McDivitt, 2011; Garner & McDivitt, 2015). Specifically as it relates to anaerobic protocols, they found a 51% reduction in cortisol levels 10 minutes post intensive bout of resistance training. This is supported in the animal literature in which stressed rats had a significantly lowered stress response while biting on a stick versus no stick (Hori, Yuyama, & Tamura, 2004; Sasaguri, Kikuchi, Hori, & et al., 2005). Yet, there is a paucity of data in the

area of mouthpiece use and effect on lactate and cortisol during resistance exercise. Thus, the purposes of this study were to determine the effect of mouthpiece use on free weight back squat performance as well as the effect on lactate and cortisol measures mid exercise, immediately post, and 30, 60 and 120 minutes post exercise.

2 Methods

2.1 Experimental Approach to the Problem

The study was conducted using a within-subject randomized treatment design. One week following an initial visit, participants reported to the lab and were randomly assigned to either a control (no mouthpiece) or treatment (mouthpiece) group. Subjects remained blind to the treatment until their first scheduled testing day and received the other treatment during their second testing day, scheduled for one week later at the same time of day controlling for diurnal variations. Subjects were instructed to refrain from intense physical activity or exercise for 24 hours prior to testing as well as food and drink except for water 2 hours prior to testing. (Stachoweicz & Lebiezinska, 2016). The influence of overall diet on cortisol secretion is inconclusive, however; the military environment of our subject pool resulted in a very homogenous diet. After arriving for testing, researchers placed an IV catheter into a superficial forearm vein for each testing sequence. Following IV insertion, subjects followed a standardized 20-minute no activity period before the first blood sample to control for the stress of IV placement. Subjects in the treatment group were individually fitted by the researcher with the boil and bite Under Armour ArmourBite® (Under Armour, Baltimore, Maryland) mandibular mouthpiece molded to mandibular characteristics before IV insertion and were instructed to wear the mouthpiece throughout the entire testing period. Six blood samples were taken during each testing protocol to analyze blood lactate and serum cortisol: Pre-testing, after set 3 (mid),

immediately post exercise (post), Post-30 minutes exercise, Post-60 minutes exercise, and Post-120 minutes exercise. Following a standardized warm-up, subjects began the standardized testing procedure, which consisted of 6 sets of 10 repetitions at 80% of the subject's respective 1 RM with 2-minutes rest between sets. Assisted repetitions were used to help subjects complete all repetitions within a set.

2.2 Subjects

Fifteen experienced resistance trained male participants (19 to 26 years of age, height: 178 ± 6.3 cm, weight: 87 ± 13.6 kg, and body fat: $16 \pm 7\%$) with no prior performance mouthpiece use volunteered to investigate the performance benefit and response of serum cortisol and blood lactate with and without the use of ArmourBite® mouthpiece during and following heavy resistance exercise. Subjects were recruited through posters, announcements, word of mouth, and referrals that briefly explained the study. The study was explained to the participants upon their initial contact with the researchers and again during their first visit. The initial meeting included: written consent approved by the institution's Internal Review Board, a completed Health Questionnaire and Physical Activity Readiness Questionnaire, one repetition maximum (1 RM) test for the free weight back squat, and anthropometric measurements (body composition by BodPod™, height, and weight).

2.3 Procedures

All subjects performed the same 1RM free weight back squat protocol: After a standardized warm up, subjects performed 2 warm-up sets with 10 repetitions at 50% and 4 repetitions at 70% of the estimated 1 RM. Subjects then made their first attempt with their estimated 1RM. Researchers sought to achieve subjects' 1RM within 4 attempts. If subjects succeeded on their attempts the load was increased. If subjects failed on their attempt,

researchers reduced the load by 15-20 pounds and a re-attempt was made. Once subjects failed twice, the subjects' 1RM was recorded with the last successful attempt. Subjects were scheduled to begin testing at least 7 days following their initial visit. Testing sessions were scheduled based on subject's availability. Testing session time remained consistent for each trial. No mouthpiece was utilized during the initial testing period to ascertain estimated 1 RM.

Before any testing began, all subjects were fitted with the Under Armour ArmourBite® (Under Armour, Baltimore, Maryland) mandibular mouthpiece following standardized fitting instructions from the manufacturer. The mouthpiece was immersed in boiling water for 1 minute then fitted to the subject's lower teeth by placing the bite pads on the teeth and then having the subjects bite down. The fit of the mouthpiece was assessed by research team members to ensure that the mouthpiece was properly placed so that the upper teeth and the lower teeth were separated by the bite pads. If the mouthpiece did not fit properly, the mouthpiece was re-boiled, and the process repeated until proper placement was achieved. In addition, during all testing which involved the mouthpiece, subjects were instructed to bite down on the mouthpiece while completing the exercise session.

The testing protocol consisted of a standardized warm-up period of 10-minutes light resistance cycling (Monark Ergonomic 828 E), 5-minutes of jumping rope, 1 set of 10 repetitions at 50% of the subject's 1 RM free weight back squat, and 1 set of 4 repetitions at 70% of the subjects 1 RM. Subjects were then given a chance to stretch *ad libitum* before initiation of the exercise protocol known to elicit increase in serum cortisol, which consisted of 6 sets of 10 repetitions at 80% of the subject's respective 1 RM with a 2-minute rest following each set (Kraemer & Ratamess, 2005; Ratamess, Kraemer, Volek, & et al., 2005). If a subject could not complete 10 repetitions on a given set, a certified strength and conditioning specialist provided

the subject the necessary assistance required to complete each subsequent repetition for that set. All assistance was administered following the National Strength and Conditioning Association standardized spotting protocol (Sands, Wurth, Hewitt, & J., 2012) If 10 repetitions were not completed on a set the load was adjusted so subjects could complete 10 repetitions for each subsequent set. Subjects completed an identical protocol for both testing days.

