International Journal of EXERCISE SCIENCE

Original Research

Evaluating the Clinical Utility of Daily Heart Rate Variability Assessment for Classifying Meaningful Change in Testosterone-to-Cortisol Ratio: A Preliminary Study

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ABSTRACT

International Journal of Exercise Science 14(3): 260-273, 2021. The study purpose was to determine the relationship of resting heart rate variability (HRV) and testosterone to cortisol (T:C) ratio, along with the diagnostic ability of HRV to assess changes in T:C ratio during a 9-week high-intensity functional training intervention. Eight recreationally-active men (n = 4, age 24.25 ± 1.75 yrs, height 181.25 ± 3.86 cm, weight 79.68 ± 11.66 kg) and women (n = 4, age 26 ± 3.6 yrs, height 164.25 ± 3.3, weight 73.4 ± 8.42) completed daily HRV measurements (HRVdaily) using photoplethysmography via a commercially-available smartphone application along with weekly saliva samples. Saliva samples were analyzed for concentrations of testosterone (T) and cortisol (C) via enzyme-linked immunosorbent assays. Upon study completion 72 data points were available, due to participant compliance and inadequate saliva sample, 67 matched pairs of HRV and T:C ratio were analyzed. A statistically significant negative relationship (n = 67, r = -.315, p < 0.05) was found between HRVdaily and saliva T:C ratio concentrations within aggregate data. Individual participant relationships showed considerable variability (r = - 0.101 – 0.665, p = 0.103 to 0.829 The model which best explained the data resulted in AIC = 130.247 with factors HRVdaily (β = -0.218, 95%CI = -0.391, -0.044, t = -2.46, p < 0.05), Sex (β = 0.450, 95%CI = -0.214, 1.114, t = 1.113, p = 0.242), and Group (β = -0.394, 95%CI = -1.089, 0.302, t = -1.11, p = 0.311). Diagnostically, HRVdaily demonstrates excellent sensitivity (95%), but poor specificity (5%) for detecting meaningful changes in T:C ratio. Assessment of HRVdaily may be a clinically valid proxy measure for monitoring hormonal changes throughout a training intervention.

KEY WORDS: Athlete monitoring, training adaptation, vagal activity, autonomic nervous system

INTRODUCTION

Exercise training represents a significant perturbation to both the human neuroendocrine and autonomic nervous systems (ANS) (2, 56). Well-planned exercise training programs attempt to balance both acute and chronic training loads (TL) in order to maximize physiological

adaptation and attenuate the risk of maladaptation (6, 16, 21). The imposed stresses from exercise TL can be viewed on a continuum with fatigue anchoring one end and recovery the other (17). As such, poor management of TL due to imbalance between training stresses and recovery may increase the risk of injury or lead to a state non-functional overreaching (5, 37). Conversely, effective TL management results in the adaptation of various physiological systems to a higher fitness level (17, 22). Practitioners have often faced difficulties in maintaining TL balance as individual responses to acute and chronic exercise are unique. Thus, the ability to monitor TL through objective measures may enhance understanding of individual training responses and reduce the risk of maladaptation (16, 21).

Monitoring strategies for quantifying internal TL (i.e., physiological responses to training) include blood lactate concentrations, heart-rate based metrics, and hormonal responses (4, 7, 21). Exercise-induced hormonal responses are controlled by the hypothalamic-pituitary adrenal axis (HPA), a key regulator of homeostasis, which responds to stress by triggering a series of endocrine changes resulting in the release of testosterone (T) and cortisol (C) (26). T is required for protein synthesis and glycogen replenishment, while C inhibits protein synthesis and can lead immunosuppression (31). Thus, the testosterone-to-cortisol ratio (T:C) can be viewed as the balance between anabolic to catabolic processes (i.e., recovery status/adaptation status) (50, 55) and has been proposed to monitor internal TL, fatigue, training stress, and adaptation (20, 33). A decrease in the T:C ratio ≥ 30% has been related to incomplete recovery from training (3). The T:C ratio is influenced by training volume (35, 50) and intensity (34, 36), often decreasing with fatigue (54). Training programs that have modulated training based on changes in the T:C ratio have shown enhanced performance outcomes in both individual (e.g., sprinting, throwing) and team sports (e.g., soccer) (23, 51). Thus, the T:C ratio may provide additional insight in both acute & chronic TL-induced stress. Despite its utility, regular assessment of T:C ratio is often infeasible in practical settings as it requires invasive serum or saliva collection and analysis (53).

