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The Effect of High-Intensity Exercise on Changes in Salivary and Serum Cortisol Proportion Dynamics

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Abstract: Typically, salivary cortisol is reported as 5–10% of total cortisol, but the stability of this proportion and the effect of exercise on the 24-h profile is unclear. Therefore, this study investigated the circadian rhythm of the proportion of serum cortisol represented by salivary cortisol, and the impact of acute high-intensity exercise. Recreationally trained males (n = 8, age = 25.7 ± 2.4 years, height = 174.7 ± 7.8 cm, mass = 69.8 ± 12.1 kg) completed two 24-h profiles (rest and exercise conditions) for serum (Q60) and salivary (Q120) cortisol. Exercise consisted of 5 × 30 s sprinting intervals on the cycle ergometer. Cortisol was assessed using commercially available assays. The proportion (C_{prop}) of serum cortisol (C_{ser}) represented by salivary cortisol (C_{sal}) was calculated as [$C_{prop} = C_{sal} / C_{ser} \times 100$]. Multilevel growth models tested for trends across the 24-h profile. The highest relation between C_{ser} and C_{sal} was observed at 08:00 AM (r = 0.90). The average C_{prop} was 5.95% and demonstrated a circadian profile characterized by a cubic model. Acute exercise did not alter C_{ser}, C_{sal}, or C_{prop}. Thus, the proportion of C_{ser} represented by C_{sal} changes across a 24-h period and should be accounted for if using salivary cortisol to reflect circadian output of cortisol.

Keywords: steroids; circadian; diurnal; cycling

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1. Introduction

Cortisol is the predominate glucocorticoid in humans. Almost all human cells contain glucocorticoid receptors [1], and thus cortisol is considered a pleiotropic hormone, activating or inhibiting a range of genes dependent on numerous factors such as cell type, total exposure, length of exposure, and presence of cofactors. Cortisol demonstrates a robust circadian and diurnal profile in humans with peak concentrations observed in the morning soon after waking and the hormonal nadir occurring in the early morning period during nocturnal sleep [2,3].

The diurnal profile is controlled via self-oscillating transcriptional loops in the suprachiasmatic nucleus of the hypothalamus. These transcriptional/translation loops consist of positive arm proteins—CLOCK and BMAL1—that form a heterodimer and promote the synthesis of the negative arm proteins—Per 1/2/3 and Cry 1/2—which inhibit the binding of the positive arm proteins [4,5]. Auxiliary loop proteins, such as REV-Erb and ROR, also provide a further level of control and stability to this loop to regulate the 24-h rhythmicity [4,6]. The suprachiasmatic nucleus becomes entrained to the external environment primarily via light signals transmitted through the optic nerves. The primary function of the circadian variation in cortisol concentration has been theorized to be a neuroendocrine synchronization of peripheral clock mechanisms [5,7]. Disruption of this hypothalamic–pituitary–adrenal axis circadian rhythm has therefore been associated with a range of physical and psychological health disorders [5,8], and is of interest to both clinicians and researchers.

Cortisol is derived from cholesterol molecules and, thus, the hormone is lipophilic and circulates in the blood primarily bound to binding proteins. Approximately 80% of circulating cortisol is bound to the high-affinity, low-capacity protein corticosteroid binding globulin (CBG) [9]. Albumin, by contrast, has a high-capacity but low-affinity for cortisol [10], and binds approximately 10% of cortisol in circulation. The remaining approximately 10% of cortisol is considered to be "free" and according to the free-hormone hypothesis, is able to be taken up by target cells and exert intracellular effects [11]. Physiological stressors such as exercise can result in elevations in circulating cortisol concentrations [12], which can also be observed in the saliva [13]. For example, Hough and colleagues demonstrated an increase in salivary cortisol following a 30-min high-intensity cycling protocol [14]. There is also evidence to suggest that an afternoon exercise protocol can impact the nocturnal serum cortisol profile [15].

The use of saliva to measure cortisol has gained popularity due to its non-invasive nature and relative ease of collection. Salivary cortisol is significantly correlated with cortisol in circulation under resting conditions [16,17] and following stimulation of the hypothalamic–pituitary–adrenal axis via physiological challenges such as exercise [18–23]. The portion of cortisol that is considered free in the serum is able to readily pass into the salivary glands [24] and, thus, salivary cortisol reflects the free portion of the hormone [23].

In order to quantify the free portion of cortisol in the blood, first the free and bound portions must be separated through techniques such as equilibrium dialysis or ultrafiltration [25]. The fluid with only free cortisol remaining can then be analyzed using analytical techniques such as liquid chromatography and mass spectroscopy [26,27]. These techniques are time consuming, costly, and unavailable to many researchers or medical practitioners, and can also be impacted by analytic choices such as incubation temperature [28]. Salivary immunoassays therefore provide a useful alternative for assessing the so-called free, biologically active portion of the hormone.

As representative of the free portion, salivary cortisol is commonly reported in the literature as being 5–10% of circulating cortisol, although the range has been reported to be as wide as 1–9% [29]. This variance is due to a number of factors including the total concentration of both the hormone and binding proteins, as well as binding affinities of the binding proteins that can be modulated by other factors [30]. Salivary cortisol has been demonstrated to approximate the circadian profile of serum cortisol [31]. However, CBG also demonstrates a circadian rhythm [32], and thus it is likely that the unbound portion of cortisol also follows a circadian rhythm and disproportionality between CBG and unbound cortisol will impact the salivary portion of the hormone.