2.4 Sample Collection and Analysis

Blood samples were collected through an 18-gauge, 1.75 inch indwelling IV catheter (Exel Safelet Cath, Exelint International Corporation, Los Angeles, California) placed into a superficial forearm vein. Researchers attached a 5 cm extension tube and a three-way stopcock with swivel male Luer-loc to the catheter. After placement of the catheter, researchers used 4 mL of heparinized saline (Heparin Lock Flush Solution 100 units/mL, BD, Franklin Lakes, New Jersey) in the extension tube to maintain patency of the line. A 2 mL heparinized saline flush was also performed at the conclusion of every blood draw except for the final. The catheter, extension tube, and stopcock were secured with paper tape (3M, St. Paul, Minnesota) and Coban wrap (3M, St. Paul, Minnesota). Following IV insertion, a standardized 20-minute no activity period before the first blood sample was utilized to control for the stress of IV placement.

Researchers collected approximately 24 mL of blood per sample in order to quantify lactate and cortisol. Using a 5 mL syringe with a Luer-loc tip, researchers drew approximately 4 mL of blood that was discarded as waste to clear the IV line of any heparinized blood. Using two 10 mL syringes with a Luer-loc tip, researchers collected 20 mL of blood. Blood samples were used to monitor hemoglobin, hematocrit and lactate, and for collection of blood serum and plasma for later examination of stress hormones. Serum tubes were placed in centrifuge (Horizon Premier, Fisher Healthcare, Philipsburg, Pennsylvania) and spun at 3000 RPM for 15 minutes.

Samples were then collected with transfer pipets (Samco Scientific Corporation, San Fernando, California) and placed in storage containers (Eppendorf North American, Hauppauge, New York) and immediately stored at -35°C (Thermo Scientific, USA) for immunoassay at a later date.

Hematocrit (Hct) and Hemoglobin (Hb) were measured to account for changes in plasma volume. Samples for Hct, Hb and lactate were taken from the remaining 6 ml of blood from the samples drawn. Blood lactate concentrations were measured using Lactate Plus distributed by Nova BioMedical (Waltham, MA). Three blood lactate concentrations were recorded and averaged together at each sampling period. Hct samples were determined in triplicate by placing blood in hemato-clad heparinized 75mm Hct tubes (Drummond Scientific Company, Broomall, Pennsylvania), packed with clay at one end, and wiped with a Kim Wipe (KimTech, Roswell, Georgia) before spinning. Hematocrit samples were centrifuged for 5 minutes at 10,000 revolutions per minute with a ZIPocrit (LW Scientific, Lawrenceville, Georgia) centrifuge. Hct values were determined using a metric ruler by the same research assistant. For Hb, blood samples were placed in HemoPoint H2 Microcuvettes and then analyzed in duplicate by a HemoPoint H2 (Stanbio, Boerne, Texas). If the two Hb values were more than 10% different from each other a third measurement was performed. Duplicate and triplicate samples values were averaged for each variable at each time point.

Enzyme-linked immunoassay analyses (ELISA) were used for detection of serum cortisol (Cortisol ELISA, ALPCO Diagnostic, Salem, New Hampshire). Samples were thawed only once and were assayed in duplicate following manufactures instructions. ELISA plates were analyzed using a Molecular Devices microplate reader (ELx808, BioTek Instruments, Winooski,

Vermont). Intra assay coefficients of variation (CV) for cortisol were 6%, and inter assay CV for cortisol were 16% $\mu\text{g/dL}$. Minimum sensitivity of serum cortisol was 0.4 $\mu\text{g/dL}$.

2.5 Statistical Analyses

Data evaluations were performed using Microsoft Excel with StatPlus supplementing analyses when needed. One way repeated measures analysis of variance (ANOVA) tests were used to determine any differences within the control and treatment groups. Dependent measures T-tests were used to compare between groups to determine if mouthpiece use has significant effects. A Bonferroni adjustment was made to protect against the chance of committing a type I error due to the low power associated with the small sample size. Mean area under the curve (AUC) analyses were performed using the trapezoidal method for three different time periods for both groups: All six sample periods (AUC All-6), Pre-testing through immediately Post (AUC Pre-Post), and immediately Post through Post-120 minutes (AUC Post- Post-120). Statistical significance was set at $p < 0.05$. All data are presented as mean \pm standard deviation.

3 Results

Based on previous research it was hypothesized that the use of the ArmourBite® mouthpiece would decrease serum cortisol levels and lactate levels during heavy resistance exercise. No significant changes in plasma volume were found between groups during the trial period. Due to the testing protocol, loads (weight lifted) were identical between groups. Loads decreased significantly between set 3 and set 4 (119 ± 21 vs. 114 ± 20 kg, $p = 0.01$ and from set 4 to set 5 (114 ± 20 vs. 109 ± 19 kg, $p = 0.028$). Loads did not decrease significantly with sets 1 through 3. However; set 4, set 5, and set 6 loads decreased significantly compared to set 1 (121 ± 24 vs. 114 ± 20 , 109 ± 19 , and 109 ± 19 kg; Set 4, $p = 0.016$; Set 5, $p = 0.013$; Set 6, $p = 0.013$).

See figure 1 for details of loads lifted. These results were expected, as the exercise protocol is known to cause muscular fatigue.

Total mean non-assisted repetitions (i.e., completed under the subject's own power) were different between control and treatment groups with the treatment group completing more non-assisted repetitions before failure (53.27 ± 0.79 vs. 54.36 ± 0.61 repetitions, $p = 0.046$).

Consequently, total mean assisted repetitions were also different between control and treatment groups with the control group requiring significantly more assisted repetitions (6.73 ± 0.79 vs. 5.64 ± 0.61 repetitions, $p = 0.046$).

Blood lactate concentrations were significantly lower in the treatment group versus control group at the Post (11.54 ± 2.23 vs. 13.07 ± 2.96 mmol/L, $p = 0.02$), Post-30 (4.45 ± 1.94 vs. 5.41 ± 1.90 mmol/L, $p = 0.02$), and Post-60 (2.07 ± 0.94 vs. 2.55 ± 0.96 mmol/L, $p = 0.048$) sampling periods as outlined in Figure 2. As expected both groups showed significant increases in blood lactate compared to rest at the Mid and Post time points; however, by Post-30 lactate concentrations no longer differed significantly from baseline levels in the treatment group while control group levels remained significantly elevated.