A promising non-invasive tool, heart rate variability (HRV), monitors internal TL and adaptation via the ANS and provides valuable insight into fatigue monitoring (1, 32, 42). HRV is estimated by measuring the time intervals between successive heartbeats, where an increase or decrease in these intervals reflects cardiac parasympathetic activity (32). Previous studies demonstrate that HRV is sensitive to individual variation in adaptation, fatigue, and overload during exercise training programs (13, 14, 42). Current assumptions are that training maladaptation is associated with reductions in cardiac parasympathetic activity and a decrease in HRV (42). Conversely, improved fitness is associated with increased cardiac parasympathetic activity and HRV (42). Unlike the T:C ratio, HRV appears to provide an easily assessed objective measure for evaluating fatigue when manipulating training prescriptions (28, 42).

As the ANS and HPA work in tandem to respond to disrupted homeostatic processes, measuring stress responses from exercise training via the highly coordinated and interconnected ANS and HPA pathways (45, 48). Due to the complex integration of these systems, to date, no single definitive marker can accurately quantify the fitness and fatigue responses to training (4, 6). Currently, the relationship between the ANS and hormonal balance throughout a multimodality exercise program such as high-intensity functional training (HIFT) is not well

understood (11). It has yet to be demonstrated if HRV and T:C ratio can identify fatigue in parallel with one another during HIFT. Therefore, the purposes of this study was to determine the relationship between daily resting HRV and pre-exercise T:C ratio and evaluate the clinical utility (i.e., diagnostic validity and reliability) of daily HRV assessment in classifying atypical T:C ratio changes throughout a nine-week HIFT intervention. It was hypothesized that HRV and T:C ratio would have a significant positive relationship and the daily HRV would be a valid surrogate measure for the body's hormonal status.

METHODS

Participants

Eight recreationally active men and women ages 18-35 were recruited for participation in the present study. All participants were currently regularly exercising, but not pursuing any specific health or fitness goal (e.g., weight loss or competition preparation) for at least six months prior to study commencement. All participants had previous experience with aerobic (9.1 ± 3.4 years) and resistance training (5.9 ± 3.4 years). All participants were considered a novice in regard to their experience with HIFT. Participant demographic characteristics are presented in Table 1. All participants were free of any physical or health limitations that might indicate a contraindication for vigorous exercise as determined by a medical history questionnaire and physical activity readiness questionnaire (PAR-Q) (52). Additionally, no participants reported taking any medications or having any physical conditions that could influence HRV. This investigation was approved by the University's Institutional Review Board (#9131) and all participants provided written informed consent prior to study commencement. This research was carried out fully in accordance with the ethical standards of the International Journal of Exercise Science (38).

Table 1. Participant characteristics.

	Men (n = 4)	Women (n = 4)
Age	24.25 ±1.75	26.00 ±3.60
Weight (kg)	79.68 ±11.66	73.4 ±8.42
Height (cm)	181.25 ±3.86	164.25 ±3.30
rHR	61.29 ±7.75	68.05 ± 6.30
LnRMSSD	8.67 ±0.63	8.86 ±1.50
T (nmol/L)	1.06 ±0.42	0.51 ±0.23
C (nmol/L)	10.55 ±6.62	9.77 ±7.32
T:C ratio (nmol/L)	0.14 ±0.12	0.08 ±0.08
LnT:C	-2.23 ±0.74	-2.75 ±0.74

rHR – resting heart rate, LnRMSSD – log of root mean squared of standard deviation, LnT:C – log of testosterone to cortisol.