Although the range of 5–10% of total cortisol that is represented by salivary cortisol appears to be a small absolute range lending validity to its use as a non-invasive marker of total cortisol, this actually represents up to a two-fold difference in the total free cortisol concentration. For example, a salivary cortisol concentration of 5 nmol.l-1 may be associated with a circulating free cortisol concentration of 50–100 nmol.l-1. Within a short time window under stable conditions, this is perhaps only a small concern, but over longer periods of time or during changes in subject allostasis, such a discrepancy may lead to erroneous conclusions regarding circulating free cortisol concentrations.

To date, no study has investigated the circadian change in the proportion of total cortisol represented by salivary cortisol, or whether high-intensity exercise may alter such a circadian rhythm. Therefore, this study aimed to investigate the relation between salivary and serum cortisol across a 24-h period, whether there was a circadian rhythm to the proportion of the two biological fluids, and the impact of high-intensity exercise on the profile. We hypothesized that (1) the proportion of total cortisol represented by salivary cortisol would be stable across the 24-h period; (2) short duration high-intensity exercise would not impact this proportion across the 24-h period; and (3) salivary and serum cortisol would be significantly correlated across the entire 24-h period, with and without mid-morning short duration high-intensity exercise.

2. Materials and Methods

2.1. Participants

Adult males (n = 8, age = 25.7 ± 2.4 years, height = 174.7 ± 7.8 cm, mass = 69.8 ± 12.1 kg) were recruited to participate in this study. Participants were required to be 18–35 years old, self-report participation in regular moderate–vigorous exercise, have a body fat percent <18%, and be free of any known metabolic, cardiovascular, or pulmonary disease. All participants gave their written informed consent for inclusion before they participated in the study. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Institutional Review Board at the University of North Carolina at Greensboro (IRB #17-0287).

2.2. Procedure

Participants completed two separate sets of visits, separated by at least 8 weeks to permit full recovery and adequate time for the replenishment of blood volume (range 8–10 weeks). Within each set of visits, participants completed a screening visit and a profile visit. At the screening visit participants completed a battery of physiological measurements including body composition via air displacement plethysmography and a maximal cycle ergometer test where maximal oxygen uptake (VO2max) was determined by indirect calorimetry (TrueOne 2400, ParvoMedics, Sandy, UT, USA).

Within a week of these screening visits, participants reported to the laboratory again for the 24-h profile. Participants arrived at the laboratory at 05:30 AM and an intravenous catheter was placed in the forearm. As part of a larger study, starting at 06:00 AM blood samples were taken at 10 min intervals (Q10) for the next 24 h (144 samples) via syringe using stopcock valves in the i.v. tubing. A saline (0.9% NaCl) drip (60 mL/h) was used to maintain line and canula patency. Saliva samples were obtained via passive drool at 06:00 AM and every 2 h thereon for the entire 24-h period. Participants retired to the sleep room at 10:30 PM where no electronic devices or other light sources were permitted and were encouraged to sleep.

Participants completed both an exercise and a control profile visit, in a randomized order. Prior to each profile visit, participants were instructed to refrain from any exercise in the 24 h prior to the visit. Participants recorded food and fluid intake during the week between the screening and profile visit, and were asked to replicate this, to the best of their ability, at the second set of visits. At the control visit, participants were permitted to complete any sedentary activities during the waking hours as desired, including reading, watching movies, or talking. Participants were required to eat breakfast, lunch, and dinner at 07:30 AM, 01:00 PM, and 08:00 PM, respectively, and were restricted to water intake only from 08:00 AM to 10:00 AM to standardize macronutrient intake prior to exercise, due to the impact of carbohydrate availability on the cortisol response to exercise [33,34].

In the exercise condition, participants completed a high-intensity sprint protocol on an electromagnetically braked cycle ergometer (Lode Excalibur Sport; Lode BV, Groningen, the Netherlands), beginning at exactly 10:00 AM (immediately following the 10:00 AM blood and saliva samples). Participants warmed-up on the cycle ergometer by cycling at a self-selected pace for 5 min and completing 2–3 high-cadence 5–10 s bouts. Participants then completed 5 sets of 30 s sprints, separated by 2.5 min of active recovery. For each sprint, participants were required to build to a maximal cadence prior to the researcher applying a resistance to the ergometer flywheel equal to 7.5% of participant body mass. Participants were then required to maintain as high a cadence as possible for the 30 s period, while receiving verbal encouragement from the research staff. This cycling protocol elicited a supramaximal power output (mean power of all repetitions = 490.0 ± 93.8 W, 6.9 ± 0.8 W/kg). This protocol was chosen to stimulate a significant physiological response across the hypothalamic–pituitary axis.

Blood (Q60) and saliva (Q120) samples were assayed for cortisol using commercially available competitive binding ELISA assays (R&D Systems). For consistency, all samples for a single individual were assayed on a single plate. The inter-assay and intra-assay CV were 20.3% and 10.1%, respectively, which were both within reported manufacturer ranges.

2.3. Statistical Analytic Plan

Mixed-effect growth models with random intercepts were first used to characterize the profile of serum cortisol (C_{ser}) and salivary cortisol (C_{sal}) cortisol separately. Profiles were first modeled for stationary, linear, quadratic, and cubic trends and model fits were determined by Bayesian Information Criterion (BIC) and change in -2LL. Models were then further assessed for improvements in fit using a continuous autoregressive correlation structure. Following the determination of the best fitting profile model, profile condition (rest or exercise) was included as a fixed effect and improvement in model fit was determined. Next, a simple Pearson correlation between C_{ser} and C_{sal} was computed for each time point throughout the profile.