Serum cortisol levels were lower at the Mid and Post-30 time points in the treatment group versus control group (19.39 ± 6.90 vs. 27.84 ± 14.56 $\mu\text{g/dL}$, $p = 0.02$; 22.91 ± 8.47 vs. 31.81 ± 10.79 $\mu\text{g/dL}$, $p = 0.04$) while the remaining time points trended lower, but not significant, for the treatment group compared to the control group as seen in Figure 3. Surprisingly, cortisol values within both groups never experienced significant increases from pre-exercise values during the Mid and Post sampling period (Treatment: 21.55 ± 9.82 , vs. Mid 19.39 ± 6.90 and 23.56 ± 4.47 $\mu\text{g/dL}$; Control: 25.58 ± 10.88 vs. 27.84 ± 14.56 and 29.06 ± 12.63 $\mu\text{g/dL}$); however, cortisol values of the treatment group returned to baseline levels by Post-30 while the

control group peaked at the Post-30 sampling period (22.91 ± 8.47 vs. 31.81 ± 10.79 $\mu\text{g/dL}$). AUC analysis for cortisol values showed significant differences within the AUC Pre-Post for control and treatment (55.16 ± 23.84 vs. 41.95 ± 12.65 $\mu\text{g/dL}$, $p= 0.02$) groups. While the AUC All-6 and AUC Post - Post-120 showed lower trend values between the control and treatment groups but no significant differences (125.81 ± 31.99 vs. 104.67 ± 33.16 $\mu\text{g/dL}$, $p= 0.053$; 77.74 ± 24.03 vs. 62.72 ± 25.39 $\mu\text{g/dL}$, $p= 0.11$). See figure 4 for differences in cortisol AUC values between groups.

4 Discussion

Previous investigations of mouthpiece use during exercise have shown reductions in salivary cortisol levels (Garner & McDivitt, 2009b; Garner et al., 2011; Garner & McDivitt, 2015), lactate concentration (Garner & McDivitt, 2009a; Garner & McDivitt, 2009b; Garner & McDivitt, 2015), and changes in respiratory kinetics (Garner, Dudgeon, Scheett, & et al., 2011; Garner, 2015). These results suggest the possible involvement of the two major stress axes: the hypothalamic-pituitary-adrenal (HPA) axis (Garner et al., 2011) and subsequently possible involvement of the sympathetic-adrenal-medullary (SAM) axis (Garner et al., 2011) due to mutual activation in the stress response (Chrousos, 2007; Fatouros, Chatzinikolaou, Paltoglou, & et al., 2010; Tsigos & Chrousos, 2002; Vanlallie, 2002). This suggests that mouthpiece use can acutely impact neuroendocrine physiology, thereby impact substrate availability and utilization, and ultimately play a role in performance outcomes. The purpose of this investigation was, firstly, to investigate the effects of performance mouthpiece use on the serum cortisol response to resistance exercise. Secondly, to investigate the effect of performance mouthpiece use on blood lactate levels before, during, and after intense resistance exercise. Finally, this study aimed at determining if performance mouthpiece use could improve resistance exercise performance.

Based on current research, it is hypothesized that serum cortisol and blood lactate concentrations will decrease as a result of performance mouthpiece use during and following a resistance exercise session and performance will be enhanced.

Total mean non-assisted repetitions completed proved to be different between groups with the treatment group completing significantly ($p < 0.05$) more repetitions before failure. Conversely, total mean assisted repetitions were significantly ($p < 0.05$) higher with the control group. Further, mean non-assisted repetitions trended higher with the treatment group during every set except sets 1 and 2. Mean weight lifted followed a similar trend with the treatment group exhibiting a trend of more weight lifted during the latter sets. The higher trend of non-assisted repetitions led to a significantly higher ($p < 0.05$) total mean weight lifted for the treatment group compared to the control group. In this application it appears that performance mouthpiece use during heavy resistance exercise has led to the completion of more repetitions before failure, thus resulting in more total weight lifted.

Lactate data from this study could provide some insight into explaining the difference in total weight lifted and non-assisted repetitions. This study showed lactate, which is released in proportion to the perceived “stress” of the activity, to be consistently lower in the treatment group compared to the control group immediately post exercise through 60 minutes post exercise. This blunted lactate response in the treatment group coincides with the trend for fewer assisted repetitions in the same group. Increases in blood lactate caused by the accumulation of hydrogen ions with high intensity exercise results in metabolic acidosis leading to fatigue, thereby hindering athletic performance (Amis, Di Somma, Bacha, & et al., 2000; Garner & McDivitt, 2015). While this study did not provide a mechanism for the lactate reduction, these

results have shown that mouthpiece use decreases blood lactate accumulation thereby possibly delaying the onset of fatigue resulting in more physical work performed.

Animal studies have been successful at linking biting and chewing mechanisms with a reduction in the stress response during stress-induced activities. A 2004 study by Hori and colleagues (Hori et al., 2004) investigated the possible suppression of the stress-induced expression of corticotrophin releasing hormone (CRH) in the paraventricular nucleus (PVN) of the rat hypothalamus through nonfunctional biting. Researchers restrained the bodies of rats, known to activate the HPA axis, for either 30 or 60 minutes to examine the expression of CRH in the cells of the PVN during biting. Rats biting on a wooden stick during the restraint period had a significant ($p < 0.05$) reduction in the expression of CRH than those not biting, regardless of the restraint time period. The secretion of CRH from the PVN results in the release of adrenocorticotrophic hormone (ACTH) from the anterior pituitary (Chrousos, 2007; Tsigos & Chrousos, 2002). ACTH secretion stimulates the release of cortisol from the adrenal cortex (Chrousos, 2007; Tsigos & Chrousos, 2002). Researchers noted the interaction of the hypothalamus with the cerebral cortex and the limbic system, thereby, integrating the autonomic and endocrine functions. Researchers speculated that it is because of this interaction that suppression of central noradrenergic transmission might be the mechanism for the suppression of CRH by biting.