Protocol

This study was a secondary analysis of a subset of participants from a larger study (8). Participants completed baseline demographic information and fitness testing prior to the start of a HIFT intervention. Following 14 days of baseline HRV assessments, participants were

randomized to either the treatment or control condition. Participants began the 60-minute exercise intervention sessions for five consecutive days (Monday-Friday) with two days of recovery (Saturday & Sunday). Two three-week training periods were interspersed between pre, mid and post-performance evaluation weeks, for a total of nine weeks (9). Full description of study intervention is provided in appendix table A1. Participants within the control condition completed all training sessions as prescribed. For participants within the treatment condition, training volume and intensity were manipulated based on potential meaningful shifts (SWC) in resting HRV as previously established (27, 28, 42). A participant HRV falling outside their SWC1 reduced their work volume and external load (i.e., absolute weight used) by 25%. A participant HRV falling outside their SWC2 completed an active recovery session (e.g., walking and light stretching) for 20 minutes (9). Participant condition was balanced so that two males and two females were within each condition. Three training times were offered each day to accommodate participant schedules, and participants were asked to attend the same training time throughout the intervention. Throughout the intervention all participants completed daily resting HRV measurements and provided weekly pre-exercise saliva samples each Friday.

Heart rate variability (HRV): Daily HRV measurements were taken via a commercially available smartphone application for both iOS and Android software HRV4Training (Amsterdam, Netherlands; seehttp://www.hrv4training.com/). The software utilized photoplethysmography to determine variability in R-R intervals from continuous heart rate data (43). In order to maintain HRV reliability, participants were instructed to, upon waking, empty their urinary bladder and return to a supine position before initiating the reading. Participant HRV was assessed in the supine position for one minute with the index finger covering the smartphone camera and the respiration rate set at 15 breaths per minute (10, 43). The methodology used for signal filtering, processing, interpolation, artifact correction, and R-R peak detection are detailed in the original reference for the applications development (43). HRV was collected for two weeks prior to the start of any testing protocol in order to establish a baseline. For day-to-day monitoring of individual recovery status (i.e., sympathovagal balance), the root mean squared of successive differences (RMSSD) of R-R intervals was used as it appears to be less influenced by breathing rate and could be assessed validly in only one minute (10, 12, 15, 42, 49). Due to the lack of normality, RMSSD was transformed using the natural logarithm (LnRMSSD), it was then multiplied by two so that LnRMSSD (HRVdaily) could be viewed on a scale of approximately six to ten for interpretation purposes and reflect application readout (57). A meaningful change in LnRMSSD was determined though smallest worthwhile change (SWC) in participants' seven-day rolling average. The SWC was set as ± 0.5 standard deviations from an individual's rolling seven-day LnRMSSD (27, 28, 41). Participants continued to measure their HRVdaily for the duration of the study following previously established protocols (9). Participants were asked to complete a total of 75 HRVdaily readings throughout this investigation, the cohort averaged a 93.3% adherence to the HRVdaily readings.

Saliva Collection and Analysis: Participants provided saliva samples 15 minutes prior to their training sessions each Friday. Approximately 2 mL of saliva was collected via an oral swab (Salimetrics LLC, State College, PA, USA) and stored in a swab storage tube at -20°C until assay. A total of 70 samples were collected across the eight participants; one sample was lost due to

insufficient saliva collection. Participants provided their pre-exercise saliva sample within 5 minutes of starting the standardized 10-minute warm-up. Concentrations of testosterone and cortisol were analyzed via a commercially available, enzyme-linked immunosorbent assay (ELISA) (Salimetrics LLC, State College, PA, USA), following the manufacturer's guidelines. All samples were analyzed in duplicate with an average coefficient of variation (CV) of (4.766%) for testosterone and (5.285%) for cortisol. A 30% or greater decrease in T:C was deemed meaningful and was determined "at risk" (3). T:C values were compared on a weekly-to-week basis.

