MONITORING THE EFFECTS OF RESISTANCE EXERCISE ON HEART RATE VARIABILITY

by

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ABSTRACT

Heart rate variability (HRV) has become a popular tool for monitoring autonomic stress responses, however, the efficacy of HRV as a valid internal training load marker for resistance exercise has not been well-established. We conducted three studies to address this gap. Study 1 compared the effects of low, moderate, and high set volumes in acute resistance exercise sessions on post-exercise parasympathetic reactivation. Three full-body resistance exercise sessions of varying set volumes were performed with HRV being measured pre- and for 30 minutes post-exercise. Statistically significant differences were observed across sessions and recording times ($p \leq .05$), but not with the session × time interaction. When comparing pre-post exercise HRV, significant differences were found across all sessions. The low volume session was significantly different from both the moderate and high volume sessions, but no differences were found between moderate and high volume sessions. Study 2 determined the relationship between pre-post changes in HRV, neuromuscular performance, and biochemical fatigue markers in response to resistance exercise. A bout of high set volume resistance exercise was performed with HRV, neuromuscular performance, and biochemical fatigue markers being measured pre- and post-exercise. Statistically significant correlations were observed with ΔHRV $Post_{5\text{-}10}$ and ΔLactate (r = -0.440, p = .036), and ΔHRV $Post_{5\text{-}10}$ and ΔLactate $Post_{30}$ (r = -0.549, p <.001). Study 3 examined the validity and reliability of HRV derived from smartphone photoplethysmography (PPG) under resting and post-resistance exercise conditions. Participants completed four resting, simultaneous ECG and PPG measurements on separate days and one measure post-resistance exercise. Significant, yet small (ES=0.2-0.6) to moderate (ES=1.14) differences were found

between simultaneous measures with moderate-to-very large correlations (r=0.41-0.76) and good agreement at rest. For the intraday reliability of PPG, ICC was “nearly perfect” (ICC=0.91) and interday reliability ICC was “very large” (ICC=0.88). The use of smartphone PPG seems to be an appropriate surrogate for ECG. However, HRV may not be a sensitive enough method for detecting all differences in set volumes. Practitioners should use an integrative approach to assess an individual’s recovery status and readiness to perform.

DEDICATION

This dissertation is dedicated to my parents, Lonnie and Cathy Holmes. I would not have made it to this point in my academic career, or my life in general, without the love and support they have provided me at every step of the way.

LIST OF ABBREVIATIONS & SYMBOLS

Δ	change
%BF	percent body percent
ANOVA	analysis of variance
BP	bench press
BS	back squat
BR	bent-over row
CE	constant error
CI	confidence intervals
cm	centimeter
CMJ	countermovement jump
ECG	electrocardiogram
ES	effect size
HR	heart rate
hr	hour
HRV	heart rate variability
HV	high volume
IHG	isometric handgrip
ICC	intraclass correlation
IL-6	interleukin-6
IP	immediately post
kg	kilogram
Lac	lactate

LnRMSSD	natural logarithm of the root mean square of successive RR interval differences
LV	low volume
M	mean
m/s	meters per second
MD	mean difference
min	minute
mm	millimeter
MPV	mean propulsive velocity
MV	moderate volume
NFOR	non-functional overreaching
PPG	photoplethysmography
PRS	perceptual recovery status
PRV	pulse rate variability
RIR	repetitions-in-reserve
RM	repetition maximum
RMSSD	root mean square of successive RR interval differences
s	second
SD	standard deviation
SEE	standard error of the estimate
SKF	skinfold

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CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

INTRODUCTION

Resistance training programs have played a fundamental role in improving sports performance for decades. The primary goal of any training program is to enhance the performance of sport-specific skills by producing certain muscular adaptations. These adaptations appear through the manipulation of dozens of training variables including load, volume, frequency, exercise selection, effort, and intra-set rest periods (1-3). The physiological responses to acute bouts of resistance exercise vary depending on the structure of the program as well as individual differences between the athletes involved. To increase muscular performance, an overloading stimulus must be applied in a periodized manner to force adaptation (4), however, accompanying any overloading stimulus is fatigue, or the disruption of physiological systems that lead to a decrement in athletic performance (5, 6). Before another overloading stimulus can be applied effectively, dissipation of the accumulated fatigue must occur through various recovery methods. Once adequate recovery has taken place, adaptation can proceed. This line of events is known as the Stimulus-Fatigue-Recovery-Adaptation theory, a sport-science modification of the General Adaptation Syndrome concept (7, 1).

Even with these concepts being generally well-known, one of the major challenges faced by sports coaches, strength and conditioning specialists, and exercise physiologists is measuring the amount of fatigue generated by resistance training. Resistance exercise is most commonly prescribed and tracked using volume-load (sets x repetitions x load lifted) (8, 9). Being able to

mathematically quantify stress imposed by resistance exercise allows coaches to periodize resistance exercise for optimal performance. Unfortunately, volume-load is an external measure of training load which is defined as the work completed by the athlete, measured independently of the individual's internal characteristics (10). Though simplistic to calculate and understand, volume-load is a poor estimate of true physiological responses to resistance training (9). To properly gauge levels of fatigue being produced within a single bout of exercise and optimize the needs of individual athletes, practitioners should seek a more integrative approach by incorporating an objective measure that takes internal responses into account.

During strenuous resistance training, tissue damage to targeted muscle groups commonly occurs resulting in muscle pain, spasms, swelling, and aches with subsequent contractions. This tissue damage during resistance exercise can stem from mechanical tension from external loading and/or metabolic stress (11). When these internal perturbations occur many biochemical markers manifest in the extracellular fluid. Inflammation occurs in response to muscle damage, releasing cytokines and other biomarkers to facilitate the repair process. Much research has been directed towards determining the most valid and reliable indicators of metabolic stress and muscle damage concerning resistance exercise (12). Though collection via blood draw and subsequent analysis of various biomarkers in the plasma is an extremely accurate measure of internal stress from resistance exercise, it is a laboratory-based method that is not easily transferred to field settings due to its invasiveness, expense, and the technical proficiency required to perform such procedures (13). For these reasons, less intrusive measures are required.

An inability to produce large amounts of force rapidly and repetitively are common signs of decreased performance capabilities. Though the ability to gauge muscle damage and inflammation through biomarker measurements is useful in tracking fatigue, being able to

quantify neuromuscular performance with techniques, such as a counter-movement jump (CMJ) and movement velocity, provides a non-invasive alternative that correlates well with weightlifting performance. Counter-movement jump has been used as a measure of athletic ability and marker of decreases in sports performance (14-19) while linear position transducers have become a popular tool utilized in strength and conditioning settings to track movement velocity (20, 21). Movement velocity utilizing a barbell is a predictor of vertical jump and an indicator of one-repetition maximum (1RM) strength (22). However, both methods have some major limitations. Much of the research on jump performance and movement velocity has been done using force plates and transducers which can be troublesome to transport and extremely expensive. Additionally, a wide variety of metrics are generated with testing that can make data analysis tedious and difficult to analyze. Finally, in team-sport settings, being able to cater to a large number of athletes may prove near impossible with limited time and staff. From a practical standpoint, alternative options may be necessary for coaches and practitioners in field-based settings.

Heart rate (HR) monitoring has been a staple of cardiorespiratory endurance training and team-sports over the decades due to its simplicity, accuracy, and objectivity as a measure of internal load. Exercise HR has been correlated strongly with increasing oxygen consumption and energy expenditure making it a valuable tool for gauging intensity and prescribing exercise (23-28). Banister and Wenger (1991) suggested that training impulse could be used as a marker of physiological stress being experienced by an individual (29). Training impulse scores are the product of HR and exercise duration which give quantifiable values of total work performed. Though it is a strong indicator of aerobic exercise intensity, the anaerobic, intermittent style of resistance training has made researchers and practitioners skeptical of its efficacy. Also, in order

to apply these methods, athletes would need to be supplied with individual HR monitoring devices, which can become extremely expensive when dealing with team sports.

A more novel and inexpensive tool that has gained popularity in recent years is heart rate variability (HRV), which has emerged as a practical tool for monitoring acute physiological responses and chronic adaptations in athletes (30, 31). Heart rate variability is defined as the time intervals that occur between successive heartbeats and is considered a noninvasive marker of the autonomic nervous control of the cardiovascular system (32). Research has supported the use of HRV as an objective indicator of stress and recovery in response to variations in training stimuli among athletes (30, 33, 34). Though traditionally used in clinical and laboratory settings, practitioners have moved to increase the effectiveness of measuring HRV in field settings with HR straps and photoplethysmography (PPG). Photoplethysmography is a noninvasive optical technique for monitoring beat-to-beat relative blood volume changes in the microvascular bed of peripheral tissues. The PPG pulsatile waveform reflects the fluctuations in finger blood volume and vasculature characteristics (35, 36). The pulse rate variability (PRV) of the PPG signal is highly correlated to both time and frequency-domain metrics from electrocardiography-derived (ECG) HRV indices (37-39). Though ECG and PPG produce similar results in HRV indices, small variations still appear. Research has demonstrated that HRV produced from PPG devices, such as ear clip pulse sensors, show good agreement and non-significant differences from traditional ECG measures (35, 40, 41). Schafer and Vagedes (2013) reviewed studies investigating the accuracy of PRV as an estimate of HRV and found a consensus in favor of its accuracy (42).