Thus, examining the clenching response may provide the key to understanding outcomes noted in mouthguard/mouthpiece use during exercise. Clenching has been shown to affect cerebral activity in activation of the cortical areas in the brain and in increasing blood flow (Hasegawa, Ono, Hori, & et al., 2007; Hori et al., 2004; Sasaguri et al., 2005; Tahara, Sakurai, & Ando, 2007; Shibusawa, Takeda, Nakajima, & et al., 2009). Specifically, researchers have cited

that restrained and stressed rats, when biting on a stick, had reductions in corticotrophin releasing factor and c-Fos in the hypothalamus which may be modulated by suppression of extracellular signal-regulated protein kinase 1/2 (pERK 1/2) in the paraventricular nucleus (Hori et al., 2004; Sasaguri et al., 2005; Kaneko, Hori, Yuyama, & et al., 2004). Human studies have also supported this link with lowered cortisol levels during biting down on a mouthpiece or chewing during stress (Garner et al., 2011; Tahara et al., 2007). Garner and colleagues (Garner et al., 2011) examined salivary cortisol levels with mouthpiece use following a 1-hour intense resistance training session in 28 trained, college-aged males. Researchers found a significant 51% difference ($p = 0.02$) in salivary cortisol levels 10-minutes post exercise between mouthpiece and no treatment groups. Perhaps the most interesting finding was that cortisol divergence did not appear between treatment and control groups until post-exercise, which researchers believe could be due to the stress response remaining unaffected by mouthpiece use during the exercise protocol (Garner et al., 2011).

This link between the hypothalamus and the involvement in the jaw musculature via clenching may be explained by neuronal projections from the lateral hypothalamus connecting to the trigeminal motor nucleus in the rat model (Mascaro, Prosdocimi, Bittencourt, & et al., 2009). In addition, it was observed that the trigeminal motor nucleus was innervated by corticotrophin releasing factor immunoreactive fibers within the amygdala (Mascaro et al., 2009). Research also suggests that activation within the dorsolateral prefrontal cortex (DLPFC, an area in the cerebral cortex) is most likely dependent on continuous teeth contact as occurs during clenching, and that intensity of the clenching most likely influences that magnitude of the cerebral activity within the sensorimotor cortex (area in cerebral cortex responsible for motor function) (Iida, Sakayanagi, Svensson, & et al., 2012; Shibusawa et al., 2009). Finally, Qin and colleagues

(2009) noted that the function of the DLPFC most likely is affected by the HPA axis by decreasing levels of the catecholamines (Qin, Hermans, van Marle, & et al., 2009). These findings are of significance as it relates to mouthpiece use during exercise as they provide potential explanation for the cited decreases in cortisol and lactate while clenching on a mouthpiece during exercise (Garner & McDivitt, 2009a; Garner & McDivitt, 2009b; Garner et al., 2011; Garner & McDivitt, 2015).

Our findings indicate a differing cortisol response to high-intensity exercise with the use of a mouthpiece. Peak cortisol levels were lower with mouthpiece use and the overall cortisol production was blunted with mouthpiece use as evidenced the AUC data. This study was the first to investigate serum cortisol levels with mouthpiece use during high-intensity resistance exercise; however, previous research has also cited salivary cortisol augmentation with mouthpiece use during exercise (Garner & McDivitt, 2009b; Garner et al., 2011). While cortisol's importance during exercise (i.e. gluconeogenesis) is an important step to provide energy during prolonged or intense exercise, its catabolic nature post-exercise could hinder or delay recovery. Our findings suggest a possible performance benefit, particularly post-exercise, as we have shown a quicker return to baseline with both salivary cortisol (Garner et al., 2011) and less overall cortisol production (present study).

Early research linking human performance and mouthpiece use was plagued by poor research methodology and called for a greater contribution and collaboration between the scientific community and clinical researchers. Research now favors proper design and analysis, with researchers beginning to investigate the mechanisms behind oral appliance use and human performance. The design of the performance mouthpiece in this study used a wedge component designed to reposition the mandible. This design results in a separation of the teeth with a more

favorable position of the mandible (Garner et al., 2011) causing a decreased amount of stress placed upon the mandible, thereby decreasing the stress response through possible actions on the motor areas of the brain (Iida, Kato, Komiyama, & et al., 2010; Tahara et al., 2007). Researchers have recently been able to map brain activity during clenching and chewing (Hasegawa et al., 2007; Momose, Niskikawa, Watanabe, & et al., 1997; Shibusawa et al., 2009; Tahara et al., 2007) and have found that clenching and chewing not only resulted in activation of the autonomic nervous system areas of the brain, but also resulted in stimulation of the hypothalamus (Tahara et al., 2007).

5 Practical Application

Due to the importance of stress hormones to provide increased blood flow, oxygen, and substrates to working muscles and the desire to suspend the stress response upon cessation of exercise to inhibit catabolic mechanisms, finding a way to decrease the stress response during the post exercise period would be valuable. Further, delaying the accumulation of lactate concentrations by prolonging the onset of muscle acidosis caused by the accumulation of hydrogen ions would thereby improve athletic performance (Garner & McDivitt, 2015; Gelb, Mehta, & Forgione, 1996). This particular study demonstrated decreases in cortisol production during and after exercise with a general trend of lower cortisol values throughout the exercise protocol with performance mouthpiece use. Further, lactate was found to be significantly lower during the post exercise time period. Much of the research in the area of mouthguard/mouthpiece use during exercise finds minimal to no acute benefits, with much of the data being conflicting. However; this research along with the earlier research related to cortisol, suggests the potential impact of mouthpiece use on recovery and subsequent training sessions. However, research related to performance mouthpiece use is still emerging, thus it is imperative researchers

continue to investigate possible mechanisms of action. Most of the research associated with mouthpiece/mouthguard use during exercise does not seek to elucidate the mechanisms of why a mouthpiece/mouthguard may be beneficial (Allen, Dabbs, Zachary, & et al., 2014). Yet our group feels it is critical to clarify the potential physiological mechanisms in order to better understand the acute effects within the specific population studied and exercise protocol chosen. Bridging the gap between exercise physiology and dental research will provide valuable knowledge for the practitioner, exercise scientist, and the athlete and avoid much of the guesswork utilized in past research.