The exercise intervention employed within this study followed a popular, community-based HIFT template (19). All training sessions were conducted as group exercise within the Functional Intensity Training Laboratory (FIT Lab) at Kansas State University. Specific details of the structure and components of each daily training session can be found in Table A1 within the previously published work by Crawford et al. (8). All training days included an instructor-led warm-up; a brief movement preparation period, daily workout, and a cool-down lasting a total of approximately one hour in duration. Thirty training sessions were programmed for participants to complete, with an adherence rate of 80% required for data inclusion. Additionally, participants were asked to not engage in any exercise training outside of the intervention.

Statistical Analysis

Data were analyzed using the R statistical computing environment and language (v. 4.0; R Core Team, 2019) via the Jamovi graphical user interface (47). Data for HRV and T:C ratio were only analyzed if a daily matched pair existed; of the 72 total time points, five were missing a matched pair resulting in an analysis of 67 time points. The HRV and T:C ratio data were checked for normality (Shapiro-Wilk test). The T:C ratio was transformed using the natural log method (lnTC) prior to statistical analysis due to excessive skewness (2.27 ± 0.28) of these data (35). Relationships between HRV and salivary hormone data were assessed using linear mixed-effects models via the GAMLj: General analyses for linear models module (18). Potential fixed effects covariates (sex and treatment group) in addition to random effects of time and the individual participant were explored. A model comparison approach was employed using the Akaike Information Criterion (AIC) goodness-of-fit metric to identify an alternative model that best explained the data for each relationship of interest (25). Missing data were treated using pairwise (i.e., available case) analyses and the resulting number of observations for each specific analysis is reported in the results section. An alpha level of 0.05 was used for all statistical inferences. Post hoc assessments were adjusted using the Bonferroni correction. A 2x2 table was constructed to allow the development of estimates for diagnostic validity (e.g., sensitivity and specificity) and reliability (e.g., positive and negative predictive value) of HRVdaily for detecting atypical changes (i.e., >30%) T:C ratio concentration (46). A posthoc power analysis conducted using G*Power 3.1 (Universität Kiel, Germany) determined that with 67 collected samples we achieved 93% statistical power for testing the relationship between resting heart rate and HRV, 80% statistical power for HRV and T:C ratio, and 98% statistical power for HRV, T and C relationships.

RESULTS

A statistically significant negative relationship was observed between HRVdaily and the T:C ratio ($p < 0.05$, $R^2 = -.315$) (Figure 1B). The model which best explained the data resulted in AIC = 130.247 with factors HRVdaily (β = -0.218, 95%CI = -0.391, -0.044, $t = -2.46$, $p < 0.05$), Sex (β = 0.450, 95%CI = -0.214, 1.114, $t = 1.113$, $p = 0.242$), and Group (β = -0.394, 95%CI = -1.089, 0.302, $t = -1.11$, $p = 0.311$). A significant main effect for sex (mean difference = -7.18, 95%CI = 2.2, 11.9, $t = 2.88$, $p < 0.05$) was observed in the rHR and HRVdaily relationship ($R^2 = .248$, $t = 2.88$, difference = 7.18, $p < 0.05$) (Figure 1A). There was no significant relationship found between C and HRVdaily ($R^2 = .249$, $t = -1.76$, difference = -5.29, $p = 0.167$) (Figure 1C), and neither sex nor group were significant factors. A significant main effect for sex (mean difference = -6.82, 95%CI = .72, 1.07, $t = -6.82$, $p < 0.05$) was observed for the T and HRVdaily relationship ($R^2 = .42$, $t = -0.56$, $p < 0.05$) (Figure 1D).

