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Effects of the Occlusal Thickness of Mouthpieces on Salivary Cortisol During **Resistance Strength Training**

Andrew J Keith^{1*}, Anne-Marie Bollen², Tar Chee Aw³ and Sucheol Gil⁴

¹University of Washington School of Dentistry, USA

²Department of Orthodontics, University of Washington School of Dentistry, USA

³Department of Restorative Dentistry, University of Washington School of Dentistry, USA

⁴Department of Oral Health Sciences, University of Washington School of Dentistry, USA

*Corresponding author: Andrew J Keith, University of Washington School of Dentistry 1959 NE Pacific St HSB D-563, Seattle, WA 98195, USA, Tel: 425-466-8544; Fax: 206-685-8163; E-mail: akeith@uw.edu

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Abstract

Background: When worn during exercise, it has been suggested that a mouthpiece may have a benefit to the athlete by increasing strength and endurance. Specifically, the use of a mouthpiece during resistance strength training has been shown to result in reduced post-exercise concentrations of cortisol when compared to no mouthpiece. The purpose of this pilot study is to determine if the thickness of the biting (occlusal) surface of the mouthpiece has an effect on the reduction of postexercise cortisol concentrations.

Methods and findings: Six male (aged 22-25 years) participants performed identical one-hour workouts of strength training exercises consisting of five sets of five repetitions of back squats, bench press, deadlifts, and military press with 90 seconds of rest between sets. Each participant completed three total workouts while wearing either a mouthpiece with an occlusal thickness of 2 mm, of 6 mm, or no mouthpiece (control). Free cortisol concentrations were measured from salivary samples collected at four times during each workout; pre-workout, 30 min into the workout, immediately following the completion of the workout, and 10 min post-workout. Statistical significance was determined at α =0.05.

Conclusion: While there was no statistical significance found amongst the results, there is a trend that follows previous research that the use of a mouthpiece during resistance strength training yields a reduced salivary cortisol concentration at 10 min post-workout when compared to no mouthpiece, suggesting that the thicker, 6 mm mouthpiece may be more effective. This finding suggests that the effect of a mouthpiece on athletic performance may be dependent on the occlusal thickness, which warrants further research into the optimal design for an athletic mouthpiece.

Keywords: Mouth guard; Stress; Exercise; Vertical height

Introduction

Since the mid-1990s, the American Dental Association (ADA) has been advocating for the use of mouth guards in sports and recreational activities due to their well-recognized protection of the orofacial region [1]. The Academy for Sports Dentistry has partnered with the ADA and other national dental organizations to dedicate the entire month of April as National Facial Protection Month in efforts to raise public awareness for the use of mouth guards and other orofacial protective devices in sports-related activities [2]. It is indisputable that mouth guards can provide physical protection during sports, but can it provide physiological protection as well?

Primal versions of ourselves relied on our body's stressinduced "fight or flight response" for survival, but now-a-days stress is referred to as "the silent killer." Stressors activate the autonomic nervous system (ANS) and hypothalamic-pituitaryadrenal (HPA) axis in efforts to trigger a release of corticosteroids and hormones to meet the pending physical demands of the body. Upon stimulation of the HPA axis, the glucocorticoid cortisol is secreted from the anterior pituitary gland [3,4]. Acute increases in cortisol can be beneficial by increasing alertness, inducing gluconeogenesis, and providing anti-inflammatory effects. However, extreme surges are associated with fatigue, impaired immune function, decreased testosterone, and reduced muscle building capacity [4,5], and decreased bone density [6].

Teeth clenching is a stress-coping behaviour [7] that has been shown to cause a localized release of catecholamine's from sympathetic nerve terminals which attenuates the stressinduced ANS activation [8-10]. Clenching also reduces HPA axis activation by diminishing secretion of adrenocorticotropic hormone (ACTH) [10,11] as well as corticotropin releasing factor (CRF) [12]. Clenching has been shown to increase cerebral circulation [13,14], which allows for better

oxygenation and glucose metabolism of the brain [15]. Compared to rats that did not bite down on a wooden stick, rats that bit down on a wooden stick while undergoing a stress inducing restraint had a reduction of CRF secretions as a result of attenuation of the HPA axis by decreased periventricular nucleus activity [12,16]. Athletes that wore a custom-fit mouthpiece during high-intensity resistance strength training had a reduction in salivary cortisol concentrations following their workout when compared to no mouthpiece use [17].