The proportion (C_{prop}) of serum cortisol (C_{ser}) represented by salivary cortisol (C_{sal}) was calculated as [$C_{prop} = C_{sal}/C_{ser} \times 100$]. C_{prop} was assessed for normality and then log-transformed. The profile of C_{prop} was then assessed in the same manner as C_{ser} and C_{sal}. Following determination of the best fitting profile model, condition (rest or exercise) was included as a fixed effect. C_{ser}, C_{sal}, and C_{prop} were all centered at 10:00 AM (start of exercise) and alpha level was set at $p < 0.05$ for all statistical inference tests.

3. Results

C_{ser} profiles demonstrated significantly improved model fit with a cubic model with continuous autoregressive correlation structure ($R^2 = 0.30$, Figure 1A). Because serum (Q60) and saliva (Q120) were sampled at different frequencies, in the post-hoc analysis serum was also modeled using only samples from the equivalent Q120 time points. These additional models also demonstrated the best fitting model was a cubic model with an autoregression correlation structure. Controlling for exercise condition did not significantly improve model fit ($p = 0.26$). Post-hoc analysis demonstrated that exercise condition also did not significantly interact with the linear ($\beta = 7.2 \times 10^{-3}$, $p = 0.99$), quadratic ($\beta = 6.0 \times 10^{-3}$, $p = 0.91$), or cubic ($\beta = 3.5 \times 10^{-4}$, $p = 0.89$) component of the C_{ser} profile model.

The C_{sal} profile demonstrated improved model fit only with the quadratic continuous autoregressive correlation structure ($R^2 = 0.28$, Figure 1B). The addition of a cubic term did not significantly improve model fit ($p = 0.71$). Model fit did not significantly improve after controlling for exercise condition ($p = 0.37$). Post-hoc analysis also demonstrated that exercise condition did not significantly interact with either the linear ($\beta = -3.2 \times 10^{-2}$, $p = 0.63$) or quadratic ($\beta = -3.4 \times 10^{-2}$, $p = 0.34$) component of the C_{sal} profile model.

The mean (standard deviation) correlation between log transformed C_{ser} and C_{sal} was r = 0.65 (0.17), and was greatest at 08:00 AM (r = 0.90, Figure 2). All correlation coefficients except at 08:00 PM (r = 0.44, $p = 0.09$), 12:00 AM (r = 0.39, $p = 0.15$), and 02:00 AM (r = 0.44, $p = 0.09$) were significant (all others: $p < 0.05$).

The grand mean of C_{prop} was 5.95% (group mean range: 3.93–8.93%). C_{prop} profiles demonstrated improved model fit with a cubic model with continuous autoregressive correlation structure (Conditional $R^2 = 0.17$, Figure 3). Controlling for exercise condition did not significantly improve model fit ($p = 0.91$). Post-hoc analysis demonstrated that exercise condition did not significantly interact with the linear ($\beta = -4.9 \times 10^{-3}$, $p = 0.65$), quadratic ($\beta = -2.2 \times 10^{-4}$, $p = 0.73$), or cubic ($\beta = -1.4 \times 10^{-5}$, $p = 0.67$) component of the C_{prop} profile model.