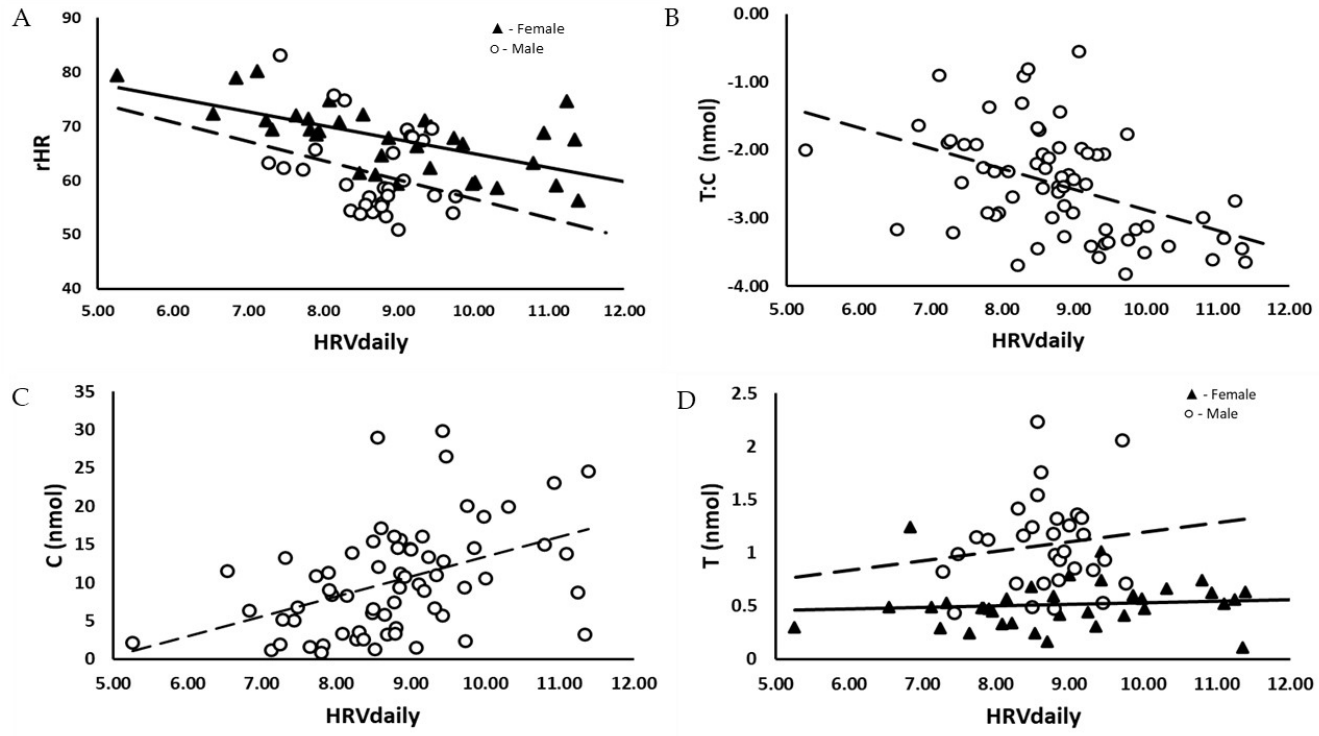

Figure 1. Regression plots – A) rHR and HRVdaily, B) T:C and HRVdaily, C) C and HRVdaily and D) T and HRVdaily. HRVdaily – log of root mean squared of successive differences.

All raw data and summary statistics are reported in Table 2. Within-participants the HRVdaily and T:C ratio relationships varied from weak to strong ($R^2 = -0.101$ to 0.665, $p = 0.103$ to 0.829).

Table 2. Individual participant data.

