
THE EFFECTS OF MOUTHPIECE USE ON CORTISOL LEVELS DURING AN INTENSE BOUT OF RESISTANCE EXERCISE

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ABSTRACT

Garner, DP, Dudgeon, WD, and McDivitt, EJ. The effects of mouthpiece use on cortisol levels during an intense bout of resistance exercise. *J Strength Cond Res* 25(10): 2866–2871, 2011—Research has suggested mouthpiece use during exercise results in an increase in muscle strength and endurance. However, the research is difficult to replicate, and the methodology suggested measures that were too subjective to determine a mouthpiece effect. Thus, the purpose of this study was to use an objective measure to determine a possible physiological mechanism occurring during and after exercise with mouthpiece use. A within-subjects design was used in which 28 division I football players, aged 18–22 years, performed 2 identical bouts of a 1-hour intense resistance exercise, with each subject being randomly assigned the use of a custom-fit mouthpiece either during the first or second session. During both exercise sessions, saliva was analyzed for cortisol at the following time points: pre-exercise, 25, 45, and 60 minutes of exercise, and 10 minutes postexercise. The results revealed a significant difference in cortisol levels with the use of a mouthpiece vs. no mouthpiece ($p = 0.019$) at 10 minutes postexercise. Additionally, although the expected increase in cortisol levels from pre to 10 minutes postexercise was present in the no-mouthpiece group ($p = 0.01$), no such increase was observed in the mouthpiece group. These observations are most likely because of the decrease in cortisol from post to 10 minutes post ($p = 0.04$) in the mouthpiece group. These data demonstrate that although cortisol continued to increase in the no-mouthpiece session, there was a significant decrease in cortisol in the no-mouthpiece condition 10 minutes postexercise.

KEY WORDS stress, hormones, oral devices

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INTRODUCTION

One of the first documented uses of mouth guards in sport was in the 1800s by a London dentist named Krause who fitted boxers' teeth with strips of rubber-like resin before their matches (19). Because the number of dental and head injuries in sports such as football, boxing, and hockey has increased, various affiliations from professional to high school athletic teams have mandated the use of mouth guards in an attempt to decrease frequency of these injuries. For example, the 2008–2009 National Collegiate Athletic Association Sports Medicine Handbook mandates the use of mouth guards during football, women's field hockey, ice hockey, and men's lacrosse (17).

In the late 1970s and early 1980s, there was some research to suggest that mouth guards could not only provide a protective effect against dental injuries but could also provide performance enhancement. Smith (22) cited that muscular strength was associated with proper alignment of the temporomandibular joint (TMJ). Specifically, he found that when football players wore a wax bite (similar to a boil and bite) mouth guard vs. no mouth guard, that isometric deltoid press testing was significantly improved. In another study Smith (23) found significant improvements in muscular strength in a small group of players ($N = 9$) from a National Football League Team. Reports of improvements have not been limited to muscular strength. Garabee (4) cited improvements in endurance athletes when subjects wore a mouthpiece vs. when they did not. Subjective data from the subjects in Garabee's study suggested an increased ability to train harder and recover from injury at a faster rate with a mouthpiece vs. without a mouthpiece. However, the research was not deemed reliable because of the possibility of a placebo effect when subjects wore the mouthpiece (12,16).

Early research in our laboratory suggests that there may be a physiological mechanism(s) contributing to the observed performance enhancements. In a study assessing airway openings during endurance exercise with and without a mouthpiece, there was a decrease in lactate levels when subjects wore a mouthpiece vs. when they did not. In addition, subjects displayed increased diameters in the upper airway cavity with a mouthpiece vs. without a mouthpiece (5).

Another study found that when subjects ($N = 24$) wore a mouthpiece while running at 75–85% of heart rate maximum, lactate levels were significantly lowered with a mouthpiece vs. no mouthpiece (4.01 and 4.92 $\text{mmol}\cdot\text{L}^{-1}$, respectively, $p = 0.024$, a 23% improvement) (6). The hypothesis is that there is some improvement in oxygen kinetics, which appears to affect lactate levels during moderate-intensity endurance exercise.