It has been proposed that daily measures of HRV using the parasympathetically-derived marker of the root mean square of successive RR interval differences (RMSSD) provide superior

results to isolated, pre-post style procedures (43, 44). However, this can be difficult to accomplish with the majority of traditionally used laboratory-based equipment. With innovations constantly being made in mobile technologies, several HRV smartphone applications that have been validated in the literature for acquiring RMSSD (43, 45). In a review by Heathers et al. (46), it was found that the smartphone pulse rate variability system was an accurate measure of HRV. One of the major advantages of RMSSD as a marker for athletic monitoring is it can be accurately measured with an ultra-shortened recording of only 1-minute (47) following a 1-minute stabilization period (48). The emerging smartphone technology has allowed convenience by following recent recommendations of acquiring daily RMSSD throughout a week then averaging to derive more meaningful information about training status compared to isolated recordings (43, 44, 49). Furthermore, smartphone applications are useful for quantifying daily fluctuation of RMSSD as assessed by the coefficient of variation which represents perturbations to cardiac-autonomic homeostasis (44). With the growing body of literature around RMSSD trends seen in athletes, practitioners can use HRV data to evaluate individual responses and potentially guide future training. Unfortunately, a controversy around its efficacy exists in the literature, specifically to the effects resistance exercise has on HRV. In a meta-analysis by Bhati et al. (50), it was found that the majority of the literature concluded that no change occurs in autonomic control following resistance training. However, many of the studies concluding no effect have used solitary recordings under standardized conditions (32) with varying metrics being utilized between studies. Though smartphone-derived HRV has been demonstrated as an objective method for examining fatigue and recovery in aerobic endurance exercise, the lack of available information regarding the effects of acute bouts of resistance exercise is a significant

problem since many team sports and individual athletes train in various forms daily, with strength training being a focal point in and out of the competitive season.

Even with the many subjective and objective fatigue and recovery measures available, there is no one perfect tool free of any error and resistant to being influenced by outside factors unrelated to resistance exercise (9, 51). Many of the criterion measures of external and internal load are ineffective or impractical for field-settings and team-sport athletes who require individualization for optimal progression of resistance exercise. Therefore, the purpose of this dissertation is to evaluate the accuracy and reliability of HRV as an objective method for measuring internal training loads of and monitoring recovery from resistance exercise. The specific study aims are as follows:

Study 1: Compare the effects of low, moderate, and high set volumes in acute resistance exercise sessions on post-exercise parasympathetic reactivation measured using RMSSD. We hypothesized that exercise sessions with higher volumes would result in significantly greater decreases in RMSSD values from pre- to immediately post-exercise. We also hypothesized greater delays in the recovery time of parasympathetic activity back to pre-exercise values would be observed following sessions of higher set numbers, with the low volume session exhibiting the fastest recovery and the high volume session displaying the slowest.

Study 2: Determine the relationship between pre and both immediately and 30-minutes post changes in HRV and neuromuscular performance and biochemical fatigue markers in response to a full-body, hypertrophic-style resistance exercise bout. Heart rate variability changes were compared to neuromuscular performance (isometric handgrip, countermovement jump, and mean propulsive velocity), metabolic stress (lactate), and inflammatory (interleukin-6) responses to resistance exercise. We hypothesized that HRV responses from pre- to immediately post-exercise

would display a strong association with lactate changes, while pre- to 30-minutes post-exercise would display significant associations with all fatigue markers.

Study 3: Examine the validity of HRV measures from a photoplethysmography (PPG) smartphone application under resting and post-full-body, hypertrophic-style resistance exercise conditions. The secondary aim was to examine the intraday and interday reliability of the PPG method. It was hypothesized that PPG and ECG measures of HRV would display strong relationships and no significant differences being found pre- or post-exercise recording times. Additionally, it was hypothesized that the PPG application would demonstrate intraclass correlations of "near-perfect" levels for intra- and inter-day reliability.

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CHAPTER 2

COMPARISON OF HEART RATE VARIABILITY RESPONSES TO VARYING RESISTANCE EXERCISE VOLUME-LOADS

ABSTRACT

Purpose: The purpose of this study was to compare the effects of low, moderate, and high set volumes in acute resistance exercise sessions on post-exercise parasympathetic reactivation measured using the root mean square of successive RR differences (RMSSD). **Methods:** Ten resistance-trained participants (25.80 ± 6.83 yrs., 173.35 ± 10.64 cm, 75.42 ± 9.88 kg) performed three full-body resistance exercise sessions (Low Volume = LV; 4 total sets, Moderate Volume = MV; 8 total sets, and High Volume = HV; 12 total sets) in a counterbalanced order at 72 hours apart. During each session, heart rate variability (HRV) was measured for 10 minutes pre- and 30 minutes post-exercise. The post-exercise HRV measurement was split into five 5-min segments: 5-10 ($Post_{5\text{-}10}$), 10-15 ($Post_{10\text{-}15}$), 15-20 ($Post_{15\text{-}20}$), 20-25 ($Post_{20\text{-}25}$), and 25-30 ($Post_{25\text{-}30}$) minutes. Repeated-measures analysis of variance (ANOVA) was used to assess differences within and between pre-post exercise natural logarithm RMSSD (LnRMSSD) values. **Results:** Significant differences were observed between sessions and when comparing pre-exercise values to post-exercise times ($p \leq .05$), but no meaningful differences with the session × time interaction for LnRMSSD. The LV session resulted in significantly higher LnRMSSD values post-exercise compared to both the MV and HV sessions while the MV and HV sessions produced fairly similar responses. When comparing pre-exercise LnRMSSD values to each post-exercise recording, significant differences were found indicating delays in recovery and no return to

baseline levels. No differences were observed with the initial drop from pre- to post-exercise or in the rate of recovery. **Conclusion:** Acute bouts of full-body resistance exercise cause reductions in LnRMSSD from pre-exercise levels and can delay parasympathetic reactivation back to baseline values, however, differences in volumes may not be fully distinguishable with LnRMSSD as the sole training load metric.

INTRODUCTION

The acute homeostatic challenges from repeating bouts of resistance exercise can eventually lead to increased muscular fitness and hypertrophy. To achieve such positive outcomes, adjustments in training stimuli must periodically occur throughout a macrocycle. Excessive training volume and load for prolonged periods may result in chronic fatigue, diminished readiness to perform, and decreased fitness levels (1). Mitigating maladaptation often requires tailored approaches when deciding appropriate recovery time. Thus, accurate methods to individually quantify fatigue and track recovery during resistance training are essential.

Heart rate variability (HRV) is defined as the oscillations that occur between successive heartbeats and is considered a noninvasive marker of cardiac-autonomic modulation (2). Traditionally considered a prognostic indicator of cardiovascular disease, HRV has recently become popular as an objective physiological indicator of stress and recovery to variations in training load among athletes (3-5). Though numerous metrics for quantifying HRV exist, the parasympathetic-mediated root mean square of successive RR interval differences (RMSSD) is most common in mobile athletic settings (3). Indeed, RMSSD appears promising as an internal indicator of training load (6) and a predictor of non-functional overreaching (7, 8). A recent literature review demonstrated that parasympathetic reactivation paralleled the recovery of

resistance exercise performance (7). Thus, post-exercise RMSSD measures seem useful for guiding exercise prescriptive decisions for subsequent sessions.

Because of the aforementioned, a number of studies to determine the specific factors that influence HRV recovery following resistance exercise have recently emerged (9, 10). Results have shown that the intensity of load (e.g., percentage of 1 repetition maximum) (11, 12), the intensity of effort (e.g. repetitions to failure) (13), set configuration (14, 15), interset rest intervals (16), and exercise type (17, 15) independently influence the recovery of HRV. Most noteworthy, higher set volumes of resistance exercise produce greater reductions in HRV (18). However, the manipulation of set volume while keeping other training variables constant has not occurred. There have been no studies to date that have examined parasympathetic activity following varying doses of set volume utilizing full-body, barbell-based movements with all other training variables remaining constant. Because volume is a key determinant of adaptations, such as muscle hypertrophy, and a major contributor to global fatigue through various mechanisms, including energy depletion and exercise-induced muscle damage (19, 20), knowledge of the autonomic responses to changes in varying set volume is needed. The lack of research on how a critical training variable, frequently manipulated in periodized strength and conditioning programs, affects a popular tool for measuring recovery must be addressed. Therefore, the purpose of this study was to compare the effects of low, moderate, and high set volumes in acute resistance exercise sessions on post-exercise parasympathetic reactivation measured using RMSSD. We hypothesized that exercise sessions with higher volumes would result in significantly greater decreases in RMSSD values from pre- to immediately post-exercise. We also hypothesized greater delays in the recovery time of parasympathetic activity back to pre-exercise values would be observed following sessions of higher set numbers, with

the low volume session exhibiting the fastest recovery and the high volume session displaying the slowest.