6 Acknowledgements

The authors would like to thank The Citadel Foundation and Bite Tech, Inc. for partial funding of this research.

7 Conflict of Interest

None of the authors in this paper have a professional relationship from any company or entity associated with this mouthpiece and will not benefit from the results of this study. The results of this study have not been fabricated in any way and are honestly and clearly presented in this article.

Reference List

Allen, C. R., Dabbs, N. C., Zachary, C. S., & et al. (2014). The acute effect of a commercial bite-aligning mouthpiece on strength and power in recreationally trained men. *J Strength Cond Res*, 28, 499-503.

Amis, T., Di Somma, E., Bacha, F., & et al. (2000). Influence of intra-oral maxillary sports mouthguards on the airflow dynamics or oral breathing. *MSSE*, 32, 284-290.

Arent, S. M., McKenna, J., & Golem, D. L. (2010). Effects of neuromuscular dentistry-designed mouthguard on muscular endurance and anaerobic power. *J Comp Physiol*, 7, 73-79.

Busca, B., Morales, J., Solana-Tramunt, M., & et al. (2016). Effects of jaw clenching while wearing a customized bite-aligning mouthpiece on strength in healthy young men. *J Strength Cond Res*, 30, 1102-1110.

Chrousos, G. P. (2007). Organization and integration of the endocrine system. *Sleep Med Clin*, 2, 125-145.

Dunn-Lewis, C., Luk, H. Y., Comstock, B. A., & et al. (2012). The effects of a customized over-the-counter mouth guard on neuromuscular force and power production in trained men and women. *J Strength Cond Res*, 26, 1085-1093.

Durante-Pereira, D. M. V., del Rey-Santamaria, M., Javierre-Garces, C., & et al. (2008). Wearability and physiological effects of custom-fitted vs self-adapted mouthguards. *Dent Traumatol*, 24, 439-442.

Ebben, W. P., Leigh, D. H., & Geiser, C. F. (2008). The effect of remote voluntary contractions on knee extensor torque. *Med Sci Sports Exerc*, 40, 1805-1809.

Fatouros, I., Chatzinikolaou, A., Paltoglou, G., & et al. (2010). Acute resistance exercise results in catecholaminergic rather than hypothalamic-pituitary-adrenal axis stimulation during exercise in young men. *Stress*, 13, 468.

Fonder, A. C. (1976). The profound effect of the 1973 Nobel Prize on dentistry. *J Am Acad Func Prosthodontics*, 1, 21-29.

Garner, D. P. (2015). Effects of various mouthpieces on respiratory physiology during steady state exercise in college-aged subjects. *Gen Dent*, 63, 30-34.

Garner, D. P., Dudgeon, W. D., & McDivitt, E. (2011). The effects of mouthpiece use on cortisol levels during an intense bout of resistance exercise. *J Strength Cond Res*, 25, 2866-2871.

Garner, D. P., Dudgeon, W. D., Scheett, T. P., & et al. (2011). The effects of mouthpiece use on gas exchange parameters during steady-state exercise in college-aged men and women. *J Am Dent Assoc*, 142, 1041-1047.

Garner, D. P. & McDivitt, E. (2009a). Effects of mouthpiece use on airway openings and lactate levels in healthy college males. *Compend Contin Educ Dent*, 30 Spec No 2, 9-13.

Garner, D. P. & McDivitt, E. J. (2009b). The effects of mouthpiece use on salivary cortisol and lactate levels during exercise. *Med Sci Sports Exerc*, 41, S448.

Garner, D. P. & McDivitt, E. J. (2015). Effects of mouthpiece use on lactate and cortisol levels during and after 30 minutes of treadmill running. *Open Access J Sci Technol*, 3, doi:10.11131/2015/101148.

Gelb, H., Mehta, N. R., & Forgione, A. G. (1996). The relationship between jaw posture and muscular strength in sport dentistry: A reappraisal. *CRANIO*, 14, 320-325.

Glendor, U. (2009). Aetiology and risk factors related to traumatic dental injuries - a review of literature. *Dent Traumatol*, 25, 19-31.

Hasegawa, Y., Ono, T., Hori, K., & et al. (2007). Influence of human jaw movement on cerebral blood flow. *J Dent Res*, 86, 64-68.

Hori, N., Yuyama, N., & Tamura, K. (2004). Biting suppresses stress-induced expression of corticotropin-releasing factor (CRF) in the rat hypothalamus. *J Dent Res*, 83, 124-128.

Hughston, J. C. (1980). Prevention of dental injuries in sports. *Am J Sports Med*, 8, 61-62.

Iida, T., Kato, M., Komiyama, O., & et al. (2010). Comparison of cerebral activity during teeth clenching and fist clenching: A functional magnetic resonance imaging study. *Eur J Oral Sci*, 118, 635-641.

Iida, T., Sakayanagi, M., Svensson, P., & et al. (2012). Influence of periodontal afferent inputs for human cerebral blood oxygenation during jaw movements. *Exp Brain Res*, 216, 375-384.

Kaneko, M., Hori, N., Yuyama, N., & et al. (2004). Biting suppresses Fos expression in various regions of the rat brain: Further evidence that the masticatory organ functions to manage stress. *Stomatologic*, 101, 151-156.

Kraemer, W. J. & Ratamess, N. A. (2005). Hormonal responses and adaptations to resistance exercise and training. *Sports Med*, 35, 339-361.

Mascaro, M. B., Prosdocimi, F. C., Bittencourt, J. C., & et al. (2009). Forebrain projections to brainstem nuclei involved in the control of mandibular movements in rats. *Eur J Oral Sci*, 117, 676-684.