Participant 1 (woman)- control	TP 1	TP 2	TP3	TP 4	TP 5	TP 6	TP 7	TP 8	TP 9
LnRMSSD	6.54	7.95	7.90	-	8.14	8.87	8.21	6.83	7.32
Testosterone (nmol/L)	0.49	0.54	0.47	-	0.57	0.42	0.34	1.24	0.53
Cortisol (nmol/L)	11.59	8.43	9.04	-	8.28	11.20	13.97	6.36	13.24
T:C ratio	0.042	0.064	0.052	-	0.069	0.038	0.024	0.195	0.040
Participant 2 (woman)- control	TP 1	TP 2	TP3	TP 4	TP 5	TP 6	TP 7	TP 8	TP 9
LnRMSSD	5.26	8.52	7.12	7.82	8.78	7.64	8.08	7.24	7.80
Testosterone (nmol/L)	0.30	0.24	0.49	0.48	0.59	0.24	0.33	0.29	0.48
Cortisol (nmol/L)	2.17	1.33	1.21	1.90	7.41	1.61	3.32	1.95	0.88
T:C ratio	0.138	0.180	0.405	0.253	0.080	0.149	0.099	0.149	0.545
Participant 3 (man)- control	TP 1	TP 2	TP3	TP 4	TP 5	TP 6	TP 7	TP 8	TP 9
LnRMSSD	7.48	8.28	9.07	8.30	8.57	8.80	8.49	8.79	8.36
Testosterone (nmol/L)	0.99	0.71	0.85	1.42	1.54	0.98	1.24	0.47	1.16
Cortisol (nmol/L)	6.76	2.62	1.48	3.53	12.07	4.15	6.58	3.32	2.60
T:C ratio	0.146	0.271	0.574	0.402	0.128	0.236	0.188	0.142	0.446
Participant 4 (man)-control	TP 1	TP 2	TP3	TP 4	TP 5	TP 6	TP 7	TP 8	TP 9
LnRMSSD	7.43	9.45	8.86	9.11	9.32	8.14	8.93	9.16	-
Testosterone (nmol/L)	0.43	0.53	0.93	1.36	0.84	-	1.01	1.33	1.09
Cortisol (nmol/L)	5.13	12.81	15.62	9.87	6.66	-	10.76	16.09	4.98
T:C ratio	0.084	0.041	0.060	0.138	0.126	-	0.094	0.083	0.219
Participant 5 (woman)- treatment	TP 1	TP 2	TP3	TP 4	TP 5	TP 6	TP 7	TP 8	TP 9
LnRMSSD	9.74	10.79	11.39	9.98	9.43	10.93	11.09	9.86	10.31
Testosterone (nmol/L)	0.41	0.74	0.63	0.57	1.01	0.62	0.52	0.60	0.66
Cortisol (nmol/L)	2.36	14.96	24.62	18.64	29.82	23.02	13.83	14.55	19.90
T:C ratio	0.174	0.049	0.026	0.031	0.034	0.027	0.038	0.041	0.033
Participant 6 (woman)- treatment	TP 1	TP 2	TP3	TP 4	TP 5	TP 6	TP 7	TP 8	TP 9
LnRMSSD	11.24	8.69	11.35	8.99	9.42	9.24	9.35	10.01	8.48
Testosterone (nmol/L)	0.56	0.16	0.11	0.79	0.74	0.44	0.31	0.47	0.68
Cortisol (nmol/L)	8.70	3.24	3.25	14.48	5.77	13.35	11.01	10.60	6.09
T:C ratio	0.064	0.049	0.034	0.055	0.128	0.033	0.028	0.044	0.112
Participant 7 (man)- treatment	TP 1	TP 2	TP3	TP 4	TP 5	TP 6	TP 7	TP 8	TP 9
LnRMSSD	8.65	9.76	9.48	8.61	9.72	9.18	9.00	8.83	-
Testosterone (nmol/L)	0.71	0.71	0.93	1.76	2.06	1.17	1.26	1.32	0.69
Cortisol (nmol/L)	5.82	20.03	26.47	17.10	9.39	8.97	14.31	14.58	4.70
T:C ratio	0.122	0.035	0.035	0.103	0.219	0.130	0.088	0.091	0.147
Participant 8 (man)- treatment	TP 1	TP 2	TP3	TP 4	TP 5	TP 6	TP 7	TP 8	TP 9
LnRMSSD	7.73	7.28	8.78	8.56	-	7.89	8.85	8.49	-
Testosterone (nmol/L)	1.15	0.82	1.18	2.23	0.85	1.12	0.74	0.49	-
Cortisol (nmol/L)	10.91	5.24	16.12	29.02	15.03	11.28	9.40	15.46	-
T:C ratio	0.105	0.156	0.073	0.077	0.057	0.099	0.079	0.032	-
Mean Data	TP 1	TP 2	TP3	TP 4	TP 5	TP 6	TP 7	TP 8	TP 9
LnRMSSD	8.01	8.84	9.24	8.77	9.05	8.84	9.00	8.65	8.46
Testosterone (nmol/L)	0.63	0.55	0.69	1.23	1.03	0.71	0.72	0.78	0.76
Cortisol (nmol/L)	6.68	8.58	12.2	13.5	11.8	10.5	10.4	10.4	7.48