METHODS

Participants

Ten healthy, resistance-trained males (n = 8) and females (n = 2) participated in this study. Descriptive statistics of physical and functional characteristics can be seen in Table 2.1. Participants were classified as "advanced" resistance-trained from having at least one year of resistance training experience consisting of at 2-3 sessions per week (21). Inclusion criteria required all participants to have engaged in regular exercise leading up to the study and be free from cardiovascular, metabolic, musculoskeletal and renal diseases or signs and symptoms during participation in the current study (22). During the screening process, blood pressure assessments were conducted with the BPM-100 automated blood pressure monitor (BpTRU Medical Devices; Coquitlam, Canada) at least three times, with two minutes apart in the dominant arm (23, 24). This project was approved by the University of Alabama Institutional Review Board and conformed to the Declaration of Helsinki.

Experimental Design

The study required participants to visit the laboratory five times over approximately two weeks. All exercise sessions began between 6 and 11 a.m. to control diurnal variations (17) and participants reported to the laboratory within the same 2 hr period for every session. During the initial visit, participants were given screening questionnaires, completed a written informed consent form and were lead through all testing procedures for familiarization purposes. During the second visit, anthropometrics and skinfold measurements were collected. Short-term (i.e. 5-

min stabilization and 5-min recording) HRV and blood pressure measurements were taken before and after 1-repetition maximum (1RM) testing for Back Squat (BS), Bench Press (BP), and Bent-over Rows (BR). During the next three visits, participants performed the full-body resistance exercise protocols (Low Volume = LV, Moderate Volume = MV, and High Volume = HV) in a counter-balanced randomized order; the full exercise protocol can be seen in Table 2.2. During the exercise sessions, HRV was measured pre- and for 30 minutes post-exercise. Briefly, the pre-exercise HRV measure was 10 minutes in length, split into a 5-min stabilization period and a 5-min recording period followed by blood pressure assessment. The post-exercise HRV measurement was split into five 5-min segments: 5-10 ($Post_{5-10}$), 10-15 ($Post_{10-15}$), 15-20 ($Post_{15-20}$), 20-25 ($Post_{20-25}$), and 25-30 ($Post_{25-30}$) minutes.

Anthropometrics and Body Composition

During the second visit, anthropometric and body composition measures were taken upon arrival. Participants were asked to come to the laboratory having refrained from eating any heavy meals (≤ 300 calories) or drinking beverages other than water (≤ 500 mL) 2 hr before arrival. Participants had nude body mass measured (to the nearest 0.1 kg) with a calibrated digital scale (Tanita BWB-800, Tanita©, Arlington Heights, IL) standing height measured (to the nearest 0.1 cm) with a stadiometer (SECA 213, Seca Ltd., Hamburg, Germany). Two measurements (within 2 mm of each other) of skinfold (SKF) thickness, were taken using calibrated skinfold calipers (Lange Skinfold Caliper, Seko, USA) across 7 standard sites on the right side of the body. Percent body fat (%BF) from SKF was calculated using the Brozek equation (%BF = [(4.57/Db) – 4.142] x 100) (25).

One-Repetition Maximum Tests

Participants completed 1RM testing for BS, BP, and BR using procedures adapted from previously reported protocols (21). For BS, participants were instructed to get to a bottom position where either the femur was parallel with the ground or the peak of the anterior portion of the thigh was perpendicular to the top of the patella. For BP, participants maintained five points of contact and touched the bar to the chest before locking out the repetition with arms fully extended. For BR, participants unracked the bar and proceeded to flex at the hip until the torso was approximately parallel with the ground. For each repetition, the participants were then instructed to touch the bar to the torso, between the umbilicus and the xiphoid process and end with arms fully extended. Each repetition began and ended with the arms fully extended and the bar elevated off the ground. The warm-up consisted of 5 minutes of cycling, ~5 minutes of self-selected dynamic stretches (i.e. arm circles, lunges, walking hamstring stretches, etc.), 10 repetitions with the unloaded Olympic barbell, 5 repetitions at 50%, 3 repetitions at 70%, and 1 repetition at 85% of 1RM. The participants then attempted their self-reported 1RM (21). Upon a successful attempt, the load was increased by 2-10 kg per attempt until failure was reached. A minimum of 180 s of rest was given between each attempt to ensure adequate recovery (26, 21).

Resistance Exercise Sessions

At least 72 hr after the 1RM testing was completed, participants performed three different resistance exercise protocols across three sessions in a randomized order to eliminate training effects. Participants were asked to come to the laboratory having refrained from the ingestion of alcohol for 24 hr, caffeine 12 hr, and any heavy meals 2 hr before the arrival. All three sessions were full-body consisting of the same exercises in the same order: BS, BP, and BR. The warm-up consisted of 5 minutes on a cycle ergometer, ~5 minutes of self-selected dynamic stretches, and 10, 5, and 3 repetitions of BS and BP with the unloaded bar, 30% and 50% of 1RM,

respectively. Sessions differed based on the number of sets (i.e. LV, MV, and HV) being performed for each exercise (see Table 2.2). The relative load was 70% of 1RM for all exercises with 120 s of rest between each set and 180 s of rest between each exercise (18, 16, 12). A participant's intensity of effort was tracked throughout each exercise session using a repetition-in-reserve (RIR) (Table 2.3) (27, 28). Failure was defined as both muscular (i.e. mechanical inability to complete a repetition) and volitional (i.e. the participant's choice to stop due to the perceived inability to continue exercise). If a participant reached failure during a set before completing the prescribed 10 repetitions, the set/rep number when it happened was recorded and 30-60 s of rest was given before continuing with the set. This process was repeated until 10 repetitions were completed. Exercise sessions were done with at least 72 hr of rest between each session (14, 15).

Heart Rate Variability

The HRV data collection procedures were based on standardized methods (29) and validated alternative methods reported in previous studies involving groups of collegiate athletes (30, 4, 5). Heart rate variability measures were taken before every exercise session in the laboratory. Participants were required to report to the laboratory within the same 2 hour period for every exercise session. Heart rate variability measures were collected using electrocardiography (ECG). The ECG signals were collected with an electronic signal acquisition system (BIOPAC MP150 Physiograph), which was connected to a Dell PC. Acknowledge software (v 4.4, BIOPAC, Goletta, CA, USA) was used to collect real-time ECG. Electrocardiogram assessment was performed with a modified lead II configuration where three surface electrodes (BIOPAC EL504 disposable Ag-AgCl) were placed on the participant: 1) near the right shoulder along the midclavicular line, 2) fifth intercostal space along the midaxillary

line, and 3) near the iliac crest of the left hip along the midclavicular line. Participants were in a quiet room maintained at a temperature of 20°-23° C. Recordings took place in the seated position to reduce any possible parasympathetic saturation which is often observed in individuals with low resting HR. Once seated in a neutral position with their feet on the ground and arms supported in a resting position by a table at approximately waist height, participants were given a 5-min stabilization period to limit any bodily movement and use normal breathing patterns.

Two separate ECG recordings were obtained throughout each session; one 10-min segment pre-exercise and one 30-min segment immediately after the exercise bouts. The pre-exercise ECG measurement consisted of 5-min stabilization followed by a 5-min recording. The post-exercise ECG measurement from the 30-min recording was separated into five 5-min segments at $Post_{5\text{-}10}$, $Post_{10\text{-}15}$, $Post_{15\text{-}20}$, $Post_{20\text{-}25}$, and $Post_{25\text{-}30}$. The HRV time-domain metric of RMSSD was solely investigated due to its strong representation of parasympathetic modulation and its ability to be unaffected by spontaneous breathing frequencies (29, 3, 31). However, it is common among HRV variables over a sample of participants to be skewed (32, 33) so to ensure a normal distribution of data, the natural logarithm of RMSSD (LnRMSSD) was evaluated instead. All ECG segments were visually inspected using the Acknowledge software for any potential motion-related artifact noted during collection not indicative of true ectopic/non-sinus beats before being transformed into a tachogram and exported into Kubios HRV Standard 3.3.0 software (Biosignal Analysis Medical Imaging Group at the Department of Applied Physics, University of Kuopio, Kuopio Finland). Occasional artifact noise was automatically replaced with the interpolated adjacent RR interval values (threshold = 0.45 s or "very low"), amounting to ≤3% of error correction. The analysis process was carried out by the same researcher to ensure consistency (34, 35).