Momose, E., Niskikawa, J., Watanabe, T., & et al. (1997). Effect of mastication on regional cerebral blood flow in humans examined by positron-emission tomography with O-labelled water and magnetic resonance imaging. *Archs Oral Biol*, 42, 57-61.

Morales, J., Busca, B., Solana-Tramunt, M., & et al. (2015). Acute effects of jaw clenching using a customized mouthguard on anaerobic ability and ventilatory flows. *Hum Movement Sci*, 44, 270-276.

Qin, S., Hermans, E. J., van Marle, H. J. F., & et al. (2009). Acute psychological stress reduces working memory-related activating in the dorsolateral prefrontal cortex. *Biol Psychiatry*, 66, 25-32.

Ratamess, N. A., Kraemer, W. J., Volek, J. S., & et al. (2005). Effects of heavy resistance exercise volume on post-exercise androgen receptor content in resistance-trained men. *J Steroid Biochem*, 93, 35-42.

Sands, W., Wurth, J., Hewit, and J. (2012). The National Strength and Conditioning Association's Basics of Strength and Conditioning Manual. Chapter 3: Technique Fundamentals and Spotting.

https://www.nscs.com/uploadedFiles/NSCA/Resources/PDF/Publications/basics_of_strength_and_conditioning_manual.pdf

Sasaguri, K., Kikuchi, M., Hori, N., & et al. (2005). Suppression of stress immobilization-induced phosphorylation of ERK 1/2 by biting in the rat hypothalamic paraventricular nucleus. *Neurosci Lett*, 383, 160-164.

Shibusawa, M., Takeda, T., Nakajima, K., & et al. (2009). Functional near infrared spectroscopy study on primary motor and sensory cortex response to clenching. *Neurosci Lett*, 449, 98-102.

Smith, S. D. (1982). Adjusting mouthguards kinesiology in professional football players. *NY State Dent J*, 48, 298-301.

Stachowicz, M. & Lebiezinska, A. (2016). The effect of diet components on the level of cortisol. *European Food Research and Technology*, 242, 2001-2009.

Stenger, J. M. (1977). Physiologic dentistry with Notre Dame athletes. *Basal Facts*, 2, 8-18.

Tahara, Y., Sakurai, K., & Ando, T. (2007). Influence of chewing and clenching on salivary cortisol levels as an indicator of stress. *J Prosthodont*, 16, 129-135.

Tsigos, C. & Chrousos, G. P. (2002). Hypothalamic-pituitary-adrenal axis, neuroendocrine factors and stress. *J Psychosom Res*, 53, 865-871.

Vanltallie, T. (2002). Stress: A risk factor for serious illness. *Metabolism*, 51, 6.

Welch, M. J., Edington, D. M., & Ritter, R. (1986). Muscular strength and temporomandibular joint repositioning. *J Orthop Sports Phys Ther*, 7, 236-239.

Yates, J. W., Koen, T. J., Semenick, D. M., & et al. (1984). Effect of a mandibular orthopedic repositioning appliance on muscular strength. *J Am Dent Assoc*, 108, 331-333.

ACCEPTED MANUSCRIPT

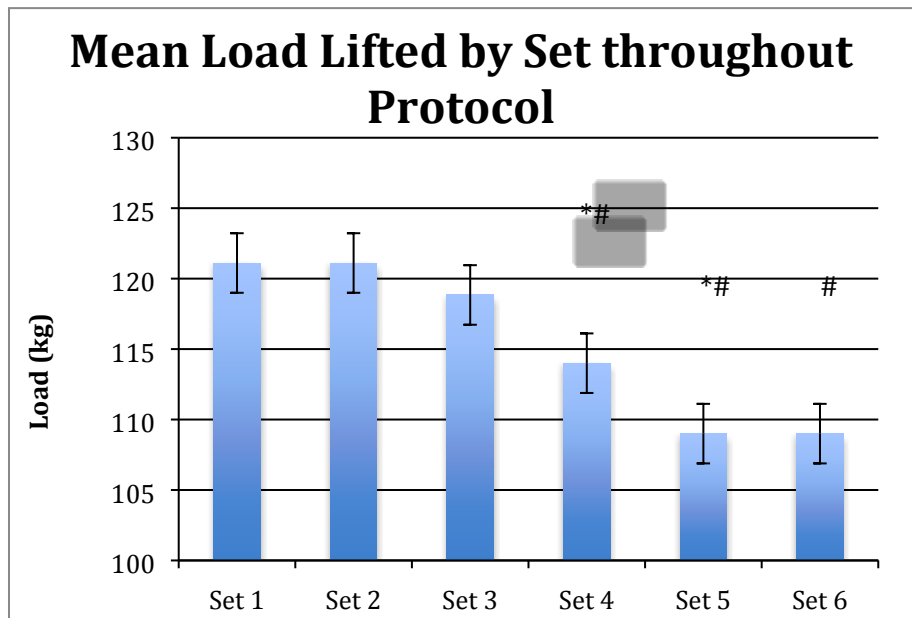
Figure 1: Mean load throughout the exercise protocol for all subjects and treatments. Identical load procedure was performed for both groups. * Indicates significant change ($p < 0.05$) from prior set. # Indicates significant ($p < 0.05$) change from set 1. Data are presented as mean \pm SE.

Figure 2: Mean repetitions completed without assistance (i.e., under the subject's own power) for control and treatment groups. No significance existed between groups ($p < 0.05$). Data are presented as mean \pm SE.

Figure 3: Mean lactate concentrations for treatment and control groups throughout testing period. *Indicates significant difference between treatment and control groups. #Indicates differences from Pre in treatment group. § Indicates differences from Pre in control group. Data are presented as mean \pm SE.