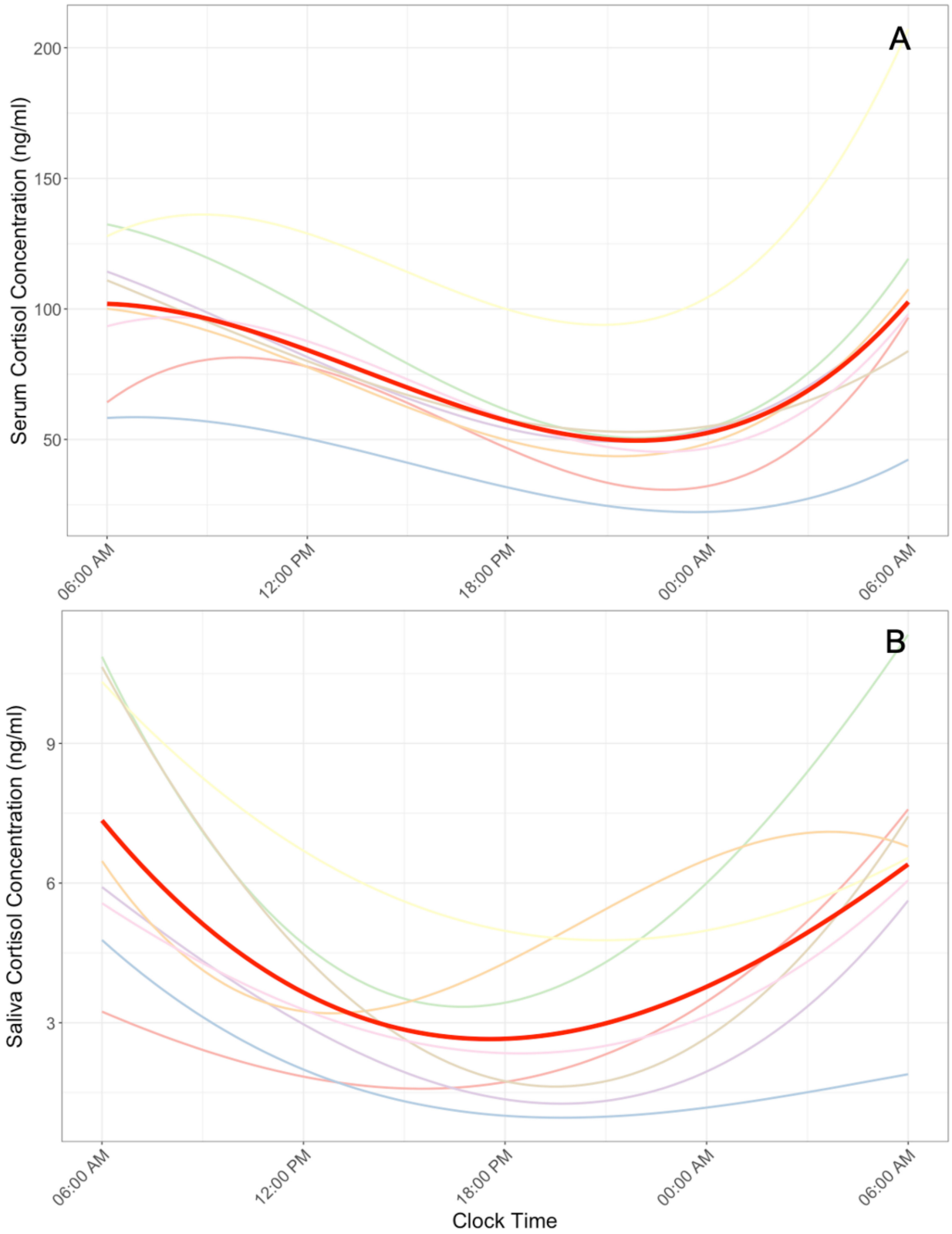

Figure 1. Serum (**A**) and saliva (**B**) cortisol profiles across 24-h. The serum and saliva profiles were best characterized via a cubic and quadratic model, respectively. Mean profiles are depicted in red.

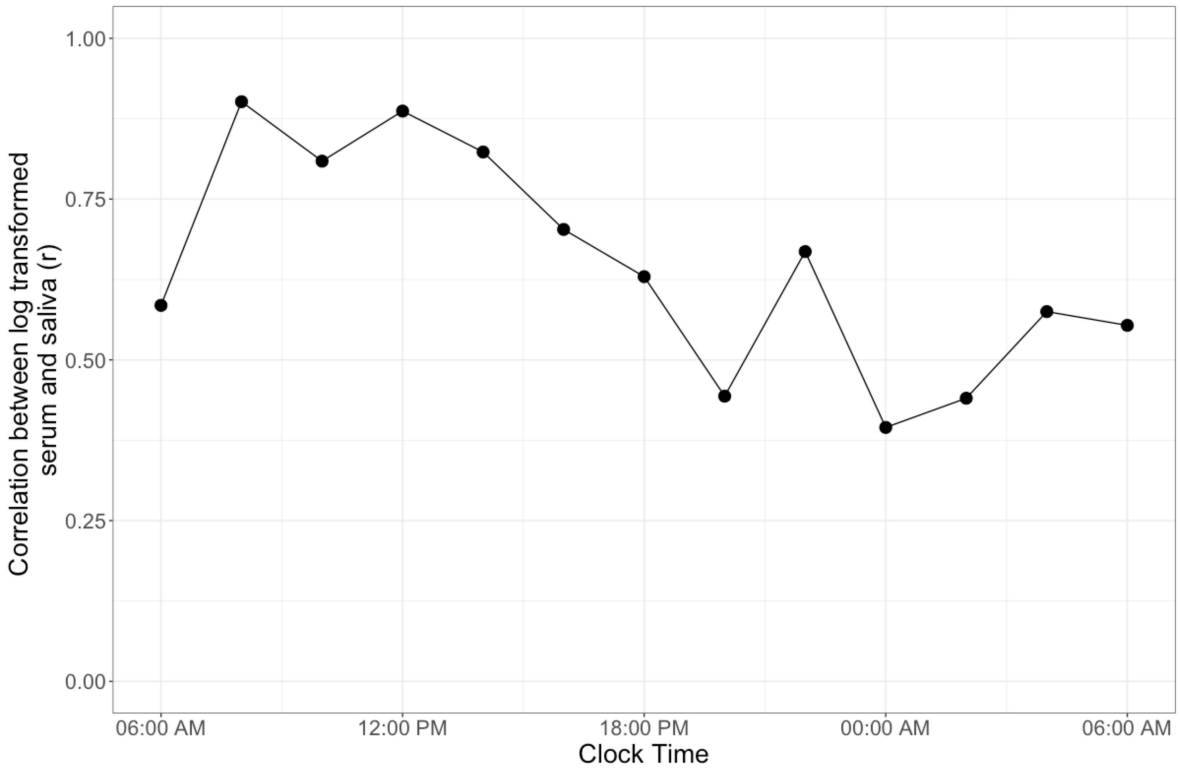

Figure 2. The correlation of log transformed saliva and serum cortisol across the 24-h profile. The correlation was greatest at 0800 (r = 0.90) but demonstrated a general decrease across the remainder of the profile.