Statistical Analysis

All data were analyzed with IBM SPSS version 25.0 for Windows (Somers, NY) and Microsoft Excel 2016 for Windows (Microsoft Corporation, Redmond, WA). A one-way analysis of variance (ANOVA) was done for all resting LnRMSSD values to determine significant differences between exercise sessions. A 3 (session) x 6 (time) repeated-measures ANOVA with a Bonferroni correction was used to assess differences between pre-exercise and post-exercise LnRMSSD values at the five separate time segments within and between exercise sessions. Follow-up paired-samples *t*-tests were used to further assess differences. To assess the initial change in LnRMSSD, the delta percent change (ΔLnRMSSD) from pre-exercise to $Post_{5\text{-}10}$ ($\Delta LnRMSSD_{pre\text{-}post}$) was calculated for each session and compared. The ΔLnRMSSD was also calculated between $Post_{5\text{-}10}$ and $Post_{25\text{-}30}$ ($\Delta LnRMSSD_{post5\text{-}30}$) to assess HRV recovery (36, 37). The magnitudes of the pair-wise differences were quantified using Cohen's *d* ES and were classified as trivial (0.0-0.2), small (0.2-0.6), moderate (0.6-1.2), large (1.2-2.0), and very large (>2.0) (38, 39). Unless otherwise stated, data were presented as mean ± standard deviation (*M* ± *SD*) and statistical significance was accepted at $p \leq .05$.

RESULTS

Each participant's highest reported RIR value for each exercise during the exercise sessions can be found in Table 2.4. The mean ± standard deviations for all LnRMSSD values can be seen in Table 2.5. The LnRMSSD values at each recording period followed a normal distribution. For session, Mauchly's *W* was not significant ($W = 0.872$, $\chi^2(2) = 1.09$, $p = .579$), but was significant for time ($W = 0.010$, $\chi^2(14) = 32.368$, $p = .005$) so the Greenhouse-Geisser correction factor was applied.

No significant differences were seen between pre-exercise LnRMSSD measures across all three sessions ($p \leq .05$). The overall F for differences in mean LnRMSSD across the three sessions was statistically significant: $F(2, 18) = 8.390$, $p = 0.003$. The corresponding estimated ES was "small" with a partial η^2 of 0.482. Using the Bonferroni correction, the adjusted p-value (.05/3) was 0.017. Pairwise comparisons demonstrated the LV session as significantly different from both the MV ($p = .013$) and HV ($p = .010$) sessions. However, there were no significant differences found between the MV and HV sessions.

The overall F for differences in mean LnRMSSD across the baseline and five separate post-exercise time points of recording was statistically significant: $F(2.550, 22.953) = 28.982$, $p < .001$. The corresponding estimated ES was "moderate" with a partial η^2 of 0.763. With an adjusted p-value of 05/6 = 0.008, pairwise comparisons demonstrated that Pre HRV values were significantly different from all post-exercise HRV recordings ($p \leq .001$). However, no other statistically significant differences were observed when comparing post-exercise HRV recordings to one another ($p > .008$). Figure 2.1 displays the differences between pre- and post-HRV recordings.

The overall F for differences in mean LnRMSSD with the session × time interaction was not statistically significant: $F(3.823, 34.408) = 2.44$, $p = .068$. The corresponding estimated ES was "small" with a partial η^2 of 0.213.

In the current study, exercise sessions were conducted in a counter-balanced randomized order. A post hoc analysis using the "order" of the sessions as a covariate revealed no statistically significant difference for session effect ($p = .235$, ES = 0.680), time effect ($p = .567$, ES = 0.539), or the interaction ($p = .606$, ES = 0.536).

When comparing $\Delta LnRMSSD_{pre\text{-}post}$ across all sessions, the overall *F* for differences was not statistically significant: F (2, 18) = 3.427, p = .055. The corresponding estimated ES was "small" with partial η^2 of 0.276. When comparing the $\Delta LnRMSSD_{post5\text{-}30}$ between sessions, the overall F for differences was not statistically significant: F (2, 18) = .975, p = .396. The corresponding estimated ES was "trivial" with a partial η^2 of 0.098.

DISCUSSION

The purpose of the present study was to compare the effects of low, moderate, and high set volumes in acute resistance exercise sessions on post-exercise parasympathetic reactivation measured using LnRMSSD. Significant differences were observed across sessions and recording times, but no meaningful differences with the session × time interaction for LnRMSSD. The LV session resulted in significantly higher LnRMSSD values post-exercise compared to both the MV and HV sessions while the MV and HV sessions produced fairly similar responses. When comparing pre-exercise LnRMSSD values to each post-exercise recording, significant differences were found indicating delays in recovery and no return to baseline levels. Finally, when comparing ΔLnRMSSD values between sessions, no differences were observed with the initial drop from pre- to post-exercise or in the rate of recovery.

Significant reductions in LnRMSSD from pre-exercise to the $Post_{5\text{-}10}$ recording were observed following all three exercise sessions. This falls in line with previous research that has concluded that acute bouts of resistance exercise can greatly reduce parasympathetic activity during the recovery period. It has also been suggested that a shift in the sympathovagal balance towards greater sympathetic dominance may be at play immediately following exercise (40). Though the LnRMSSD $Post_{5\text{-}10}$ measure was significantly lower from the pre-exercise HRV measure across all sessions, no statistically significant differences were found between

$\Delta LnRMSSD_{pre\text{-}post}$ values across sessions. These results demonstrate that resistance exercise at both low and higher volumes can elicit significant reductions in vagal activity and the effects of varying set volumes cannot be distinguished using LnRMSSD, within the first 10 minutes post-exercise.

Additionally, the comparison of overall differences in mean LnRMSSD showed that the LV session resulted in significantly higher LnRMSSD values within the 30-min recovery period. Conversely, no statistically significant pair-wise differences could be found between the HRV recovery periods of the MV and HV sessions, however, the effect sizes of change pre-post differed between sessions until the 20-min mark. Figueiredo et al. (18), when comparing varying set volumes (i.e. 1 vs. 3 vs. 5 sets), found that multiple set resistance-exercise volumes had a stronger influence on HRV than the single set protocol. Similar to the current study, throughout the 60-min post-exercise recovery period, RMSSD remained reduced after both the 3-set and 5-set sessions but the magnitudes of the reductions differed; reductions were significantly different from pre-exercise values at the 10- (ES = -1.43 and -1.07), 20- (ES = -0.74 and -1.02) and 30- (ES = -0.47 and -0.82)-minute time points (18). Based on the findings of Figueiredo et al. in conjunction with our own, there seems to be a certain threshold volume in which statistically significant differences in post-exercise LnRMSSD values can no longer be observed within the first 30 minutes following exercise. Longer recording periods post-exercise may be needed for practitioners to properly gauge how fatiguing a higher set volume bout of resistance exercise (e.g. $\geq$ 3 sets) is for individuals.

The protocol by Figueiredo et al. (18) utilized machine-based exercises that isolated muscle groups. The current study implemented a full-body, free-weights routine composed of compound exercises for greater practical application to athletes involved in college and

professional level strength training programs. Previous research has shown that whole-body routines elicit greater reductions in RMSSD than split routines (i.e. upper- and lower-body) (17) and that the use of free-weight exercises results in decreased vagal modulation for up to 30 minutes (10). With LnRMSSD being a measure of parasympathetic activity, in cases of high sympathetic dominance, it may be difficult for any parasympathetic metric of HRV to distinguish differences in effects. The multijoint exercises chosen for the current study are dynamic and target large muscle areas, recruiting a higher number of motor units than single joint and/or machine-based movements (21). The high degree of muscle activation can result in a large magnitude of stimulation of mechanoreceptors and chemoreceptors sending sensory information via group III/IV muscle afferents to the central nervous system leading to more sympathetic drive (41). The implementation of multi-joint, free-weight exercises done over higher set volumes may have adjusted the sympathovagal balance towards sympathetic dominance making measures of parasympathetic activity (e.g. LnRMSSD) so low that detecting significant differences between protocols difficult, especially during the acute recovery timeframe of the current study.

Across all sessions, LnRMSSD never returned to pre-exercise values during the entire 30-min recovery period for all sessions. This slow response parasympathetic reactivation is in agreement with previous research (9, 42). Chen et al. (9) examined HRV recovery up to 72 hr following a 2-hour bout of heavy resistance exercise using compound movements in seven weightlifters. The study was found that parasympathetic activity was at its lowest at 3 hr post and did not rebound back to baseline levels until ≥48 hr post (9). More recently, Gambassi et al. (42) examined the effects of 10 sets of 10 repetitions at 50% of 1RM for bench press and leg press and found the largest reductions in post RMSSD were seen at the 30-40-minute segment,

indicating a continuous decrease in parasympathetic modulation even after the cessation of exercise (42). Though our exercise protocol differed from those previously used, it seems that greater total volume (set x reps) and multi-joint exercises are key factors in parasympathetic reactivation.

