Effect of Caffeine on Salivary Cortisol during 10-Mile Cycling Time Trial

Anne Roshong
Lauren Clune
H.Scott Kieffer
Jodie Haak

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Abstract

Cortisol is a steroid hormone that is produced in the body as a response to stressors, including acute bouts of exercise. One of the roles of cortisol related to exercise involves the enhanced metabolism of fat, carbohydrates, and proteins. Caffeine is the most commonly-used ergogenic aide and has been suggested to improve performance in high-intensity and endurance exercise. However, it is unclear whether the mode used to deliver the caffeine has an effect on performance or whether that effect would be related to the release of cortisol from the adrenal glands. The goal of this double-blind study was to test the differences in performance and cortisol levels between caffeine gum and caffeine pills. Due to the non-invasive nature of saliva collection, and because cortisol is known to accumulate in the saliva in a predictable pattern, this study measured salivary cortisol. Trained cyclists completed three separate 10-mile time-trials, and each time they were given either placebo pill/placebo gum (mean cortisol concentration change of 0.1763±0.2522 nmol/L), placebo pill/caffeine gum (0.0880±0.0689 nmol/L), or caffeine pill/placebo gum (0.1068±0.0901 nmol/L; p = 0.34). Saliva was collected five minutes before and immediately following each time trial. Saliva samples were stored at -80°C. Samples were analyzed using an ELISA test and a high-sensitivity salivary cortisol kit from Salimetrics, Inc. (State College, PA). No evidence of a relationship was found between caffeine use of either type and change in cortisol level, and evidence of a relationship was observed between each subject’s individual trial times and change in cortisol level for that trial.

Introduction

Cortisol is a steroid hormone that is released from the adrenal cortices into the bloodstream when the body is placed under stress, particularly if that stress is acute in nature. This includes bouts of exercise, injury, and the “fight or flight” response. Cortisol has several roles within the body, the most pronounced of which involves nutrient metabolism. It is a potent stimulant of lipolysis in adipose tissue,
increasing blood levels of free fatty acids; this happens at a relatively equal rate in adipose tissue throughout the body.\textsuperscript{2,3} Cortisol also stimulates protein degradation, raising blood levels of amino acids, and ketogenesis.\textsuperscript{3,4} Cortisol inhibits glucose uptake/use by most body tissue, except the brain—this spares glucose for use by the brain and forces muscles and other tissues to use the amino acids and free fatty acids that have been released into the blood as fuel instead.\textsuperscript{1,3}

The elevation of cortisol has been shown following many different types of exercise, most notably in high-intensity and/or endurance testing. Hill et al showed that there may be a threshold for cortisol levels, which increased proportionately to exercise intensity up to 20 minutes/60\% of VO\textsubscript{2}\text{max} but then increased at greater rates at intensities and durations longer/higher than that.\textsuperscript{5} VanBruggen et al indicated a lack of significant increase in pre- to post-exercise salivary cortisol levels for intensities/durations less than 80\% of VO\textsubscript{2}\text{max} and 30 minutes but did show significant concentration increases in 30 minutes/80\% VO\textsubscript{2}\text{max} and above.\textsuperscript{6} Usui et al determined that salivary cortisol increased significantly after subjects completed 60 minutes of cycling on a stationary ergometer at 75\% of their previously-measured VO\textsubscript{2}\text{max}.\textsuperscript{6} In addition, acute salivary cortisol increases were shown by Rahman et al following a Bruce protocol (a way to measure VO\textsubscript{2}\text{max} using a treadmill and increasing both speed and incline at each stage).\textsuperscript{8} These near-maximum tests are all similar conditions to the protocol used for the present study, so it is therefore predicted that the saliva analyses will show an increase in salivary cortisol concentrations from pre- to post-exercise trial.

Cortisol is released directly into the blood, but recent studies have shown that it can also be found in measurable, comparable quantities in saliva.\textsuperscript{6,9,10,11,12,13} Saliva has been increasingly utilized as a method of sample collection for several reasons. The first is that it is a non-invasive technique; subjects simply are handed a tube and are asked to spit (or “passive drool”) into it. This is an advantage over using serum (blood), which requires using needles to draw it out, which often increases subject anxiety and therefore an increase in stress response; this could lead to an inadvertent increase in cortisol
production, skewing data results. It has also been suggested that saliva may provide a more accurate picture of a particular stress response than serum because the concentration of cortisol found in saliva represents the unbound, active form of the hormone (while most of the cortisol in blood is bound to carrier molecules and thus inactive). Because of the non-invasive nature of saliva collection, and because collection does not require a license (blood requires a phlebotomy license), it is the method used in this study for measuring cortisol levels.