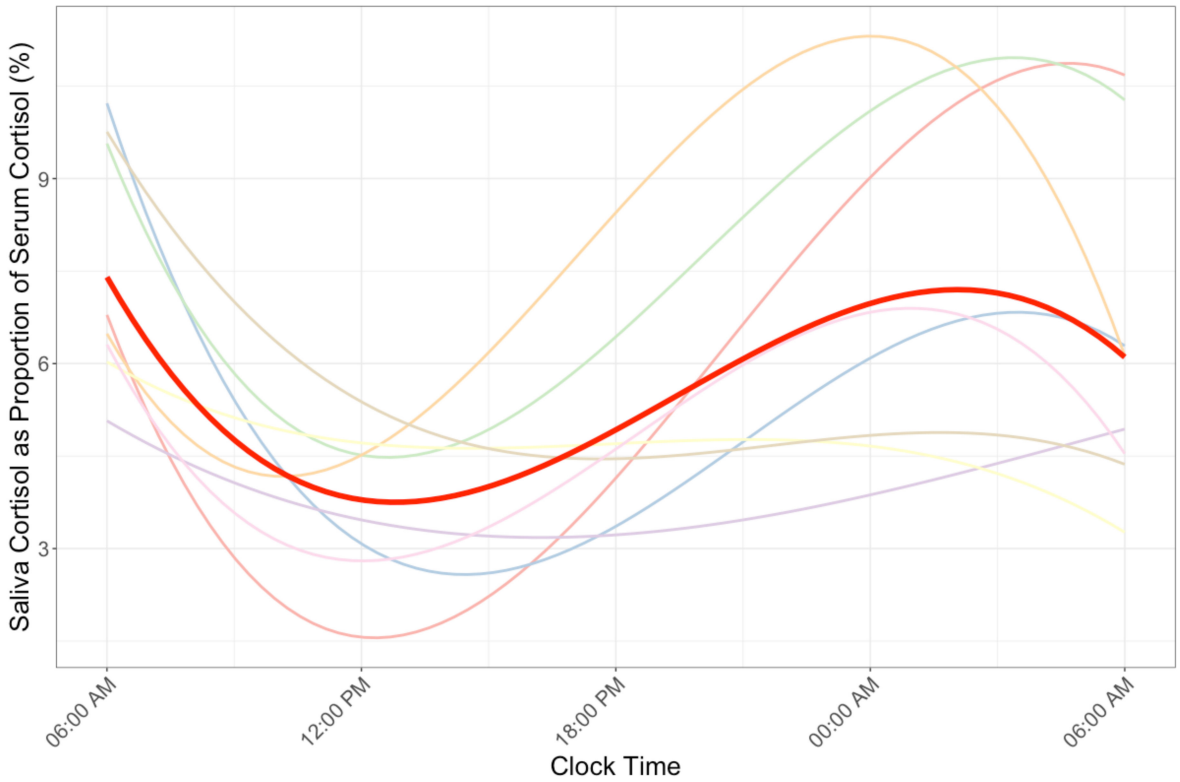

Figure 3. Saliva cortisol as a proportion of serum cortisol across the 24-h profile. The best fitting model demonstrated a cubic circadian rhythm. The mean profile is depicted in red.

4. Discussion

The purpose of this study was to assess the nature of any circadian rhythm to the proportion (C_{prop}) of serum total cortisol (C_{ser}) as represented by salivary cortisol (C_{sal}), and to investigate whether any such rhythm was altered by mid-morning high-intensity exercise. The present results demonstrate a circadian rhythm in C_{prop} that was best characterized by a cubic growth model and appears to follow the general trend of the circadian and diurnal profile of cortisol itself. Moreover, it appears that the variability in this proportion is significantly greater during the nocturnal period, which is also characterized by a reduction in the correlation between cortisol concentrations from these two biological fluids. Lastly, the results do not support the hypothesis that high-intensity exercise in the mid-morning period affects the circadian rhythm of C_{ser}, C_{sal}, or C_{prop}.

The free portion of cortisol is able to pass into the salivary gland and then the saliva, and thus it is believed that C_{sal} represents the free portion of the total C_{ser}. The circadian rhythm observed in C_{sal} in the present study may therefore reasonably represent a circadian rhythm in the amount of free cortisol in circulation. A majority of cortisol is bound by CBG, which has a half-life of approximately 10 h [35]. Given this relatively static concentration of the binding protein and thus cortisol binding capacity, changes in the total cortisol concentration would lead to alterations in the free cortisol concentration. This may explain the reduction in the C_{prop} as total cortisol decreased across the diurnal period, and increases in C_{prop} when total cortisol concentrations in C_{ser} are expected to be greatest. Of particular note, one participant had higher cortisol levels compared to other participants, but cortisol concentrations were higher in both serum and saliva, thus not affecting the analysis of C_{prop} for this individual.

It is also conceivable, however, that other factors that affect the free portion of cortisol beyond the total cortisol concentration contribute to the observed C_{prop} profile. There is some evidence to suggest that while concentrations of CBG do not vary substantially, there is a circadian rhythm to CBG concentrations [32], and thus the binding capacity fluctuates throughout a 24-h period. Further, CBG affinity for cortisol is known to be highly temperature sensitive [36]. Core body temperature also demonstrates a robust circadian rhythm that is integral to sleep onset [37]; given the tight thermocouple of cortisol and CBG, decreases in core body temperature during the nocturnal period may impact free cortisol and thus the C_{prop}. This in vitro temperature dependence of CBG should be investigated further both for understanding HPA-axis circadian rhythms and regulation, as well as potentially being an elegant local targeting mechanism [30], particularly during exercise. The apparent mismatch between serum total and salivary free cortisol that generates the cubic model across time for C_{prop} suggests that, for at least some portion(s) of the circadian profile, the cross-correlation between these measures should be greatest at a lag >1 unit. In fact, researchers recently reported a maximal cross-correlation between serum cortisol and subcutaneous free cortisol of five units (50 min) [38]. We therefore completed post-hoc analyses in which we computed the cross-correlation of the differenced log transformed serum and saliva values across the 24-h period. This analysis demonstrated a maximal correlation at a 4-h lag, possibly contributing to the profile in C_{prop}. However, this should be investigated further in saliva using more frequent sampling procedures (e.g., Q30) or via the promising microanalysis technique for ambulatory free cortisol monitoring [39].