In addition to assessing lactate levels in our laboratory, we have sought to understand if a cortisol connection exists with the use of a mouthpiece. The purpose for the pursuit of this hypothesis is based on the work by Hori et al. (11) in which rats bit down on a wooden stick during a stress-induced restraint. They found that when rats bit down on the stick, there was a decrease in corticotrophin-releasing factor (a precursor to the release of cortisol) vs. when rats did not bite down on the stick during stress. Sasaguri et al. (20) followed up with a study on rats and the effect of biting on stress indicators and found a decrease in Fos proteins (a marker of neuronal activity), which are induced in the hypothalamic paraventricular nucleus (PVN) during stress. They suggested that biting on a stick decreases activity in the PVN, thereby, reducing the stress response. However, little data exist to support a decreased stress response with the use of a mouthpiece in humans.

Tahara et al. (24) cited that during psychological stress, clenching and biting could decrease cortisol release in humans. They found that during a 20-minute mental stress activity, subjects displayed a decreased cortisol response when they were able to clench their teeth or chew on paraffin wax. Both of these situations (clenching and chewing during a stressful situation) would require the use of the masticatory muscles. The authors suggest that the activity of these muscles resulted in a cascade of events leading first to the activation of the motor area of the cerebrum, which then resulted in a decreased hypothalamic–pituitary response and thereby a reduction in cortisol release, similar to the theory suggested by Hori et al. (11). Yet, little data exist to support or deny this response during a physiological stressor such as high-intensity exercise. Thus, the purpose of this study was to determine if the use of a mouthpiece would alter cortisol levels during the physical stress of resistance training and could thereby begin to explain some of the performance enhancements that have been cited with those who use a mouthpiece during exercise.

Numerous researchers have shown a more robust cortisol response with higher intensity resistance exercise (13,14,21). McGuigan et al. (14) found a 145% difference in salivary cortisol between low- (3 sets) and high- (12 sets) intensity resistance training, with the cortisol levels being elevated 97% after high-intensity exercise. Thus, in designing our study, we wanted to better understand the mouthpiece effects on cortisol levels during a high-intensity group exercise session, as is typically used by most amateur and professional team sports.

METHODS

Experimental Approach to the Problem

The focus of this study was to determine if the use of a mouthpiece during intense resistance training resulted in a decrease in cortisol levels. Previous research has suggested improvements in recovery and improved strength performance when wearing a mouthpiece, but there has been no study to elucidate the possible mechanism(s) for this improvement. Thus, a within-subjects design was used in which each subject was randomly assigned the use of mouthpiece during 1 of 2 identical bouts of exercise that were separated by 2 weeks. Saliva samples were collected for cortisol analysis at 5 different time points before, during, and after each session.

Subjects

Twenty-eight division I male football players, between the ages of 18 and 22 years, were recruited for this study. Each subject had at least 4 months (3–4 sessions per week) of training with his team and was therefore familiar with all the training protocols used by The Citadel Strength and Conditioning staff. All subjects were informed of the procedures of the study and signed a consent form before their participation in the study. The consent form informed subjects of their ability to withdraw from the study at any time with no negative consequence. The Internal Review Board approval for this study and all procedures was obtained through The Citadel Institutional Review Board for use of Human Subjects.

Procedures

Before the exercise testing, subjects were fitted with a customized mouthpiece by a sports dentist (Under Armour Performance Mouthwear, Baltimore, MD in cooperation with Bite Tech Corporation, Minneapolis, MN, USA) (Figure 1). The mouthpiece was noninvasive and fit snugly on the

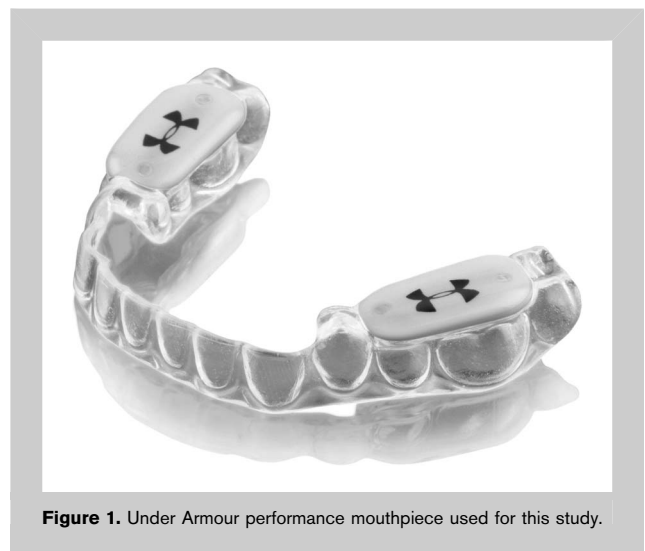


Figure 1. Under Armour performance mouthpiece used for this study.

individual's lower set of teeth. When mouthpieces arrived, individuals placed them in their mouths and all fit with ease and were comfortable. No issues of discomfort were noted or stated by any of the subjects. Subjects were instructed to wear the mouthpieces during routine exercise sessions before the testing days.