Table 3 presents the diagnostic ability of HRVdaily for assessing unfavorable changes in the T:C ratio concentration. This method of HRVdaily assessment demonstrated strong sensitivity (95%), but poor specificity (5%) for detecting meaningful changes in T:C ratio. Further, HRVdaily showed an excessively high false-positive rate (95%) yet a preferable rate of false negatives (5%). HRVdaily also demonstrated a reasonable degree of negative diagnostic accuracy; successfully classifying 41 out of 58 (71%) "no risk", less than a 30% change, T:C ratio days.

Table 3. Clinical utility of HRV and T:C.

	T:C Ratio (At Risk)	T:C Ratio (No Risk)
HRV (At Risk)	1	2
HRV (No Risk)	17	41
Measures of Validity and Reliability		
Diagnostic Accuracy	74%	
Sensitivity	95%	
Specificity	5%	
False Positive Rate	95%	
False Negative Rate	5%	
Positive Predictive Value	33%	
Negative Predictive Value	71%	

DISCUSSION

This study evaluated the relationship between HRVdaily and pre-exercise T:C ratio as well as the clinical utility of HRVdaily to detect changes in T:C ratio during a 9-week HIFT intervention. Our results do not fully support our primary hypothesis that HRVdaily and T:C ratio will respond in parallel; however, they do reaffirm the relationship between the ANS and HPA axis in response to physiological stress. This was demonstrated by elevated parasympathetic outflow resulting in an increase in LnRMSSD while the stimulated HPA axis increased cortisol secretion. However, the association between the ANS and HPA axis has high individual variability with men displaying a better HRVdaily and T relationship. Further, in support of our secondary hypothesis, we demonstrate for the first time that a commercially available HRV monitoring application could be used as a proxy measure to evaluate clinically meaningful fluctuations in T:C ratio throughout a high-intensity exercise intervention, particularly for men.

We found that the ANS regulated changes in HRVdaily were negatively associated with the T:C ratio throughout 9-weeks of HIFT. Previous work by Huovinen et al. revealed a significant positive association between change in HRV, using standard deviation of normal to normal, and T:C ratio (24). The difference in findings may, in part, be due to the inability of Huovinen et al. to collect baseline hormone values resulting in the authors being unable to report the relationship on a single day (24). Despite Houvinen et al demonstrating that improvements in HRV over time may occur with a greater T:C ratio, the single-day relationship is still unclear (24).

Our findings differ from previous research due to increased cortisol levels displayed by our participants. This is due in part to the applied stressor. Huovinen et al.'s participants were primarily under psychological strain, whereas our subjects completed regular bouts of HIFT that placed them under physiological strain (24). Previously, HIFT has been shown to produce a physiological overload that increases C levels and significantly reduces the T:C ratio (33). The physiological overload is result of the high training volume, short rest intervals and varied exercise selection design of HIFT.

The observed positive relationship between C and HRVdaily also is in contrast to findings of Kuorelahti who studied junior endurance athletes (30). The elevated cortisol levels we found may in part be due to the lack of HIFT experience within our participants. In contrast, Poderoso et al showed decreasing cortisol levels in experienced HIFT participants over a six month period (44). Additionally, hormone samples were collected within 15-minutes prior to the start of training therefore it could be speculated that the increase in cortisol may have been due to anticipatory response to enable an improved performance (39, 40).

Finally, our observed difference in HRV response may be the result of the specific training modality. Kliszczewicz et al. demonstrated that despite an acute HRV depression following a bout of HIFT, resting HRV values were not altered (29). It is possible that the ANS stress from HIFT is not sufficient to cause long term disruptions in HRV. Our results demonstrate that during 9-weeks of HIFT the activity of the ANS and HPA-axis were not matched across all individuals. The previously demonstrated positive relationship between HRV and T:C ratio may not hold true across all exercise training programs (30).