Salivary glands may also exhibit circadian properties that modify the C_{prop} value over time. For example, salivary glands have a high concentration of 11β-HSD2, an enzyme that readily converts cortisol to cortisone [40]. The assay used in the present study reports a cross-reactivity with cortisone of only 0.2%, and thus it is possible that the large variance in C_{prop} observed in the early morning period was due to differences in the conversion of cortisol to cortisone. Indeed, in one of only several studies of circadian relations between serum and saliva steroids, a higher correlation was reported to salivary cortisone than salivary cortisol [39]. Although cortisol does not appear to be flow dependent and cross-correlations have demonstrated a similar lag (e.g., ~10 min) between serum adrenocorticotropin hormone and C_{ser} and C_{sal} [41], saliva production is markedly reduced

during the evening period [42], providing another potential confounding factor. In sum, due to these considerations, C_{sal} may not always accurately represent free cortisol in circulation across the circadian profile.

The lack of effect of this high-intensity exercise protocol may be a result of several factors. First, and most importantly, the exercise session was likely not of sufficient duration to elicit an acute cortisol response, and thus has minimal effect on the post-exercise period. Although the intensity of the activity was supramaximal relative to the power output achieved during the VO2max test, the participants only engaged in this activity for a total of 2.5 min, with an additional 18 min of low-intensity active recovery between these efforts. Cortisol has a known intensity [43] and duration [44] threshold for acute responses. Despite the intensity of the exercise meeting this threshold, the total work of the exercise session was not likely great enough to cause sufficient metabolic and thermoregulatory disturbances to dramatically increase cortisol output. Secondly, the circadian rhythm of the HPA-axis and cortisol specifically is robust and plays a role in the circadian regulation of a variety of other cells and systems [45]. It is possible that such a robust circadian profile requires a greater perturbation than the exercise session employed here. Lastly, the effect of the exercise employed in this specific study on the cortisol dynamics may be small and/or variable, and was therefore unable to be statistically detected with a relatively small sample size.

Although this study provides novel insights into the utility of salivary cortisol and its interpretation, there are several limitations to acknowledge. As discussed, the sample size was small (n = 8), and thus the present analyses may be underpowered. Notably however, since this is a repeated measures, randomized control study, it is important to highlight that each participant contributed 48 blood samples and 24 saliva samples across two 24 h periods to these analyses, underscoring the logistical and financial challenge of conducting research of this kind. Larger studies with more frequent sampling should be conducted in the future while understanding these challenges. Secondly, participants were awoken to provide the saliva samples during the night sample collection (the blood draws were completed with care to not wake the participant). Disrupting the sleep cycle in this manner was a methodological choice that may have perturbed the regular HPA-axis activity. There is a need therefore to devise saliva collection devices that can be used without disrupting regular sleep. Lastly, the exercise protocol employed, whilst of a maximal intensity in the work interval, likely did not elicit a robust enough metabolic or thermoregulatory challenge to the participants to disrupt the underlying circadian cortisol profile. Attempts should be made to replicate these findings while employing an exercise protocol specifically structured to maximize the acute cortisol response to exercise. Future studies should also analyze salivary cortisone in addition to C_{ser} and C_{sal}, as an indirect measure of 11β-HSD2 activity across the circadian profile.

5. Conclusions

These results demonstrate that the collection of saliva for the quantification of cortisol is a useful, non-invasive method, but has essential considerations if being used to describe the circadian rhythm of the HPA-axis. Many researchers are aware of the need to standardize the time of day for sample collection to account for the circadian rhythm of cortisol. However, it appears that change in salivary cortisol does not linearly scale across a 24-h period with total circulating cortisol, and so practitioners and researchers should also control for the time of day when making inferences to the total cortisol concentration. Finally, saliva collection via passive drool may not be an appropriate fluid for examining cortisol during sleep periods due to its low correlation with total circulating cortisol.