In conjunction with the effects of varying set volumes, the intensity of effort measured via proximity to muscular failure during an exercise set may have played a huge role in post-exercise autonomic modulation (13). It has been demonstrated that sets performed to failure can result in greater protein accretion and recruitment of high-threshold motor units, which can lead to higher levels of parasympathetic withdrawal and increases in sympathetic activity with more skeletal muscle tissue being contracted (19). Badillo et al. (13) analyzed the time course of recovery following two different resistance exercise protocols based on the proximity to failure. The study found that significantly lower HRV occurred following a higher repetition within the set configuration protocol immediately post-exercise (13). In the current study, participants either reached failure or reported being 1-2 repetitions from failure in both the HV and MV sessions, but not in the LV session. Participants who reached failure were given only 30 s to recover before continuing with the set. Repeated exercise sets taken to or near muscular failure followed by relatively short rest periods can lead to greater fatigue accumulation, specifically through metabolic stress due to the occlusion of blood vessels creating a hypoxic environment for the working muscles (43). Differences in HRV responses across various set volumes may be attributed to decreasing levels of stored phosphocreatine, rapid glycolysis and greater lactate production with higher repetitions (13, 14). The possible metabolic stress generated from the present study's protocol could have lead to extremely high levels of sympathetic dominance causing the effects on HRV seen post-exercise. The continual drive of sympathetic activity could

have also lead to the release of a large number of circulating catecholamines, which might not have been cleared in the 30-min recovery period.

Though set volume was the primary independent variable being examined in the current study, the combined effect of all the other resistance training variables may have superseded the ability of LnRMSSD to detect differences between the HV and MV sessions. Figueiredo et al. (12), when comparing loads at 60%, 70%, and 80% of 1RM, found that post-exercise RMSSD was reduced pre-post with the largest effect sizes being observed within the first 30 minutes following the 70% session (12). In a follow-up study, Figueiredo et al. (16) compared the effects of 1-min and 2-min interset rest periods during resistance exercise and observed that both produced large effect sizes and no significant difference between periods during 30 minutes of HRV recovery (16). The current study's protocol involved higher set volumes, moderately heavy load (i.e. 70%), relatively short rest periods (i.e. 2 min). and full-body, barbell exercises. The lack of clear distinction between sessions and the prolonged delay in parasympathetic reactivation may be associated with the exercise stimulus being too high even with the lower set volume (e.g. LV session).

Limitations

Several limitations of the present study should be emphasized. There was no control session in which participants came to the laboratory and had measurements taken for the same duration of time absent exercise intervention. However, considering the main objective of the study was to investigate the effects of varying exercise volumes on HRV, a control session was not essential. Though the current study was adequately powered due to its experimental design, a larger sample size might have proven beneficial in detecting greater set volume differences with LnRMSSD. Though both males and females were recruited for this study, the female-to-male

ratio was too small to run a comparative analysis. Finally, the breathing frequency and tidal volume were not controlled; however, this was done to simulate field conditions for athletes (i.e. the target population).

CONCLUSION

In conclusion, acute bouts of full-body resistance exercise cause reductions in parasympathetic activity and can delay reactivation for at least 30 minutes following exercise. Significant differences can be observed in LnRMSSD between resistance exercise sessions of varying set volumes. However, these differences are only significant between low volume and moderate-to-high volume sessions and produce small effect sizes. These results suggest that there may be a volume threshold at which parasympathetic reactivation is blunted similarly, even with further increases in volume set numbers. Differences in LnRMSSD responses to varying set volumes may be masked when used in conjunction with moderately heavy loads, short rest periods, and multiple compound exercises done in succession. Though HRV is a useful measure of autonomic modulation in response to resistance exercise, sports coaches, strength and conditioning specialists, and exercise physiologists should look to incorporate multiple methods of monitoring fatigue and recovery to assess an athlete's readiness to perform more effectively.

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Table 2.1

Physical and Functional Characteristics of the Participants (n = 10)

Descriptives	Participants
Age (yrs)	25.80 ± 6.83
Height (cm)	173.35 ± 10.64
Body mass (kg)	75.42 ± 9.88
Body fat (%)	21.30 ± 4.82
Systolic (mmHg)	112.67 ± 6.41
Diastolic (mmHg)	68.97 ± 7.28
Resting Heart Rate (bpm)	64.17 ± 8.43
Back Squat (kg)	127.27 ± 38.12
Bench Press (kg)	94.32 ± 28.97
Bent-over Rows (kg)	78.18 ± 19.59

Notes: Data displayed as means ± standard deviations

Table 2.2

Resistance Exercise Protocol

Exercises	LV	MV	HV	All Sessions	
	Volume (set x repetitions)			Load (%1RM)	Interset Rest (s)
Back Squat	2 x 10	4 x 10	6 x 10	70	120
Bench Press	1 x 10	2 x 10	3 x 10	70	120
Bent-over Rows	1 x 10	2 x 10	3 x 10	70	120

Notes: LV = low volume; MV = moderate volume; HV = high volume; %1RM = the percentage of one-repetition maximum; s = seconds

Table 2.3

Rating of Perceived Exertion Based on Repetitions-in-Reserve

Rating	Description of Perceived Exertion
10	Maximum Effort
9	1 repetition remaining
8	2 repetitions remaining
7	3 repetitions remaining
5-6	4-6 repetitions remaining
3-4	Light effort
1-2	Little to no effort

Table 2.4

Individual Reports of Failure and Repetitions-in-Reserve

Participants	Low Volume				Moderate Volume				High Volume			
	Failure (Y/N)	BS	BP	BR	Failure (Y/N)	BS	BP	BR	Failure (Y/N)	BS	BP	BR
001	N	7	7.5	5.5	Y	7.5	10	7.5	Y	10	10	7
002	N	10	10	9	N	10	10	10	N	10	10	10
003	N	7	5.5	5.5	N	7	5.5	5.5	N	7	5.5	5.5
004	N	8.5	9	8.5	N	10	10	10	N	10	10	10
005	N	8.5	8.5	7.5	Y	10	10	8.5	Y	10	10	8.5
006	N	8.5	8.5	7	N	8.5	8.5	7	Y	9.5	10	8
007	N	7.5	8.5	5.5	N	9	9	8	Y	9	10	10
008	N	8.5	8.5	9.5	N	10	9.5	10	Y	9	9.5	10
009	N	8.5	5.5	7.5	N	9	8	10	Y	10	10	10
010	N	8	8	9	N	9	8.5	10	Y	9	8.5	10

Notes: Y/N = yes/no; BS = Back Squat; BP = Bench Press; BR = Bent-over Row

Table 2.5

Comparison of LnRMSSD Changes from Pre to Post Measures Across Three Exercise Sessions

	M ± *SD*	Δ (%)	MD	ES	SE	*t*	*p*
LV							
Pre	4.32 ± 0.59	-	-		-	-	-
$Post_{5-10}$	3.38 ± .076	-21.75*	0.94	Moderate	0.21	4.49	.001
$Post_{10-15}$	3.43 ± 0.66	-20.53*	0.89	Moderate	0.18	4.84	.001
$Post_{15-20}$	3.51 ± 0.55	-18.76*	0.81	Moderate	0.15	5.26	.001
$Post_{20-25}$	3.47 ± 0.49	-19.60*	0.85	Moderate	0.16	5.21	.001
$Post_{25-30}$	3.62 ± 0.47	-16.25*	0.70	Moderate	0.16	4.46	.002
MV							
Pre	4.01 ± 0.80	-	-		-	-	-
$Post_{5-10}$	2.77 ± 0.78	-31.07*	1.25	Large	0.24	5.00	.001
$Post_{10-15}$	2.88 ± 0.74	-28.17*	1.13	Moderate	0.21	5.30	<.001
$Post_{15-20}$	2.94 ± 0.82	-26.79*	1.08	Moderate	0.20	5.27	.001
$Post_{20-25}$	2.91 ± 0.76	-27.49*	1.10	Moderate	0.22	4.99	.001
$Post_{25-30}$	3.19 ± 0.70	-20.49*	0.82	Moderate	0.22	3.69	.005
HV							
Pre	4.16 ± 0.43	-	-		-	-	-
$Post_{5-10}$	2.62 ± 0.73	-36.92*	1.54	Large	0.24	6.19	<.001
$Post_{10-15}$	2.60 ± 0.56	-37.53*	1.56	Large	0.18	8.47	<.001
$Post_{15-20}$	2.69 ± 0.54	-35.39*	1.47	Large	0.16	8.80	<.001
$Post_{20-25}$	3.02 ± 0.43	-27.48*	1.14	Moderate	0.15	7.32	<.001
$Post_{25-30}$	3.04 ± 0.42	-26.92*	1.12	Moderate	0.16	6.61	<.001

Notes: LnRMSSD = natural log transformation of the square root of the mean of the sum of the square of differences between successive RR intervals; M = mean; SD= standard deviation; Δ (%) = percent change from pre-exercise measure; MD = mean difference; ES = effect size; SE = standard error; *significantly different between sessions ($p \leq .05$).