Apart from reducing the stress response, mouthpieces have been shown to increase endurance and expedite recovery in runners [18], decrease cyclist's breathing rates [19], enlarge the airway, and reduce lactate levels in athletes [20]. Mandibular advancement with the use of a mouthpiece has been shown to increase the diameter of the upper airways through several studies in the field of sleep apnea, suggesting that their use in sports can be beneficial in oxygen delivery during endurance exercise and by decreasing lactate production [21-24].

An imbalance in the jaw by placement of an isolateral mouthpiece has been shown to decrease arm strength. This contralateral loss of arm strength was linearly related to the vertical heights of the mouthpieces, suggesting that the thickness of the mouthpiece plays a role in the mouthpiece's effect on strength [25]. The variations in mouthpiece thickness have had little investigation with their effects of suppression on the HPA axis. By observing the effects of mouthpiece use during resistance strength training we may be able to better control the increase of cortisol in the body following exercise. The purpose of this study is to act as a pilot study to investigate if the variations in the occlusal thickness of the mouthpiece have any effect on the reduction of salivary cortisol. Furthermore, this pilot study will reaffirm previous findings that the use of a mouthpiece during resistance strength training leads to a decrease in post-workout salivary cortisol levels.

Materials and Methods

Six (n=6) college-aged men (22-25 years) who have physically active lifestyles were recruited for this study. Prior to the start of the study, informed consent was obtained from the participant. The procedures for this study were reviewed and approved by the Institutional Review Board of the University of Washington Subjects Division.

Mouthpieces were fabricated by using Plackers[®] Grind No-More[®] Dental Night Protectors. The unaltered Dental Guard has an occlusal thickness of 2 mm. Additional mouthpieces were constructed by adhering separated bite pads of mouthpieces to an unaltered mouthpiece so that the occlusal thickness of the altered mouthpieces measured 6 mm.

At least three days before the first workout, each participant was tested for their one repetition maximum (1RM) on each of the following barbell exercises: back squat, seated military press, deadlift, and bench press. Each participant conducted three separate, but identical, one-hour workouts under the supervision of the lead researcher. Workouts for the individual

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participant were performed at least 72 hours apart from one another. Participants were randomly assigned to one of the six different sequences of mouthpiece conditions (e.g. workout 1: no mouthpiece; workout 2: 2 mm mouthpiece; workout 3: 6 mm mouthpiece).

The workout started with a warm-up of a 10-min incline walk on the treadmill while maintaining 50% maximum heart rate (220-Age). The strength training portion of the workout consisted of a series of 5 sets of 5 repetitions for the following barbell exercises: back squats, seated military press, deadlifts, and bench press. The 1st set was 70% of the athlete's 1RM, the 2nd set was 80% of 1RM, and the 3rd/4th/5th sets were 85% of 1RM, with 90 seconds of rest between sets. Five full sets of an exercise were completed before advancing to the next, with three min of rest between exercises.

Salivary samples (1 mL) were collected in 1.5 mL micro centrifuge tubes (SciMart, St. Louis, MO) immediately before the start of the workout (pre-workout), 30 min into the workout (mid-workout), immediately at the conclusion of the workout (post-workout), and 10 min after the workout (10 min +post-workout). Saliva samples were frozen until analyzed as instructed by the protocol of the Free Cortisol in Saliva ELISA Kit (IBL-America, Minneapolis, MN), which was used to measure the free cortisol levels in the collected saliva samples. Participants were instructed not to consume caffeine or dairy on the days of testing, or alcohol within 24 hours of testing. The last meal was more than 60 min prior to the start of the workout.

Microsoft Excel was used to compile the data. A one-way analysis of variance (ANOVA) was used to compare the salivary cortisol concentrations at each time point. At ten min post-workout, a paired sample t-test was used to compare the salivary cortisol concentrations between the different mouthpiece conditions. Statistical significance was determined at a=0.05. Percent change among the means of the different conditions was calculated using the formula: $(\mu_2-\mu_1/\mu_1) \times 100$.

Results

A total of six males (mean age=23.5 years) participated in the study with all six participants having complete data sets.

The differences in mean salivary cortisol concentrations between the various conditions are illustrated in **Figure 1.** At 10 min post-workout, there was a 29.4% difference between the no mouthpiece and 2 mm mouthpiece condition, and a 36.0% difference between the no mouthpiece and 6 mm mouthpiece condition (p=0.38 and 0.08, respectively). Whereas, there is only a 6.8% difference between the 2 mm mouthpiece and 6 mm mouthpiece condition at 10 min post-workout (p=0.82).