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Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data presented in this study are available on reasonable request from the corresponding author.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. De Kloet, E.R.; Van Acker, S.A.; Sibug, R.M.; Oitzl, M.S.; Meijer, O.C.; Rahmouni, K.; de Jong, W. Brain Mineralocorticoid Receptors and Centrally Regulated Functions. *Kidney Int.* **2000**, *57*, 1329–1336. [CrossRef]
2. Hellman, L.; Nakada, F.; Curti, J.; Weitzman, E.D.; Kream, J.; Roffwarg, H.; Ellman, S.; Fukushima, D.K.; Gallagher, T.F. Cortisol Is Secreted Episodically by Normal Man. *J. Clin. Endocrinol. Metab.* **1970**, *30*, 411–422. [CrossRef]
3. Weitzman, E.D.; Fukushima, D.; Nogeire, C.; Roffwarg, H.; Gallagher, T.F.; Hellman, L. Twenty-Four Hour Pattern of the Episodic Secretion of Cortisol in Normal Subjects. *J. Clin. Endocrinol. Metab.* **1971**, *33*, 14–22. [CrossRef]
4. Nader, N.; Chrousos, G.P.; Kino, T. Interactions of the Circadian CLOCK System and the HPA Axis. *Trends Endocrinol. Metab.* **2010**, *21*, 277–286. [CrossRef]
5. Oster, H.; Challet, E.; Ott, V.; Arvat, E.; de Kloet, E.R.; Dijk, D.-J.; Lightman, S.; Vgontzas, A.; Van Cauter, E. The Functional and Clinical Significance of the 24-Hour Rhythm of Circulating Glucocorticoids. *Endocr. Rev.* **2016**, *38*, 3–45. [CrossRef]
6. Andreani, T.S.; Itoh, T.Q.; Yildirim, E.; Hwangbo, D.-S.; Allada, R. Genetics of Circadian Rhythms. *Sleep Med. Clin.* **2015**, *10*, 413–421. [CrossRef]
7. Spencer, R.L.; Chun, L.E.; Hartsock, M.J.; Woodruff, E.R. Glucocorticoid Hormones Are Both a Major Circadian Signal and Major Stress Signal: How This Shared Signal Contributes to a Dynamic Relationship between the Circadian and Stress Systems. *Front. Neuroendocr.* **2018**, *49*, 52–71. [CrossRef]
8. Adam, E.K.; Quinn, M.E.; Tavernier, R.; McQuillan, M.T.; Dahlke, K.A.; Gilbert, K.E. Diurnal Cortisol Slopes and Mental and Physical Health Outcomes: A Systematic Review and Meta-Analysis. *Psychoneuroendocrinology* **2017**, *83*, 25–41. [CrossRef]
9. Dunn, J.F.; Nisula, B.C.; Rodbard, D. Transport of Steroid Hormones: Binding of 21 Endogenous Steroids to Both Testosterone-Binding Globulin and Corticosteroid-Binding Globulin in Human Plasma. *J. Clin. Endocrinol. Metab.* **1981**, *53*, 58–68. [CrossRef]
10. Levine, A.; Zagoory-Sharon, O.; Feldman, R.; Lewis, J.G.; Weller, A. Measuring Cortisol in Human Psychobiological Studies. *Physiol. Behav.* **2007**, *90*, 43–53. [CrossRef]
11. Mendel, C.M. The Free Hormone Hypothesis: A Physiologically Based Mathematical Model. *Endocr. Rev.* **1989**, *10*, 232–274. [CrossRef]
12. Hackney, A.C. Stress and the Neuroendocrine System: The Role of Exercise as a Stressor and Modifier of Stress. *Expert Rev. Endocrinol. Metab.* **2006**, *1*, 783–792. [CrossRef] [PubMed]
13. VanBruggen, M.D.; Hackney, A.C.; McMurray, R.G.; Ondrak, K.S. The Relationship between Serum and Salivary Cortisol Levels in Response to Different Intensities of Exercise. *Int. J. Sports Physiol. Perform.* **2011**, *6*, 396–407. [CrossRef]
14. Hough, J.; Corney, R.; Kouris, A.; Gleeson, M. Salivary Cortisol and Testosterone Responses to High-Intensity Cycling before and after an 11-Day Intensified Training Period. *J. Sports Sci.* **2013**, *31*, 1614–1623. [CrossRef] [PubMed]
15. Hackney, A.C.; Ness, R.J.; Schrieber, A. Effects of Endurance Exercise on Nocturnal Hormone Concentrations in Males. *Chronobiol. Int.* **1989**, *6*, 341–346. [CrossRef]
16. Aardal, E.; Holm, A.-C. Cortisol in Saliva-Reference Ranges and Relation to Cortisol in Serum. *Eur. J. Clin. Chem. Clin. Biochem.* **1995**, *33*, 927–932. [CrossRef]
17. Reid, J.D.; Intrieri, R.C.; Susman, E.J.; Beard, J.L. The Relationship of Serum and Salivary Cortisol in a Sample of Healthy Elderly. *J. Gerontol.* **1992**, *47*, P176–P179. [CrossRef]
18. Cadore, E.; Lhullier, F.; Brentano, M.; Silva, E.; Ambrosini, M.; Spinelli, R.; Silva, R.; Kruel, L. Correlations between Serum and Salivary Hormonal Concentrations in Response to Resistance Exercise. *J. Sports Sci.* **2008**, *26*, 1067–1072. [CrossRef]
19. Gozansky, W.; Lynn, J.; Laudenslager, M.; Kohrt, W. Salivary Cortisol Determined by Enzyme Immunoassay Is Preferable to Serum Total Cortisol for Assessment of Dynamic Hypothalamic–Pituitary–Adrenal Axis Activity. *Clin. Endocrinol.* **2005**, *63*, 336–341. [CrossRef]
20. O'Connor, P.J.; Corrigan, D.L. Influence of Short-Term Cycling on Salivary Cortisol Levels. *Med. Sci. Sports Exerc.* **1987**, *19*, 224–228.
21. Paccotti, P.; Minetto, M.; Terzolo, M.; Ventura, M.; Ganzit, G.; Borrione, P.; Termine, A.; Angeli, A. Effects of High-Intensity Isokinetic Exercise on Salivary Cortisol in Athletes with Different Training Schedules: Relationships to Serum Cortisol and Lactate. *Int. J. Sports Med.* **2005**, *26*, 747–755. [CrossRef]
22. Stupnicki, R.; Obminski, Z. Glucocorticoid Response to Exercise as Measured by Serum and Salivary Cortisol. *Eur. J. Appl. Physiol. Occup. Physiol.* **1992**, *65*, 546–549. [CrossRef] [PubMed]