A within-subjects design was used in which subjects were randomly assigned to wear the mouthpiece during 1 of the 2 exercise sessions that took place between 3:00 and 4:10 PM on both days of testing. All subjects were instructed to bring their mouthpieces to the training facility, because they were not told before the testing day to which group they had been assigned. At the beginning of each exercise session and upon arriving at the training facility, subjects were reminded to ingest no dairy products during the exercise session, because dairy has been shown to interfere with proper salivary cortisol assessment. Additionally, before the exercise testing, subjects had been instructed to have no caffeine, dairy on the day of testing or alcohol 24 hours before testing. On the testing day, subjects were questioned as to their adherence to the following guidelines: no dairy or caffeine consumption immediately before exercise, no alcohol consumption within 24 hours of testing, and no eating of a major meal within 60 minutes of testing. All subjects stated adherence to these dietary guidelines. Subjects were then instructed on the saliva sample collection and how often the samples would be taken during the exercise sessions. Passive drool saliva samples were collected from each of the subjects at the following time points: (time point 1: immediately before exercise, 3:00 PM; time point 2: 25 minutes into exercise at 3:25 PM; time point

3: 45 minutes into exercise at 3:45 PM; time point 4: the end of session (60 minutes) at 4:00 PM; and time point 5: 10 minutes postexercise at 4:10 PM). Samples were collected in 2-ml cryovials with the total volume averaging between 1.0 and 1.5 ml for all samples, as recommended by manufacturer's guidelines (Diagnostic Systems Laboratories, Webster, TX, USA).

Salivary cortisol samples were used for this study because of the ease of collecting saliva during the 5 time points of pre, during, and postexercise. Salivary cortisol samples have been cited to have a strong correlation with blood samples and provide an accurate assessment of unbound cortisol levels (18,25). Salivary samples were taken and stored in the freezer for a period of <2 months. When ready for assay, samples were removed from the freezer and saliva was analyzed for cortisol concentrations by enzyme-linked immunoassays (Diagnostic Systems Laboratories). Manufacturer's guidelines were followed for all salivary cortisol assay procedures. The minimum detection limit for this assay is 0.0065 $\mu\text{mol}\cdot\text{L}^{-1}$, and we observed intraassay variations of 3.44% and interassay variations of 6.01% during our analysis.

The samples for this study were collected during 2 identical group exercise sessions led by The Citadel Strength and Conditioning staff. These exercise sessions took place during the in-season football strength maintenance program, which focused on high-intensity and low repetition resistance exercise. Specifically, both sessions began with a 10-minute warm-up that consisted of the following exercises: wood chops, reverse lung and twists, chest press (all with a medicine ball), line hops, push-ups, and straight leg dead lifts. The

resistance training component began with 3 sets of 3 repetitions of hang cleans at a light weight, with side lunges in between sets. Three sets of 3 repetitions were completed at 70–75% of each subject's 1 repetition maximum (RM), with approximately 60–90 seconds rest between sets. Narrow grip dead lifts were performed next at 70–75% of 1RM for 3 sets of 5 repetitions, with shoulder mobility exercises performed during the 60- to 90-second recovery period. Subjects then performed 3 sets of 5 repetitions of single leg overhead shoulder presses at the maximum weight possible with good form, whereas trunk rotation exercises were performed during the 60- to 90-second recovery period. The final 10 minutes of the workout sessions