Figure 1 Salivary cortisol concentrations measured at the start, middle, immediately after, and ten min after a 60-min high-intensity resistance strength training workout (n=6).

When comparing the salivary cortisol concentrations immediately post-workout to 10 min post-workout, there is a 35.6% difference for the no mouthpiece condition, a 9.9% difference for the 2 mm mouthpiece condition, and a 1.8% difference for the 6 mm mouthpiece condition (p=0.08, p=0.36, and p=0.92, respectively).

Table 1 Means and standard deviations of salivary cortisol concentrations at various time intervals during a 60-min high-intensity resistance strength training workout under different mouthpiece conditions (N=6).

	[Salivary Cortisol] (ng/mL)			
Period of Collection	No mouthpiece	2mm mouthpiece	6mm mouthpiece	
Pre-workout	4.15 ± 1.83	3.45 ± 1.43	3.69 ± 1.31	
Mid-workout	3.59 ± 1.37	3.73 ± 2.36	3.44 ± 0.91	
Post-workout	3.64 ± 1.16	3.52 ± 2.32	3.69 ± 1.09	
10min + Post-workout	5.22 ± 2.68	3.88 ± 2.47	3.63 ± 1.31	

Table 2 Means and standard deviations of changes from baseline salivary cortisol concentrations at various time intervals during a 60-min high-intensity resistance strength training workout under different mouthpiece conditions (n=6).

	Change in [Salivary Cortisol] (ng/mL) from baseline			
Period of Collection	No mouthpiece	2mm mouthpiece	6mm mouthpiece	
Pre- workout	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	
Mid- workout	-0.56 ± 1.10	0.28 ± 1.99	-0.25 ± 1.17	
Post- workout	-0.50 ± 2.26	0.07 ± 1.72	0.00 ± 1.35	
10min +	1.07 ± 3.02	0.44 ± 2.21	-0.06 ± 2.26	

Post- workout			
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The change in mean salivary cortisol concentrations (ng/mL) from baseline to 10 min post-workout increased by 1.07 ± 3.02 for the no mouthpiece condition, 0.44 ± 2.21 for the 2 mm mouthpiece, and decreased by 0.06 ± 2.26 for the 6 mm mouthpiece condition (Table 2). The mean salivary cortisol concentrations (ng/mL) increased from 3.64 ± 1.16 immediately post-workout to 5.22 ± 2.68 , 10 min post-workout for the no mouthpiece condition, and 3.52 ± 2.32 to 3.88 ± 2.47 for the 2 mm mouthpiece condition, and 3.52 ± 2.32 to 3.88 ± 2.47 for the 2 mm mouthpiece condition, and decreased from 3.69 ± 1.09 to 3.63 ± 1.31 for the 6 mm mouthpiece condition (Table 1). When comparing the change in salivary cortisol concentrations (Figure 2) from baseline levels to 10 min post-workout, there is a 224.2% difference between no mouthpiece and 6 mm mouthpiece condition (p=0.11).



Figure 2 Changes from baseline salivary cortisol concentrations at various time intervals during a 60-min high-intensity resistance strength training workout (n=6).

Discussion

This pilot study suggests that mean salivary cortisol concentrations of the athlete wearing a 6mm mouthpiece during resistance strength training, when compared to the no mouthpiece condition, and the difference of mean salivary cortisol concentrations at 10 minutes post-exercise between the same conditions are not quite statistically significant. However, there is a trend that follows previous research [20] that the use of a mouthpiece during resistance strength training yields a reduced salivary cortisol concentration 10 min post-workout when compared to no mouthpiece. The no mouthpiece group had an increase in salivary cortisol of 25.8% from the start of the workout to 10 min post-workout, which is consistent with previous research [26,27]. The 2 mm mouthpiece group had an increase of 12.5% and the 6 mm mouthpiece group had a decrease of 1.6% over the same range, suggesting that the increased occlusal thickness of the mouthpiece may further enhance its effectiveness. This finding relates to previous research that has shown that creating a jaw imbalance by using a unilateral increase in occlusal thickness

of a mouthpiece holds a nearly linear relationship with loss of contra-lateral arm strength [25]. Further research needs to be done in order to determine if there is an optimal design for mouthpieces to be used during exercise in order to minimize the duration of elevated cortisol concentrations following exercise. Although this study did not examine the effects of varying occlusal thicknesses on lactate concentrations, breathing rates, or airway diameter, the findings warrant that continued research should be conducted to determine if there is an optimal occlusal design for these parameters as well.