The body’s response to the acute stress of exercise can sometimes be altered through use of ergogenic aids, such as caffeine, which is perhaps the most commonly-used ergogenic aid. Caffeine has been shown to improve performance in endurance exercise, especially those of the time trial variety. McNaughton et al found that cyclists rode significantly farther during a 60-minute time trial after being given 6 mg/kg of caffeine in the form of a pill one hour prior to beginning the trial. Hodgson et al showed an significant increase in distance during a 45-minute cycling time trial after subjects were given 5 mg/kg of caffeine, either as coffee or a pill, one hour prior to beginning the trial compared to the placebo/decaffeinated coffee trials. These are similar conditions to the protocol used in this study, so it can be theorized that a decrease in time-to-completion for time trials in which the subject is given caffeine will be seen.

Caffeine alters performance through several different proposed mechanisms, including central nervous system stimulation (theoretically leading to improved reaction time), but the mechanism that pertains specifically to the present study is that of increased mobilization of free fatty acids. An increased mobilization of free fatty acids during endurance exercise would theoretically enable the body to rely more on fats, which are more calorie-dense, for fuel rather than stored muscle glycogen; this could allow athletes to run or cycle for longer distances and cover those distances in a shorter amount of time. A study by Pesta et al showed increases in fatty acid oxidation; they also proposed that increased lipolysis led to a decreased reliance on glycogen use in muscles, as indicated by a reduced
respiratory exchange rate (RER) in caffeine trials. McNaughton et al determined that levels of free fatty acids in serum were significantly elevated in subjects who had taken caffeine pills before completing a cycling time trial. Caffeine has also been shown to increase cortisol production both at rest and during stress, although the exact mechanism by which this happens is not yet known. Because cortisol, as stated above, has been shown to also work in this way, it has been posited that cortisol is the mechanism by which caffeine exerts its fat-mobilizing effect. Therefore, in theory, cortisol levels are expected to be elevated in a subject who has taken some form of caffeine prior to or during their exercise bout.

The purpose of this study was to assess salivary cortisol levels in trained cyclists before and after completion of three separate 10-kilometer time trials, in which they were given a placebo pill/placebo gum, caffeine pill/placebo gum, or placebo pill/caffeine gum.

Methods

**Participants.** Each of the subjects whose saliva was analyzed for cortisol was a trained, adult cyclist who completed all three of the time trials. The subjects were not divided into control and testing groups because each served as their own control. A total of ten subjects, one female and nine males, were tested.

**10-mile cycling protocol.** Each subject was allowed to set his or her own gear and cadence on the stationary cycle and was instructed to finish the 10-mile distance as quickly as possible (with maximum exertion). The three trials for each subject were completed on three separate occasions with multiple days of rest in between.
**Caffeine administration.** Both gum and pills were used as methods of administering 5 mg/kg of caffeine, an amount shown in literature to alter performance. The study was done such that both the researcher and the subject were blinded to whether a placebo or caffeine was given. Each subject completed three trials that had three different conditions: placebo pill/placebo gum, placebo pill/caffeine gum, and caffeine pill/placebo gum. The conditions were set up in a random order. Pills (placebo or caffeine) were given to the subjects 60 minutes prior to testing, and gum was given five minutes prior to testing and also at the 5-mile mark, in equal (2.5-mg/kg) doses (see Figure 1).

**Sample collection and analysis.** Saliva samples were collected five minutes prior to testing and immediately after testing using passive drool and were frozen at -80°C. Analysis for salivary cortisol was done using a high-sensitivity enzyme-linked immunosorbent assay (ELISA) kit from Salimetrics, Inc. (State College, PA). This has been shown to be an acceptable and accurate method for saliva sample analysis. Samples were thawed on ice prior to analysis, and 100-µL aliquots were taken and centrifuged at 3000 rpm for 15 minutes. Each sample was assayed in duplicate. The assay was completed per protocol provided with the kit. The ELISA plate-reader (BIO-TEK Synergy HT) was programmed to read the plate at 450 and 750 nm, and KC4 software was used to collect the absorbance data. Data was analyzed using repeated measures ANOVA.
Results

The saliva of ten subject was analyzed for salivary cortisol for this study, specifically for the change in cortisol concentration pre- to post-exercise. Saliva from each of the three time trials completed by each subject was analyzed separately, and the mean change in concentration for each condition (placebo pill/placebo gum, placebo pill/caffeine gum, and caffeine pill/placebo gum) was calculated (see Figure 2). It was found that the mean change for the placebo pill/placebo gum condition was 0.1763±0.2522 nmol/L; for placebo pill/caffeine gum was 0.0880±0.0689 nmol/L; and for caffeine pill/placebo gum was 0.1068±0.0901 nmol/L ($p = 0.34$). No evidence of a relationship was found between caffeine or placebo use and the change in salivary cortisol pre- to post-trial.