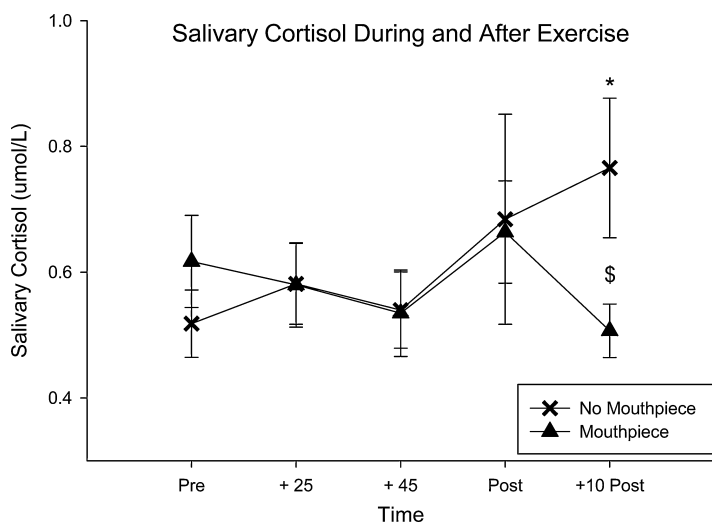


Figure 2. Salivary cortisol levels collected before, during, and after 60-minute, high-intensity resistance training sessions in collegiate football players, within-subjects design ($N = 28$). *Indicates an increase ($p = 0.02$) in cortisol from pre to 10 minutes post in the no-mouthpiece group and a difference in cortisol levels between the no-mouthpiece and mouthpiece groups. \$Indicates a decrease in cortisol ($p = 0.04$) from post to 10 minutes post in the mouthpiece group. $N=28$ per group.

TABLE 1. Descriptive statistics for salivary cortisol, within-subjects design ($N = 28$).*

Sample time	Salivary cortisol ($\mu\text{mol}\cdot\text{L}^{-1}$)	
	Mouthpiece	No mouthpiece
Pre-exercise	0.62 ± 0.07	0.52 ± 0.05
25 min into exercise	0.58 ± 0.07	0.58 ± 0.06
45 min into exercise	0.54 ± 0.07	0.54 ± 0.06
Immediately postexercise	0.66 ± 0.08	0.68 ± 0.17
10 min postexercise	0.51 ± 0.04	0.77 ± 0.11

*Values are given as mean \pm SE.

consisted of core exercises aimed at strengthening the abdominal, oblique and lumbar musculature.

Statistical Analyses

All data was entered into Microsoft Excel for management and exported to SigmaStat 3.5 (Point Richmond, CA, USA) for statistical analysis. Comparison of cortisol levels at individual time points between groups was done using a paired t -test. If normality tests failed, a Wilcoxon Signed Rank Test was performed to assess differences between groups. Percent change scores between group means were calculated using the following formula: $(\mu_2 - \mu_1 / \mu_1) \times 100$. Statistical significance was set at $p \leq 0.05$. All data are presented as mean \pm SE.

RESULTS

Twenty-eight subjects took part in this study, with a total of 22 subjects having complete data. The remaining 6 subjects had 2 or less of the 10 data points missing, thus mean substitution was used for these data points (3), and these subjects were included in the final analysis. There was a 51% difference ($p = 0.02$) in cortisol levels 10 minutes postexercise between mouthpiece and no-mouthpiece conditions (Figure 2). Mean cortisol levels of the mouthpiece condition were $0.51 \pm 0.04 \mu\text{mol}\cdot\text{L}^{-1}$, whereas mean levels without the mouthpiece were $0.77 \pm 0.11 \mu\text{mol}\cdot\text{L}^{-1}$ at 10 minutes postexercise (Table 1). Interestingly, there was also a 29% decrease in cortisol between 60 minutes exercise and 10 minutes postexercise in the mouthpiece condition (0.66 ± 0.08 vs. $0.51 \pm 0.04 \mu\text{mol}\cdot\text{L}^{-1}$), respectively ($p = 0.04$) (Table 1, Figure 2). Finally, results indicated a 48% increase in cortisol ($p = 0.02$) from baseline $0.52 \pm 0.05 \mu\text{mol}\cdot\text{L}^{-1}$ to 10 minutes post $0.77 \pm 0.11 \mu\text{mol}\cdot\text{L}^{-1}$ in the no-mouthpiece condition (Table 1 and Figure 2). There was no significant difference in baseline to 10 minutes post in the no mouthpiece condition (Table 1).