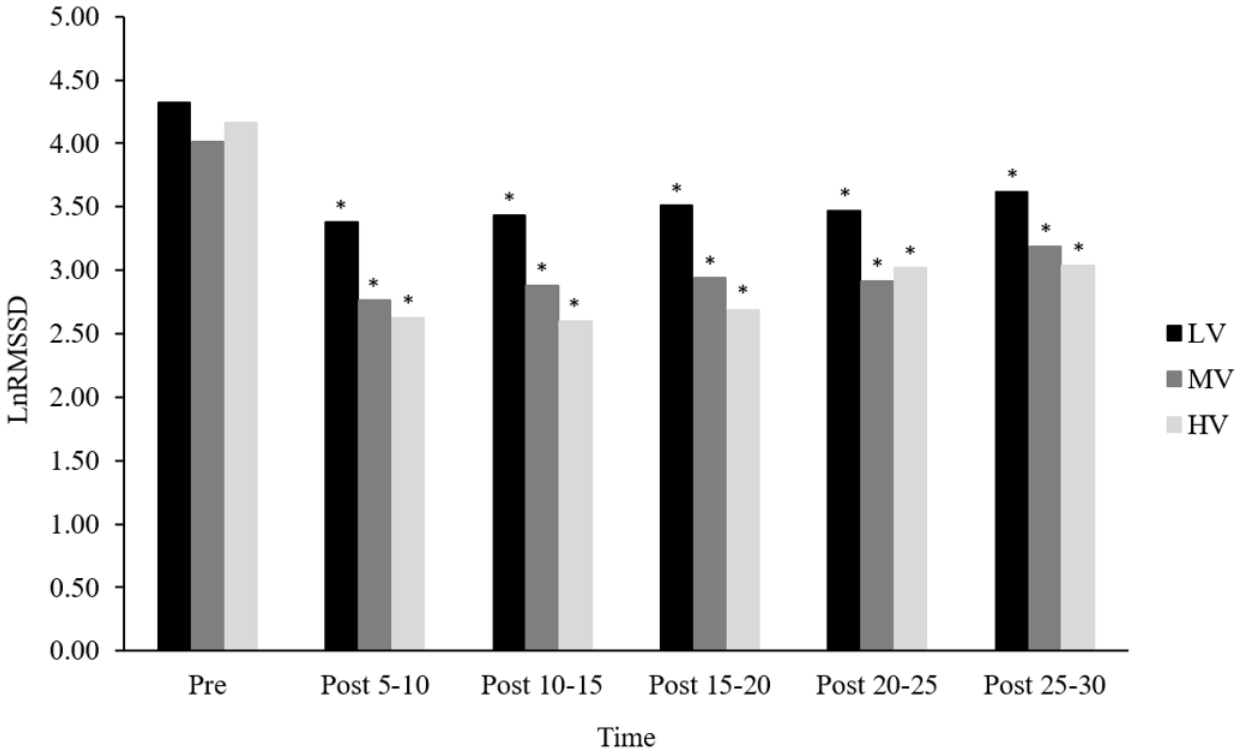

Figure 2.1. LnRMSSD response to three resistance training exercise sessions. LnRMSSD = log-transformation of the root mean square of successive RR differences; LV = low volume; MV = moderate volume; HV = high volume; *significant difference from resting pre-exercise in the same session; †significant difference between the same post-exercise measure of different sessions ($p \leq .05$).

CHAPTER 3

COMPARISON OF HEART RATE VARIABILITY CHANGES TO MEASURES OF TRAINING LOAD FOLLOWING RESISTANCE EXERCISE

ABSTRACT

Purpose: The current study aimed to determine the relationship between pre-post changes in heart rate variability and neuromuscular performance and biochemical fatigue markers in response to resistance exercise. HRV changes via the root mean square of successive RR interval differences (RMSSD) were compared to neuromuscular performance (IHG, CMJ, and MPV), metabolic stress (Lac), and inflammatory (IL-6) responses to resistance exercise. **Methods:** Thirty resistance-trained participants (30% female, 23.77±5.44 yrs., 171.47±21.69 cm, 76.36±12.84 kg) performed a resistance exercise protocol consisting of Back Squat (BS), Bench Press (BP), and Bent-over Rows (BR). A 10-min pre- and 30-min post-exercise HRV measure were taken using an electrocardiogram. Pre-post exercise HRV was compared to changes in blood lactate (Lac), interleukin-6 (IL-6), IHG, CMJ, and MPV for BS (MPV_{BS}) and BP (MPV_{BP}). Paired-samples *t*-tests were used to assess pre-post exercise differences. Pearson's correlations were used to compare pre-post exercise delta values. **Results:** Lac concentrations were only obtained from 23 participants and plasma IL-6 concentrations were only obtained from 16 participants. Statistically, significant mean differences were found between all pre- and post-exercise variables, except for IL-6 ($p = .296$) and MPV_{BP} ($p = .678$). Statistically significant correlations were observed with ΔLnRMSSD $Post_{5\text{-}10}$ and ΔLac ($r = -0.440$, $p = .036$), and ΔLnRMSSD $Post_{5\text{-}10}$ and ΔLac $Post_{30}$ ($r = -0.549$, $p < .001$). **Conclusion:** Metabolic and

cardiovascular alterations demonstrate significant associations in response to resistance exercise. However, practitioners should avoid using indirect methods to represent the responses of an alternate variable but instead, use a battery of testing protocols to gain a more accurate depiction of an individual's recovery status and readiness to perform.

INTRODUCTION

Resistance exercise is an established method for aiding in these abilities through enhanced muscular hypertrophy, power, and strength over time (1). It is well-incorporated into most athletic programs at all levels to enhance sports performance. To see these adaptations, athletes must experience a certain level of stress that disrupts homeostasis, while maintaining an appropriate balance with recovery to avoid potential non-functional overreaching (NFOR) or overtraining syndrome (1). For these reasons, objective methods for quantifying training loads from resistance exercise are essential for sports coaches, strength and conditioning specialists, and exercise physiologists.

Heavy resistance exercise is regarded as an exclusively anaerobic activity, characterized by its high energy requirement, provided by the catabolism of phosphocreatine and glucose (2). The reliance on fast glycolytic pathways increases with repeated sets, which in turn results in blood lactate (Lac) accumulation. In addition to metabolic stress from by-products, exercise-induced muscle damage (EIMD) can occur, especially when in individuals that are unaccustomed to this type of exercise stimulus. Exercise-induced muscle damage has been identified as a mechanism of skeletal muscle adaptations specifically regarding hypertrophy training (3, 4). Following a damaging exercise bout, a cascade of cellular inflammatory signals is triggered to facilitate the repair and regeneration of damaged muscle tissue through increased

protein synthesis and satellite cell mobilization for myonuclear domain synthesis (5). Within this cascade of cellular signals is the release of inflammatory biomarkers know as cytokines with interleukin-6 (IL-6) one of the most notable. Due to these various physiological responses to heavy resistance exercise, criterion internal training loads are determined by the significant or non-significant increases of these biomarkers associated with metabolic stress (6), muscle damage (7), and inflammation (8). However, the blood draws required to collect samples are highly invasive and the analysis of them requires expensive equipment, time, and knowledgeable personnel. For these reasons, more practical alternatives are needed for practitioners in conditions outside of traditional laboratory settings.

Because resistance exercise and sport-specific movements rely on high rates of force development (9), being able to measure force production and movement velocity is desirable when monitoring neuromuscular performance. Isometric handgrip (IHG), countermovement jump (CMJ), and mean propulsive velocity (MPV) are common tools for gauging neuromuscular performance and are indirect indicators of both peripheral and central fatigue (10, 11). Coaches and physiologists alike have begun using IHG, CMJ, and MPV as external training load markers reflecting fluctuations in sports performance (12-14). Though these measures have shown promise as indicators of neuromuscular fatigue and predictors of performance, some major limitations still exist including expense, the complexity of data analysis, and an inability to cater to a large number of athletes in team-sport settings. Also, it has been proposed that these measures may only be reflective of the isolated muscle groups involved during their testing, which calls into question their ecological validity as markers of neuromuscular fatigue with the functional, compound movements (13). Since IHG, CMJ, and MPV are all external measures of

fatigue, an internal training load marker may be necessary for a more integrative approach of monitoring resistance exercise (15).