![Figure 2](image_url). Average change in salivary cortisol concentration, pre- to post-time trial. For each of the three different conditions, the average change in salivary cortisol concentration was calculated and graphed, along with standard deviation. No evidence of a relationship was found between caffeine administration, either pill or gum form, and change in cortisol concentration when compared to the trials in which the subjects only received placebos.

The change in salivary cortisol concentration for each trial for each subject was then plotted and compared to the individual performance times for each time-trial (see Figures 3a and 3b). No relationship was observed between time-trial performance and caffeine or placebo use, and there was
no evidence of a relationship between time-trial performance and change in salivary cortisol concentration.

Figure 3a. Change in salivary cortisol concentration, pre- to post-time trial, for individual subjects. The change in concentration values (nmol/L) in the duplicates for each sample were averaged, and the change in concentration from pre- to post-trial was graphed for each of the three conditions for every subject. No evidence was observed to show a relationship between caffeine and non-caffeine trials or between caffeine gum and caffeine pill trials.

Figure 3b. Time-trial performance under three separate conditions. This graph is meant to be compared to Figure 2 and shows how long it took each subject to finish each trial under the three different conditions. The goal of these two graphs, together, was to compare the time trial performance with the change in salivary cortisol levels within the same trial and subject. No relationship was observed between change in cortisol levels and time trial performance, even within individual subjects.
Discussion

The purpose of this study was to assess the changes in salivary cortisol concentrations in 10 trained cyclists between pre- and post-exercise during the completion of three separate 10-kilometer cycling time trials, in which they were given a placebo pill/placebo gum, caffeine pill/placebo gum, or placebo pill/caffeine gum. The mean salivary cortisol concentration changes from pre- to post-trial for each of the three conditions were 0.1763±0.2522 nmol/L for the placebo pill/placebo gum condition; 0.0880±0.0689 nmol/L for the placebo pill/caffeine gum condition; and 0.1068±0.0901 nmol/L for the caffeine pill/placebo gum condition (p = 0.34; see Figure 2). It was found that there was not a relationship between change in cortisol concentration and the presence or absence of caffeine.

Because of the lack of relationship between cortisol concentration change and caffeine when the subjects’ trials were averaged, the change in salivary cortisol concentration for each subject’s three trials were graphed (see Figure 3a). Again, there was no evidence of a relationship between salivary cortisol concentration changes from pre- to post-exercise and caffeine use. This was compared to a graph of each subject’s time trial performance in each of the three conditions (see Figure 3b), and there was no apparent relationship between cortisol concentration changes and time-trial performance. No relationship between time trial performance and caffeine use was observed, either.

Though it was predicted that, based on previous studies, that a greater change in cortisol would happen in trials in which the subject had been given caffeine and that caffeine would improve time trial performance, these findings are not necessarily uncommon. Slivka et al found that caffeine had no effect on performance during a 12.4-mile time trial, and that cortisol levels were raised only in the subjects who had not ingested any kind of carbohydrates before the trial.20 Lovallo et al found that cortisol concentrations were not significantly increased following exercise with caffeine if the subject
was a chronic caffeine user. In other words, caffeine can have vastly different effects on individuals based on a number of factors, and because its mechanism for altering cortisol levels is not yet fully understood, it cannot be determined with certainty why these variations occurred. Many of the studies looked at before the design of this study involve longer, time-controlled time trials, rather than a set distance to be finished as quickly as possible; this often meant that the subjects in the present study were only cycling for half the time of the other studies. It is therefore possible that the reduced time was not long enough to induce the full effects of caffeine on both performance and salivary cortisol concentrations.

The saliva samples themselves could also have had some impact on the findings for this study. Some were more viscous than others, possibly indicating differences in hydration status of the subjects and therefore differences in how concentrated the samples were. This is particularly important for loading the samples onto the assay plate; 25 µL of each sample was loaded, but if one subject was dehydrated and had incredibly viscous saliva, it may appear that they have more cortisol simply because their saliva is more concentrated than a subject whose saliva was more liquid and diluted. This could be remedied through using more standardized collection methods; several studies recommended having the subjects rinse their mouths three times for 30 seconds with distilled water at least ten minutes before saliva collection. It was also suggested by Hansen et al that caffeine ingestion prior to saliva collection may alter the binding of substances within the saliva when it is assayed, though this phenomenon has not been widely studied and it is not clear to what degree binding is affected (Hansen). However, if this is the case, this could have altered the binding of cortisol to the assay plate in samples from trials in which the subject was given caffeine, giving slightly skewed results.

Overall, the presented studied showed that there is no relationship between changes in salivary cortisol from pre- to post-exercise in trained cyclists and caffeine use during 10-mile cycling time trials. There is also no evidence from this study that there is a relationship between time-trial performance
and change in salivary cortisol concentrations pre- to post-time trial. Caffeine use results in highly varied responses from different individuals, and further research is needed to determine exactly what factors play a role in these differing reactions.