We initially hypothesized there would be a significant relationship between HRV and T:C ratio. If true, this relationship would allow for daily measurement of HRV to serve as a non-invasive proxy for hormonal changes throughout an exercise intervention. As with all diagnostic assessments, we hoped HRVdaily would demonstrate a high degree of both sensitivity (i.e., an ability to rule-out meaningful change in T:C ratio) and specificity (i.e., an ability to rule-in meaningful change in T:C ratio). However, as is common with clinical tests, we were confronted with limitations of HRVdaily for monitoring fluctuations in T:C ratio. In particular, we found a lack of specificity (i.e., 5%) in HRVdaily accurately identifying negative changes in T:C ratio. That is, using resting HRV status missed 95% of the actual "at risk" T:C ratios. However, HRVdaily did demonstrate a high degree (i.e., 95%) of diagnostic sensitivity. Meaning, that if an individual's resting HRV status indicated "no risk" there was a high likelihood that same person's T:C ratio also indicated "no risk." We argue that this finding highlights partial efficacy of HRVdaily to be utilized when a practitioner desires to monitor the hormonal status of his or her athletes in response to a training intervention.

While a test that can both identify "at risk" and "no risk" would be preferred, tests that are good at one or the other still have clinical utility. For example, using HRVdaily, a person can reasonably infer that when the application indicates they are not at risk they are, in fact, not at risk for maladaptation on that particular day. However, if HRVdaily indicates they are at risk, the person might want to employ a secondary assessment that is more specific to help confirm

a meaningful change in T:C ratio. For the recreational athlete, the use of HRVdaily might be able to serve as the sole monitoring strategy of recovery status. As these individuals are typically not concerned with optimal performance and/or adaptation, the consequences of missing a potential training day due to a false-positive "at risk" classification are almost non-existent. Conversely, in high performance environments, a false-positive "at risk" HRVdaily classification would result in an athlete missing a training day and potentially produce suboptimal performance and/or adaptation.

The present study is not without limitations. Throughout the investigation, we asked participants to not engage in any additional exercise outside of the intervention and we were not able to prevent or record additional activities that could have influenced measured variables (i.e., HRV and T:C ratio). Additionally, sleep quality and duration were not recorded and participant nutrition was not controlled nor standardized, all of which could affect T:C ratio and HRV responses. Furthermore, there is the potential that the use of an orthostatic assessment of HRV could have altered our findings as the positive relationship reported by Huovinen et al. was present during the standing portion of an orthostatic test (24). Strengths of this investigation included a high (≥80%) adherence rate to training and HRVdaily recordings. Additionally, no participants reported injuries.

Future investigations should attempt to quantify individual and weekly training in order to account for HPA-axis and ANS changes influenced by exercise intensity and volume. Individual T:C ratio should be recorded daily in order to determine if fluctuations in the ANS and HPA-axis are better related opposed to the summative response to a week of training. As our participants were novices with HIFT, future examinations should determine if these findings are consistent with more experienced participants. As more experienced individuals are typically the ones looking for advanced monitoring strategies to optimize training responses, future results may provide more practical application to this group.

The present study demonstrates an association between the ANS and the HPA axis during 9-weeks of HIFT. Additionally, we demonstrated that HRVdaily is sensitive enough to accurately classify 95% of positive T:C ratios. While these findings indicate HRVdaily may be a useful tool for recreational athletes to monitor recovery status, in more high-performance settings, a more specific secondary test may be needed to ensure valuable training time is not lost due to missing that the athlete was actually "at risk". These observations further emphasize the potential of HRV for the guidance of training, however, as hormonal responses to training are highly individual the creation of individual ANS and hormonal profiles would increase the accuracy of training stress modulation.

ACKNOWLEDGEMENTS

The authors would like to acknowledge the contributions of Blanca De LaTorre and Jason Sartor. This study received funding from the Mindlin Foundation and the Kansas State University Office of Undergraduate Research and Creative Inquiry.

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