DISCUSSION

The literature shows a paucity of data related to mouthpiece use and improved performance. Previous research has

suggested an effect of mouthpiece use on performance, but there has been little to no evidence to elucidate the reasons for improvements. In the research by Smith (22,23) the suggestion of the performance improvements were cited as being related to a better alignment of the TMJ. Jakush (12) cited that the improvements in TMJ were because of improved proprioceptive function of ligaments, muscle spindles, and Golgi tendons. However, this concept has been difficult to study, and many have cited this as controversial (12,16). Moore (16) stated that the practice of fitting athletes with mouthpieces to improve malocclusions and TMJ imbalances was considered a technique that has many critics. He referred to the treatment of TMJ as largely subjective and difficult to pinpoint medically or scientifically. Possibly because of the vagueness of the theories and the difficulty in defining the exact mechanisms for the improvements, mouthpiece and exercise research virtually stopped in the 1980s and has only recently begun to resurface.

In our laboratory, we have initiated studies to determine if any physiological mechanisms could be associated with the performance improvements during mouthpiece use (5–7). Research in other laboratories has cited that when animals bite down on a wooden stick during stress, there is a lowered stress response (11,20). Specifically, Hori et al. (11) stated that when rats bit down on a stick during stress, there was a decrease in corticotrophin releasing hormone, a hormone in the chain of the hypothalamo-pituitary-adrenocortical (HPA) axis that is essential to cortisol production. In humans, Tahara et al. (24) cited a decreased stress response when human subjects bit down or chewed during a stressful mental exercise. These researchers cited a mechanism that connected the masticatory muscles to the HPA axis. Specifically, they suggested that a reduction in masticatory stress, as created by biting down or chewing, would lead to stimulation of the motor area in the cerebrum. This then would cascade to the hypothalamus and lead to decreased cortisol response. However, it is unclear whether this outcome would hold true in humans during a physical stress such as resistance training.

Acevedo et al. (1) cited differences in the psychological and physical stressors, which result in cortisol changes, specifically that mental stressors elicit a quicker response in cortisol vs. cortisol changes that occur with physical stress. They suggest that physical stressors must be at least 60 minutes in duration to elicit changes in cortisol. Therefore, in choosing the physical stress, we sought to use a protocol that would likely elicit a significant increase in salivary cortisol levels. Kraemer and Ratamess (13) proposed that the greatest increases in cortisol were seen in resistance training studies where there was high to moderate intensity, little rest between sets, and a higher number of sets. In this study, we sought to examine a comparable stress-inducing protocol, but this time in a setting that is applicable to most team sport athletes. The goal of this study was to examine the effects of

wearing a mouthpiece on cortisol release during high-intensity training (low repetitions and high weights as recommended by Kraemer and Ratamess [13]).

The increases in cortisol we found without a mouthpiece are similar to those in other studies that assessed cortisol changes after resistance exercise. McGuigan et al. (14) in a study of nonathletes found a 97% increase in cortisol from baseline after 6, 10 repetition sets at 75% of 1RM. Ahtiainen et al. (2) had strength athletes follow a maximal repetition protocol (4 sets of squats with 2 minutes of recovery, 12 repetitions) and found that cortisol increased 54% from baseline with values of $0.41 \mu\text{mol}\cdot\text{L}^{-1}$ at baseline to $0.63 \mu\text{mol}\cdot\text{L}^{-1}$, 15 minutes postexercise. These results are similar to the 48% increase in cortisol ($0.52 \mu\text{mol}\cdot\text{L}^{-1}$ at baseline to $0.77 \mu\text{mol}\cdot\text{L}^{-1}$, 10 minutes postexercise) we saw with athletes in the no-mouthpiece condition from baseline to 10 minutes postexercise.

The results of this study demonstrated decreased cortisol levels with a mouthpiece 10 minutes after a high-intensity bout of resistance exercise. The intriguing aspect of this research is that cortisol levels with the mouthpiece condition returned to baseline levels 10 minutes postexercise, whereas the no mouthpiece condition continued to elicit increased cortisol levels 10 minutes postexercise. In addition, it is interesting to note that cortisol levels between conditions trended similarly throughout the exercise and then demonstrated a divergence 10 minutes postexercise. This may indicate that metabolism during exercise was not negatively affected by the mouthpiece condition, that is, cortisol was available for gluconeogenesis. Yet elevated cortisol has been linked to decreased ability to recover from exercise via increased skeletal muscle protein degradation (13). Enhancements in skeletal muscle protein postexercise may lead to improvements in exercise recovery. Potential improvements in recovery may support the cited increased recovery as noted in the endurance runners in the study by Garabee (4).