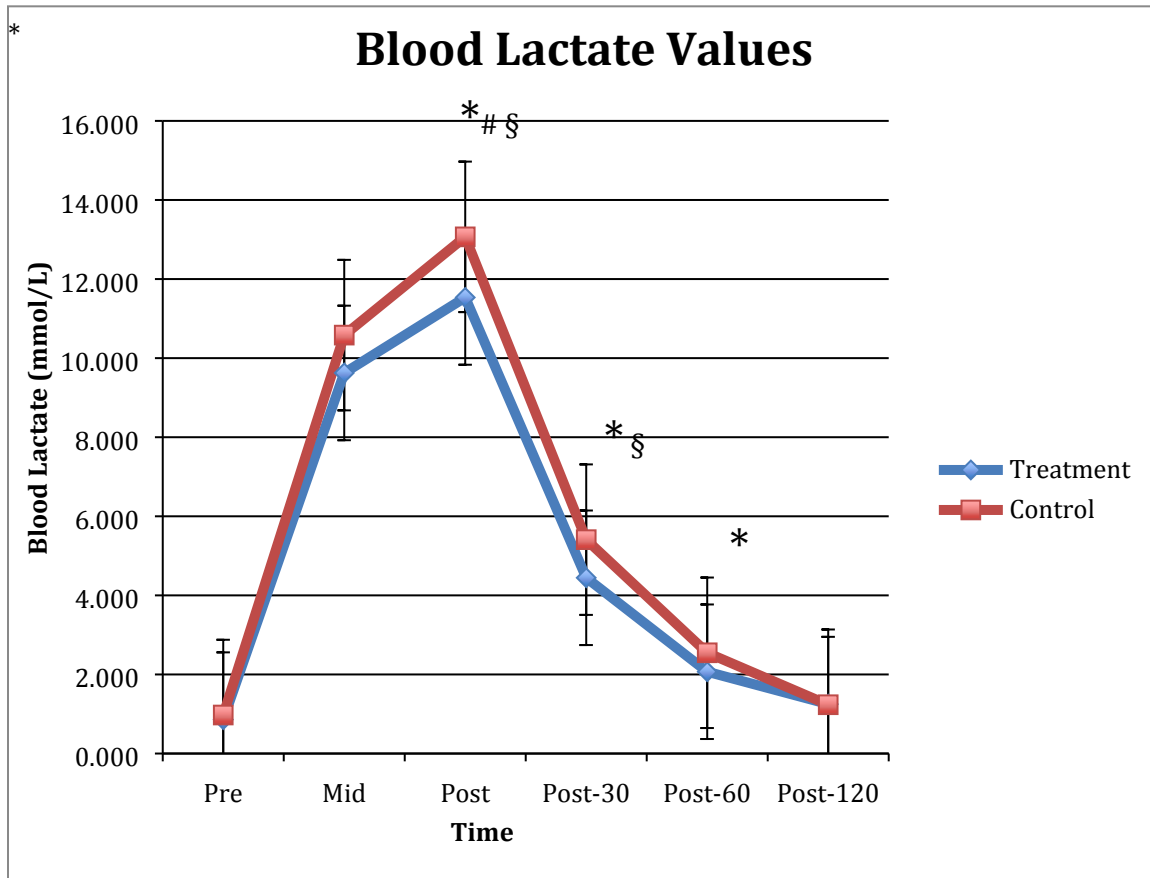
Figure 4: Mean cortisol values for treatment and control groups during and after exercise. *Indicates significant difference exists between treatment and control groups ($p < 0.05$). Data are presented as mean \pm SE.