Heart rate variability (HRV) has become a popular tool for monitoring autonomic responses to stressors which demonstrates the potential for predicting NFOR (16, 17). Through the use of HRV, post-exercise parasympathetic reactivation has been shown as a potential marker of internal training load (18). The root mean square of successive R-R interval differences (RMSSD), a parasympathetically-derived marker of HRV, is a highly used variable as a potential marker of internal training load due to its lower coefficient of variation compared with other indices (15). However, research is still lacking in regards to short-term RMSSD measures being a valid internal marker of fatigue from resistance exercise. Therefore, the aim of the present study was to determine the relationship between pre and both immediately and 30-minutes post changes in HRV and neuromuscular performance and biochemical fatigue markers in response to a full-body, hypertrophic-style resistance exercise bout. Heart rate variability changes were compared to neuromuscular performance (isometric handgrip, countermovement jump, and mean propulsive velocity), metabolic stress (lactate), and inflammatory (interleukin-6) responses to resistance exercise. We hypothesized that HRV responses from pre- to immediately post-exercise would display a strong association with lactate changes, while pre- to 30-minutes post-exercise would display significant associations with all fatigue markers.

METHODS

Participants

Thirty healthy, resistance-trained males (n = 21) and females (n = 9) participated in this study. Descriptive statistics of physical and functional characteristics can be seen in Table 3.1.

Participants were classified as well resistance-trained (i.e. advanced) if they had at least one year of resistance training experience consisting of at 2-3 sessions per week (19). Inclusion criteria required all participants to have engaged in regular exercise leading up to the study and be free from cardiovascular, metabolic, musculoskeletal and renal diseases or signs and symptoms during participation in the current study (20). This project was approved by the University of Alabama Institutional Review Board and conformed to the Declaration of Helsinki.

Experimental Design

The study required participants to visit the laboratory three times over approximately two weeks. All testing sessions began between 6 and 11 a.m. to control diurnal variations (21) and participants reported to the laboratory within the same 2 hour period for every session. During the initial visit, participants were given screening questionnaires, completed a written informed consent form, and were familiarized with all testing procedures. During the second visit, anthropometrics and skinfold measurements were collected. Short-term (i.e. 5-min stabilization and 5-min recording) HRV and blood pressure measurements were taken before and after 1-repetition maximum (1RM) testing for Back Squat (BS), Bench Press (BP), and Bent-over Rows (BR). During the third visit, participants performed a high-volume, full-body resistance exercise protocol. A 10-min pre-exercise HRV and 30-min post-exercise HRV measure were taken during this session. Pre- and post-exercise HRV measurements were compared to pre-post changes in the following measures: Lac, IL-6, IHG, CMJ, and MPV for BS (MPV_{BS}) and BP (MPV_{BP}). A summary graphic illustration of the experimental design can be seen in Figure 3.1.

Anthropometrics and Body Composition

Upon arrival at the laboratory, anthropometrics and body composition measures were taken during the second visit. Participants were asked to come to the laboratory having refrained

from eating any heavy meals (≤300 calories) or drinking beverages other than water (≤ 500 mL) 2 hr before arrival. Nude body mass was measured (to the nearest 0.1 kg) with a calibrated digital scale (Tanita BWB-800, Tanita©, Arlington Heights, IL) and standing height was measured (to the nearest 0.1 cm) with a stadiometer (SECA 213, Seca Ltd., Hamburg, Germany). Two measurements (within 2 mm of each other) of skinfold (SKF) thickness were taken using calibrated skinfold calipers (Lange Skinfold Caliper, Seko, USA) across 7 standard sites on the right side of the body. Percent body fat (%BF) from SKF was calculated using the Brozek equation (%BF = [(4.57/Db) – 4.142] x 100) (22).

One-Repetition Maximum (1RM) Tests

Participants completed 1RM testing for BS, BP, and BR using procedures adapted from previously reported protocols (19). For BS, participants were instructed to get to a bottom position where either the femur was parallel with the ground or the peak of the anterior portion of the thigh was perpendicular to the top of the patella. For BP, participants maintained five points of contact and touched the bar to the chest before locking out the repetition with arms fully extended. For BR, participants unracked the bar and proceeded to flex at the hip until the torso was approximately parallel with the ground. For each repetition, the participants were then instructed to touch the bar to the torso, between the umbilicus and the xiphoid process and end with arms fully extended. Each repetition began and ended with the arms fully extended and the bar elevated off the ground. The warm-up consisted of 5 minutes of cycling, ~5 minutes of self-selected dynamic stretches (i.e. arm circles, lunges, walking hamstring stretches, etc.), 10 repetitions with the unloaded Olympic barbell, 5 repetitions at 50%, 3 repetitions at 70%, and 1 repetition at 85% of 1RM. The participants then attempted their self-reported 1RM. Upon a successful attempt, the load was increased by 2-10 kg per attempt until technical/volitional

failure was reached. A minimum of 180 s of rest was given between each attempt to ensure adequate recovery (19).

Resistance Exercise Protocol

At least 72 hr after the 1RM testing was completed, participants performed a high set volume bout of resistance exercise. Participants were asked to come to the laboratory having refrained from the ingestion of alcohol for 24 hr, caffeine for 12 hr, and any heavy meals 2 hr before the arrival. The warm-up consisted of 5 minutes on a cycle ergometer, ~5 minutes of self-selected dynamic stretches, and 10, 5, and 3 repetitions of BS and BP with the unloaded bar, 30% and 50% of 1RM, respectively. Upon completion of the warm-up, participants completed a full-body, free-weight protocol consisting of 6 sets of 10 repetitions of BS, 3 sets of 10 repetitions of BP, and 3 sets of 10 repetitions of BR. The relative load was 70% of 1RM for all exercises with 120 s of rest between each set and 180 s of rest between each exercise (23, 24). If a participant reached failure during a set before completing the prescribed 10 repetitions, 30-60 s of rest was given before continuing with the set. This process was repeated until 10 repetitions were completed.

Biomarkers

Blood samples were collected via antecubital region venipuncture pre- and 30 minutes post-exercise (~75 minutes after the initiation of exercise) following the collection of HRV measures. Approximately 10ml of venous blood was drawn during both collections to measure the production of Lac and expression of plasma IL-6 concentrations. Additionally, capillary blood samples were drawn from the fingertip immediately following exercise. Lactate measurements for statistical analysis occurred pre-exercise, immediately post-exercise (IP) and approximately 30-min post-exercise ($Post_{30}$), while IL-6 measures were pre- and 30-min post.

All Lac concentrations were analyzed using the Lactate Plus handheld analyzer (Nova Biomedical, USA). All plasma samples were stored at -80° C until ready for analysis. High-sensitivity enzyme-linked immunosorbent assay (Abcam, Cambridge, MA USA) was used to measure the plasma concentration of IL-6, following the manufacturer's instructions and using reagents from the cytokine reagent kit.

Neuromuscular Performance Measures

The collection of all neuromuscular performance variables took place pre- and post-exercise following the recording of HRV and the collection of blood samples. Isometric handgrip was assessed using a Jamar dynamometer (G.E. Miller, Inc., Yonkers, New York). Participants were in a sitting position with a neutral grip and the elbow flexed at a 90° angle. Three attempts were allowed for each hand with 1-min of rest between attempts. The average of the three attempts was calculated and the peak force (kg) between the hands was used for further analysis. Next, CMJ was measured using portable force plates (Kistler 9286ba 10kn, Switzerland). Participants performed three jumps with 180 s of rest between each jump. Participants were instructed to stand at the center of the force plates and jump as high as possible while maintaining their hands on their hips. The average jump height from the three attempts was calculated and used for further analysis (25). Finally, movement velocity was monitored using a linear position transducer (GymAware PowerTool, Kinematic Performance Technology in Canberra, Australia). Mean propulsive velocity (m/s^{-1}) of Back Squat (MPV_{BS}) and Bench Press (MPV_{BP}) was measured using 60 and 50% of 1RM, respectively (26, 27). The MPV was averaged from the three repetitions of both exercises (26).

Heart Rate Variability

The HRV data collection procedures were based on standardized methods and validated alternative methods reported in previous studies involving groups of collegiate athletes (17, 16). Heart rate variability measures were collected using electrocardiography (ECG). The ECG signals were collected with an electronic signal acquisition system (BIOPAC MP150 Physiograph), which was connected to a Dell PC. Acknowledge software (v 4.4, BIOPAC, Goletta, CA, USA) was used to collect real-time ECG. Electrocardiogram assessment was performed with a modified lead II configuration where three surface electrodes (BIOPAC EL504 disposable Ag-AgCl) were placed on the participant: 1) near the right shoulder along the midclavicular line, 2) fifth intercostal space along the midaxillary line, and 3) near the iliac crest of the left hip along the midclavicular line. Participants were in a quiet room maintained at a temperature of 20°-23° C. Recordings took place in the seated position to reduce any possible parasympathetic saturation which is often observed in individuals with low resting heart rates. Once seated in a neutral position with their feet on the ground and arms supported in a resting position by a table at approximately waist height, participants were given a 5-min stabilization period to limit any bodily movement and establish normal breathing patterns.