The underlying question remains as to how a mouthpiece can elicit changes in cortisol postexercise. In a rat and human stress model, researchers have suggested there is HPA axis involvement (11,20,24). The HPA axis is vital to homeostatic response during stress, with the end result being activation and release of glucocorticoids such as cortisol. The HPA axis is partially regulated by the hypothalamic PVN neurons that are positioned to rapidly activate the HPA axis (9). Herman et al. (10) cited that neurons within the PVN directly synthesize the transmitter nitric oxide. Nitric oxide has been shown to increase vasodilatation of blood vessels at the onset of exercise (8) in addition to functioning as a neurotransmitter that affects the HPA axis (9,10). Thus, involvement of the HPA axis via a mouthpiece may involve the activation of the PVN. To better answer these questions of decreased cortisol levels postexercise when one uses the mouthpiece, our laboratory is currently assessing the hypothalamic pituitary axis mechanisms associated with depressed cortisol levels before, during, and after an intensive exercise protocol.

As we examine the possible involvement of the HPA axis and its potential interaction of the mouthpiece, the design of the mouthpiece must be further explained. The mouthpiece used was a custom-fit mouthpiece that fit snugly to the lower set of teeth in each subject. The mouthpiece has an acrylic material that fits in front of the bottom front teeth, with 2 wedge pads that create a separation between the upper and lower teeth (Figure 1). The design of the product causes a separation of the teeth and a more forward repositioning of the mandible because of the placement of the bite pads. Although assessment of the TMJ position was not evaluated with this study, the length of time that the wearers used this product (<70 minutes) would not contribute to any permanent issues or problems with the TMJ. In addition, it should be noted that no subjects complained of joint pain postexercise, which would indicate issues with TMJ occurring with mouthpiece use during exercise. Earlier findings from our laboratory showed that this product specifically creates a forward movement and increased space between the upper and lower teeth leading to improved airway dynamics (5). Thus, one theory for a partial improvement in the cortisol response may lie in the repositioning of the mandible and the improved airway response.

Another possible explanation for the improved cortisol response may be attributed to a decreased stress response while biting down on a mouthpiece. Tahara et al. (24) suggested an increased ability to clench leads to decreased mandibular stress that is channeled through the motor area of the cerebrum in turn affecting the HPA axis and a decreased stress response. During intense resistance exercise, it is often noted that subjects clench their teeth to power through the movement with a possible improvement in motor performance. Specifically, Miyahara et al. (15) cited that improved motor performance may be modulated by the H reflex, which is activated when one clenches their teeth. They cited that as one improves their strength of teeth clenching, this leads to improved H reflex response, which in turn improves motor performance. Therefore, we suggest that with the mouthpiece in place, a mouthpiece with padded wedges separating the teeth that there is improved ability to clench, resulting in positive motor performance and an overall decreased stress response. These studies imply that improved clenching mechanism results in changes in motor performance and decrease mandibular stress, which may both lead to an overall decreased stress response as indicated by a decreased cortisol response.

PRACTICAL APPLICATIONS

Many strength athletes will clench down during a power movement with some studies suggesting that mouthpieces may improve motor performance during strength exercises. However, to date, little has been known about any physiological mechanisms that may be associated with the exercise response and mouthpiece use. The results of this study suggest that mouthpiece use during intensive resistance training results were similar in both mouthpiece and no mouthpiece conditions. This

is important because it suggests that metabolic needs were not compromised during the activity in either condition. However, an interesting finding of this study was the difference in cortisol levels 10 minutes postexercise, in which the mouthpiece condition displayed baseline levels of cortisol, whereas no mouthpiece condition exhibited increased levels of cortisol. Understanding the cortisol response postexercise is an important step in explaining any physiological mechanisms that may respond to mouthpiece use during exercise. Elevated cortisol levels result in an anabolic state of the skeletal muscle; therefore, a lowered cortisol response postexercise may indicate an improved protein response. In summary, these findings support the use of a noninvasive performance mouthpiece during intensive training sessions to lower the cortisol response postexercise, while not inhibiting cortisol during exercise which is necessary to maintain fuels for exercise.

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