Figure 1



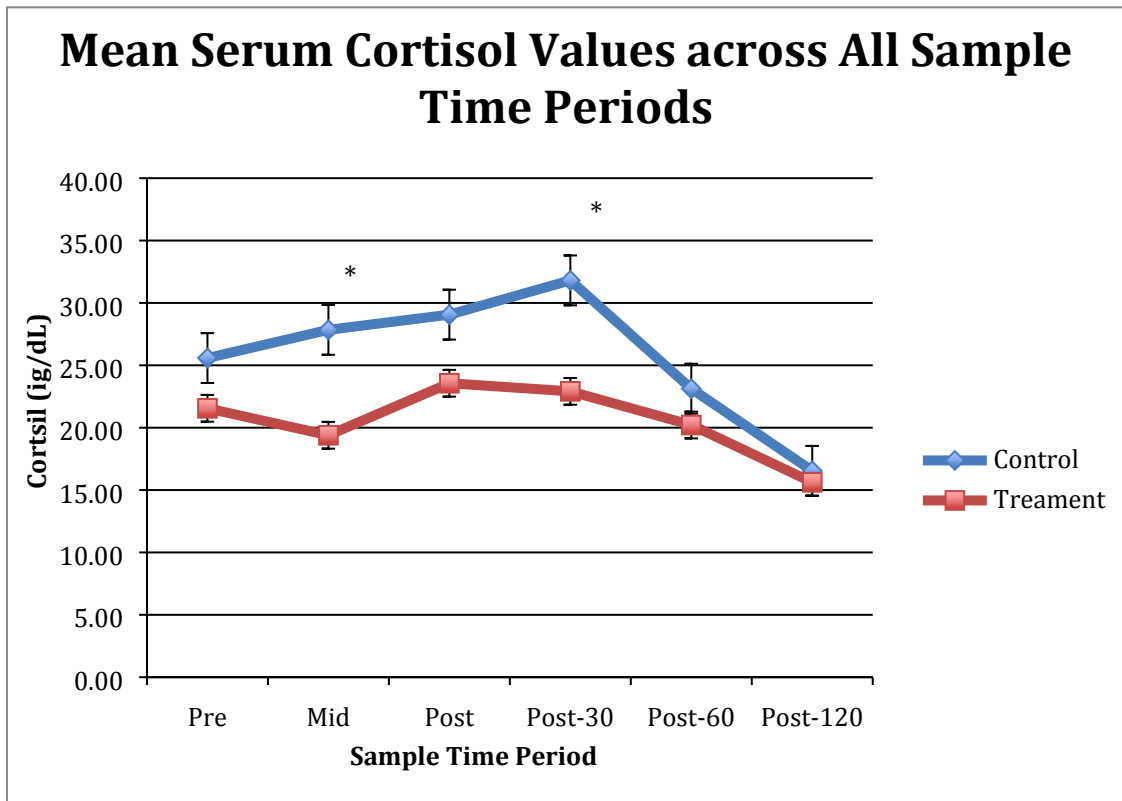
ACCEPTED MANUSCRIPT

Figure 2



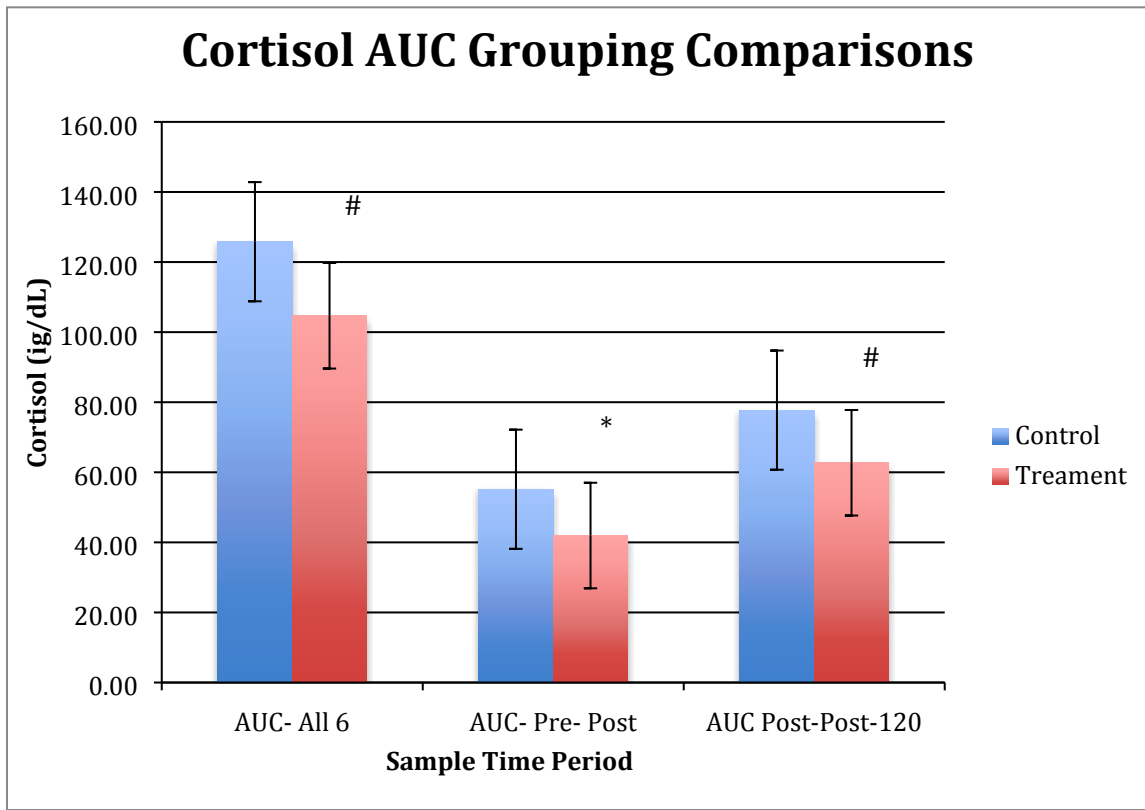
ACCEPTED

Figure 3



ACCEPTED MANUSCRIPT

Figure 4



ACCEPTED MANUSCRIPT

FIGURE CAPTIONS

Figure 1: Mean load throughout the exercise protocol for all subjects and treatments. Same load procedure was performed for both groups. *Indicates change from prior set. Significant difference was found from set 3 to set 4 ($p < 0.05$) and from set 4 to set 5 ($p < 0.05$). #Indicates significant change from set 1. # Indicates difference between set 1 and set 4, set 5, and set 6 ($p < 0.05$). Data are presented as mean \pm SE.

Figure 2: Mean lactate concentrations for treatment and control groups. *Indicates significant difference between treatment and control groups. #Indicates differences from Pre in treatment group. § Indicates differences from Pre in control group. All significance levels set at $p < 0.05$. Data are presented as mean \pm SE.

Figure 3: Mean cortisol values for treatment and control groups during and after exercise. *Indicates significant difference exists between treatment and control groups ($p < 0.05$). Data are presented as mean \pm SE.

Figure 4: Comparison of AUC analysis between control and treatment groups. *Indicates significant difference exists between treatment and control groups. #Indicates lower trend between control and treatment groups. Data are presented as mean \pm SE.

ACCEPTED MANUSCRIPT

Public Interest Statement

Mouthguards/mouthpieces have traditionally been used for protection during collision sports. However, our research finds that mouthpieces may also provide a performance benefit to the user during strenuous exercise. This article finds that individuals who used a lower custom-fitted mouthpiece experienced improvements during intensive resistance exercise. More specifically, with mouthpiece use, their blood lactate levels were 13% lower immediately after exercise and they had a 39% reduction in cortisol levels 30 minutes post exercise. What does this mean? For those who take part in strenuous anaerobic exercise, this improvement in both lactate and cortisol could benefit recovery mechanisms which would then improve subsequent training session. Thus, it is evident from this study that wearing a mouthpiece promoted positive physiological changes. More exploration into why this occurred is needed in order to understand the effect of mouthpiece use during exercise.

ACCEPTED MANUSCRIPT

About the authors and their research focus

The Human Performance Laboratory at The Citadel has spent the last 13 years assessing the effect of mouthpiece use on human performance parameters during exercise. Parameters assessed have included **oropharyngeal** width and diameter, cortisol (blood and salivary), lactate levels, respiratory changes and human work output. The research in our laboratory has suggested a physiological effect during both aerobic and anaerobic performance. Theories which support these changes may include increased cerebral blood flow that occurs during clenching on a mouthpiece and improved placement of the genioglossus which reduces the respiratory rate during aerobic exercise. The latest research presented in this paper demonstrates blood marker changes in both lactate and cortisol after a bout of intense resistance exercise. Future research needs must assess the causes for these physiological changes with mouthpiece use during exercise.

ACCEPTED MANUSCRIPT