Other studies have shown that cortisol concentrations increase either immediately following commencement of exercise [28-31] or at 15 min into rest [32], with cortisol concentrations returning to baseline anywhere from 30 min [32] to 3 hours following commencement of exercise [31]. Larger increases in post-exercise cortisol concentrations and elongated cortisol recovery times were associated with highintensity exercise of a greater duration. Acute increases in cortisol can be beneficial following exercise in order to promote recovery through glycogenolysis and gluconeogenesis [33]. However, the prolonged elevation of cortisol can be detrimental to the athlete's recovery by causing fatigue, decreased testosterone levels, reduced muscle building capacity [17,34], and decreased bone density [6]. The use of a mouthpiece during high-intensity exercise may provide the benefit of a greatly reduced exposure to the catabolic state of elevated cortisol concentrations, enabling the athlete to recover faster and train more efficiently. This advantage may be more applicable to workouts of a prolonged duration and may not be observed in shorter bouts of exercise.

Measuring cortisol levels in a participant is difficult due to a high number of factors affecting biologic and procedural analytic variation. Multiple factors relating to the large fluctuations in the cortisol secretions include diet, sleep, and both physical and psychological stress, making it extremely difficult to control amongst participants. Furthermore, cortisol is secreted on a diurnal cycle, peaking in the morning shortly after waking and decreasing to about half of the morning levels by evening [35]. Although this study was designed to ensure that the workouts were performed at the same time of day for each individual participant, it did not have all the participants performing their workouts at the same time of the day as each other. This could result in a greater range of salivary cortisol concentrations, leading to an increase in the variance of data and less significant findings. Future studies involving measurement of salivary cortisol concentrations should take these factors into account when designing their research.

An alternative data analysis compared the pre-workout measurement as the baseline salivary cortisol concentration and the change in salivary cortisol concentration from the baseline was calculated for each time measurement (Figure 2). The change in salivary cortisol concentrations was less in both the 2 mm and 6 mm mouthpiece conditions when compared to the control (no mouthpiece condition), 84.5% and 224.2% difference, respectively. Between the two conditions using a mouthpiece, the 6 mm mouthpiece yielded a decreased

change in salivary cortisol concentrations from pre-workout to 10 min post-workout when compared to the 2 mm mouthpiece, with a 265.4% difference [36]. These findings suggest that the use of a mouthpiece may result in a reduced response to salivary cortisol concentrations 10 min postworkout when compared to non-use of a mouthpiece, and that the 6 mm mouthpiece may lead to a greater reduction than a 2 mm mouthpiece. However, no statistically significant differences between the groups were found.

When wearing a mouthpiece during high-intensity resistance strength training, athletes had a reduction in concentrations of salivary cortisol 10 min post-workout relative to when they did not wear a mouthpiece. The occlusal thickness of the mouthpieces used in this study, 2 mm and 6 mm, had similar effects at reducing salivary cortisol concentrations 10 min post-workout with the 6 mm mouthpiece being slightly more effective. The change in salivary cortisol concentrations from baseline to 10 min post-workout was the least for the workouts where the athletes wore mouthpieces, with the 6 mm mouthpiece yielding the least amount of change.

In order to obtain more significant findings, further research should include a larger sample size and a stronger control of other factors that affect cortisol regulation, such as consistent timing of the workout amongst all participants, daily stressors, and quality of sleep during the study.

Conclusion

Resistance strength training increases muscle mass and improves athletic performance but can also result in an increased cortisol response following the conclusion of a workout. Since cortisol can create a catabolic state, which can be detrimental to the athlete's performance and recovery, it is important to minimize the exposure of cortisol to the athlete. In addition to the physical protection of the orofacial region, the use of a mouthpiece during exercise has been proposed to provide an improvement in athletic performance and endurance. This pilot study supports previous findings that the use of a mouthpiece during resistance strength training may reduce post-exercise exposures to cortisol, and that the thickness of the mouthpiece may have a role in this effect. This information is valuable for the design of athletic mouthpieces as it suggests that the occlusal thickness can optimize the physiologic benefits of mouthpieces, resulting in improved athletic performance and recovery.

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Competing and Conflicting Interests

There are no professional or financial relationships between the authors and any products used in this study.

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