23. Vining, R.F.; McGinley, R.A.; Maksvytis, J.J.; Ho, K.Y. Salivary Cortisol: A Better Measure of Adrenal Cortical Function than Serum Cortisol. *Ann. Clin. Biochem.* **1983**, *20*, 329–335. [CrossRef]
24. Umeda, T.; Hiramatsu, R.; Iwaoka, T.; Shimada, T.; Miura, F.; Sato, T. Use of Saliva for Monitoring Unbound Free Cortisol Levels in Serum. *Clin. Chim. Acta* **1981**, *110*, 245–253. [PubMed]
25. Vogeser, M.; Möhnle, P.; Briegel, J. Free Serum Cortisol: Quantification Applying Equilibrium Dialysis or Ultrafiltration and an Automated Immunoassay System. *Clin. Chem. Lab. Med.* **2007**, *45*, 521–525. [CrossRef]
26. Pretorius, C.J.; Galligan, J.P.; McWhinney, B.C.; Briscoe, S.E.; Ungerer, J.P. Free Cortisol Method Comparison: Ultrafiltation, Equilibrium Dialysis, Tracer Dilution, Tandem Mass Spectrometry and Calculated Free Cortisol. *Clin. Chim. Acta* **2011**, *412*, 1043–1047. [CrossRef]
27. Kirchhoff, F.; Briegel, J.; Vogeser, M. Quantification of Free Serum Cortisol Based on Equilibrium Dialysis and Isotope Dilution-Liquid Chromatography–Tandem Mass Spectrometry. *Clin. Biochem.* **2011**, *44*, 894–899. [CrossRef]
28. Vogeser, M.; Briegel, J. Effect of Temperature on Protein Binding of Cortisol. *Clin. Biochem.* **2007**, *40*, 724–727. [CrossRef]
29. Kirschbaum, C.; Hellhammer, D.H. Salivary Cortisol in Psychoneuroendocrine Research: Recent Developments and Applications. *Psychoneuroendocrinology* **1994**, *19*, 313–333. [CrossRef]
30. Henley, D.; Lightman, S. New Insights into Corticosteroid-Binding Globulin and Glucocorticoid Delivery. *Neuroscience* **2011**, *180*, 1–8. [CrossRef]
31. Dorn, L.D.; Lucke, J.F.; Loucks, T.L.; Berga, S.L. Salivary Cortisol Reflects Serum Cortisol: Analysis of Circadian Profiles. *Ann. Clin. Biochem.* **2007**, *44*, 281–284. [CrossRef]
32. Lewis, J.; Möpert, B.; Shand, B.; Doogue, M.; Soule, S.; Frampton, C.; Elder, P. Plasma Variation of Corticosteroid-Binding Globulin and Sex Hormone-Binding Globulin. *Horm. Metab. Res.* **2006**, *38*, 241–245. [CrossRef]
33. Bishop, N.C.; Gleeson, M.; Nicholas, C.W.; Ali, A. Influence of Carbohydrate Supplementation on Plasma Cytokine and Neutrophil Degranulation Responses to High Intensity Intermittent Exercise. *Int. J. Sport Nutr. Exerc. Metab.* **2002**, *12*, 145–156. [CrossRef]
34. Galbo, H.; Holst, J.; Christensen, N. The Effect of Different Diets and of Insulin on the Hormonal Response to Prolonged Exercise. *Acta Physiol. Scand.* **1979**, *107*, 19–32. [CrossRef]
35. Lewis, J.G.; Saunders, K.; Dyer, A.; Elder, P.A. The Half-Lives of Intact and Elastase Cleaved Human Corticosteroid-Binding Globulin (CBG) Are Identical in the Rabbit. *J. Steroid Biochem. Mol. Biol.* **2015**, *149*, 53–57. [CrossRef]
36. Cameron, A.; Henley, D.; Carrell, R.; Zhou, A.; Clarke, A.; Lightman, S. Temperature-Responsive Release of Cortisol from Its Binding Globulin: A Protein Thermocouple. *J. Clin. Endocrinol. Metab.* **2010**, *95*, 4689–4695. [CrossRef]
37. Kräuchi, K.; Wirz-Justice, A. Circadian Clues to Sleep Onset Mechanisms. *Neuropsychopharmacology* **2001**, *25*, S92–S96. [CrossRef]
38. Bhake, R.; Kluckner, V.; Stassen, H.; Russell, G.; Leendertz, J.; Stevens, K.; Linthorst, A.; Lightman, S. Continuous Free Cortisol Profiles—Circadian Rhythms in Healthy Men. *J. Clin. Endocrinol. Metab.* **2019**, *104*, 5935–5947. [CrossRef]
39. Bhake, R.; Russell, G.M.; Kershaw, Y.; Stevens, K.; Zaccardi, F.; Warburton, V.E.; Linthorst, A.C.; Lightman, S.L. Continuous Free Cortisol Profiles in Healthy Men: Validation of Microdialysis Method. *J. Clin. Endocrinol. Metab.* **2020**, *105*, e1749–e1761. [CrossRef] [PubMed]
40. Chapman, K.; Holmes, M.; Seckl, J. 11β-Hydroxysteroid Dehydrogenases: Intracellular Gate-Keepers of Tissue Glucocorticoid Action. *Physiol. Rev.* **2013**, *93*, 1139–1206. [CrossRef] [PubMed]
41. Schlotz, W.; Kumsta, R.; Layes, I.; Entringer, S.; Jones, A.; Wüst, S. Covariance between Psychological and Endocrine Responses to Pharmacological Challenge and Psychosocial Stress: A Question of Timing. *Psychosom. Med.* **2008**, *70*, 787–796. [CrossRef] [PubMed]
42. Dawes, C. Circadian Rhythms in Human Salivary Flow Rate and Composition. *J. Physiol.* **1972**, *220*, 529–545. [CrossRef] [PubMed]
43. Hill, E.; Zack, E.; Battaglini, C.; Viru, M.; Viru, A.; Hackney, A. Exercise and Circulating Cortisol Levels: The Intensity Threshold Effect. *J. Endocrinol. Investig.* **2008**, *31*, 587–591. [CrossRef] [PubMed]
44. Duclos, M.; Corcuff, J.; Rashedi, M.; Fougere, V.; Manier, G. Trained versus Untrained: Different Hypothalamo-Pituitary Adrenal Axis Responses to Exercise Recovery. *Eur. J. Appl. Physiol.* **1997**, *75*, 343–350. [CrossRef]
45. Nicolaides, N.C.; Charmandari, E.; Kino, T.; Chrousos, G.P. Stress-Related and Circadian Secretion and Target Tissue Actions of Glucocorticoids: Impact on Health. *Front. Endocrinol.* **2017**, *8*, 70. [CrossRef]