Two separate ECG recordings were obtained during the session; one 10-minute segment pre-exercise and one 30 minute segment immediately after the exercise bout. The pre-exercise ECG measurement consisted of 5-min stabilization followed by a 5-min recording. For comparative analysis against fatigue markers, two different time points were extracted from the 30-min post-exercise ECG measurement: the 5-10-min segment ($Post_{5\text{-}10}$) and 25–30-min segment ($Post_{25\text{-}30}$). The HRV time-domain metric of RMSSD was solely investigated due to its strong representation of parasympathetic modulation and its ability to be unaffected by spontaneous breathing frequencies (28). However, it is common among HRV variables over a

sample of participants to be skewed (29) so to ensure a normal distribution of data, the natural logarithm of RMSSD (LnRMSSD) was evaluated instead. All ECG segments were visually inspected using the Acknowledge software for any potential motion-related artifact noted during collection not indicative of true ectopic/non-sinus beats before being transformed into a tachogram and exported into Kubios HRV Standard 3.3.0 software (Biosignal Analysis Medical Imaging Group at the Department of Applied Physics, University of Kuopio, Kuopio Finland). Occasional artifact noise was automatically replaced with the interpolated adjacent RR interval values (threshold = 0.45 s or "very low"), amounting to ≤3% of error correction. The analysis process was carried out by the same researcher to ensure consistency (30, 31). Blood pressure assessments were conducted following baseline HRV, immediately after fingerprick Lac collection, and after the 30-min recovery period. Assessments were done with the BPM-100 automated blood pressure monitor (BpTRU Medical Devices; Coquitlam, Canada) at least three times, two minutes apart in the dominant arm (32, 33).

Statistical Analysis

All data were analyzed with IBM SPSS version 25.0 for Windows (Somers, NY) and Microsoft Excel 2016 for Windows (Microsoft Corporation, Redmond, WA). A Shapiro-Wilk test was used to determine if the collected variables were normally distributed. Paired-samples *t*-tests were used to compare pre-post exercise IHG, CMJ, MPV, Lac, IL-6, and LnRMSSD ($Post_{5-10}$ and $Post_{25-30}$) values. The magnitudes of the pair-wise differences were quantified using Cohen's *d* effect size (ES) and were classified as trivial (0.0-0.2), small (0.2-0.6), moderate (0.6-1.2), large (1.2-2.0), and very large (>2.0) (34, 35). Additionally, The coefficient of variation (CV%) and the smallest worthwhile change (SWC) were calculated for each dependent fatigue variable to detect meaningful changes from pre- to post-resistance exercise. The delta change

score (Δ) from pre-post exercise was calculated for LnRMSSD, neuromuscular performance and fatigue markers using the following equation: pre-post = Δ. Pearson's product-moment correlation coefficients (*r*) were calculated to assess the association between delta values of LnRMSSD and biochemical fatigue markers (i.e. Lac and IL-6). Correlation values between 0 to 0.30 were considered small, 0.31 to 0.49 was moderate, 0.50 to 0.69 was large, 0.70 to 0.89 was very large, and 0.90 to 1.00 was near perfect (35). Unless otherwise stated, data were presented as mean ± standard deviation (*M* ± *SD*) and statistical significance was accepted at $p < .05$.

RESULTS

The mean ± standard deviation values for all fatigue measures are provided in Table 3.2. Thirty participants volunteered for the current study, however, Lac concentrations were only obtained from 23 participants and plasma IL-6 concentrations were only obtained from 16 participants. Due to the vast difference in data points between the biomarkers and other variables, separate statistical analyzes were done with participants with completed blood sample collections. Statistically, significant mean differences were found between all pre- and post-exercise variables, except for IL-6 ($p = .296$) and MPV_{BP} ($p = .678$) (see Table 3.3). Statistically significant correlations were observed with ΔLnRMSSD $Post_{5\text{-}10}$ and ΔLac IP ($r = -0.440$, $p = .036$), and ΔLnRMSSD $Post_{5\text{-}10}$ and ΔLac $Post_{30}$ ($r = -0.549$, $p < .001$) (see Table 3.3).

DISCUSSION

Heart rate variability is a valid tool for measuring autonomic modulation and has been regarded as a global marker of fatigue. The present study aimed to determine the relationship between pre-post changes in HRV and neuromuscular performance and biochemical fatigue markers in response to resistance exercise. Heart rate variability changes via LnRMSSD were

compared to neuromuscular performance (IHG, CMJ, and MPV), metabolic stress (Lac), and inflammatory (IL-6) responses to resistance exercise. We hypothesized that HRV would display significant associations with criterion fatigue markers. The current study found significant correlations between ΔLnRMSSD and ΔLac. Although significant reductions were observed in IHG, CMJ, and MPV_{BS}, no significant associations were found with LnRMSSD.

In the current study, significant reductions in parasympathetic activity following a high set volume bout of resistance exercise were observed with ΔLnRMSSD $Post_{5\text{-}10}$ displaying a 38.0% decrease and remaining lower than baseline values after the 30-min recovery period. This falls in line with previous research that has concluded that acute bouts of resistance exercise can greatly reduce parasympathetic activity during the recovery period (36). Similar to the current study, high set volume protocols imitating hypertrophying workouts have been shown to greatly reduce RMSSD measures pre-post and delay parasympathetic reactivation during 30 minutes of recovery (23, 37). The significant reduction and delayed reactivation of parasympathetic activity displayed in previous research as well as the current study, demonstrate the potential ability of HRV to detect accumulated stress from resistance exercise through autonomic modulation and cardiorespiratory responses. Based on this cause-effect relationship, it was thought that HRV may have a strong connection with neuromuscular performance. Chen et al. (36) had seven weightlifters complete a 2-hour bout of heavy resistance exercise and found that HRV recovery patterns followed a similar trend as weightlifting performance with both reaching peaks at the 72-hour time point. It was suggested that HRV may mirror changes in neuromuscular fatigue due to parallels with weightlifting performance (36). Though only an acute recovery period was utilized in the current study, the findings conflict with the previous conclusion with no strong link being observed between HRV and neuromuscular responses.

In the current study, the general concept of fatigue was denoted by changes in neuromuscular performance assessments and biomarkers for metabolic stress and inflammation attributed to skeletal muscle damage. These measures were chosen due to their relationships with different resistance exercise protocols displayed in previous literature (26, 38). Medina et al. (26) saw significant decreases in MPV for back squat (6.1-21.3%), bench press (5.2-32.8%) and CMJ height (5.7-19.3%), as well as strong correlations between Lac responses and the loss in MPV pre-post exercise for both squat (r = 0.93) and bench press (r = 0.97) following resistance exercise (26). Custodio et al. (38) examined the percent loss in velocity, jump height, and increases in lactate following four different back squat protocols. Significant differences from pre- to post-exercise were seen in all protocols for MPV (9.1-27.4%), CMJ (13.6-21.5%) and Lac (2.6-6.7 mmol/L) (38). Although Medina et al. and Custodio et al. did not incorporate HRV measures, considering the significant changes in HRV following resistance exercise displayed other literature, the assumption of strong associations between the variables was thought to exist.

The present study found significant pre-post changes in all neuromuscular performance metrics, except for MPV_{BP}, with exposure to a high volume bout of resistance exercise. However, ΔLnRMSSD values were not significantly associated with Δneuromusclar performance changes. Though these findings contradict our original hypothesis, recent studies corroborate the current study's results (39, 40). Flatt et al. (39) examined the associations between changes in HRV, perceptual recovery status (PRS) and neuromuscular performance measures (i.e. CMJ and bar velocity) at immediately, 24 hr, and 48 hr post-resistance exercise and observed significant differences from pre- to immediately post-exercise in all measures but found no strong correlations between ΔLnRMSSD and all other metrics (39). Thamm et al. (40) compared the HRV responses immediately post, 30 min, 1, 24, and 48 hr, following two different

resistance exercise protocols, to other recovery measures included maximal isometric voluntary contraction and rate of force development on the leg press, Lac and creatine kinase concentrations, and muscle pain scores using a visual analog scale. Measures of RMSSD significantly decreased immediately post-exercise but returned to baseline values within 30 min. for both protocols. Statistically significant correlations ($p < .06$) with RMSSD were only found between pre- to immediately post-exercise changes in maximal isometric voluntary contraction ($r = 0.433$, $p = .056$) and rate of force development ($r = 0.55$, $p = .012$), but not at any other time point (40). Based on the results of the current study in conjunction with the previous literature, it can be inferred that stress generated from resistance exercise can disrupt various physiological systems to different magnitudes and the time course of a return to homeostasis is not a singular, general concept but is instead comprised of multiple levels.

Moderate-to-large correlations were observed between LnRMSSD and Lac measures in the present study. Heavy resistance exercise is regarded as an exclusive anaerobic activity with a high rate of energy utilization through the breakdown of phosphocreatine and glucose (41, 42). As volume increases through the manipulation of the number of sets and repetitions, the reliance on glycolytic pathways increases, which in turn results in blood lactate accumulation. Due to this metabolic response, blood lactate concentrations have been used as a primary means of quantifying internal training load (41, 42). The increased levels of metabolic stress seen with resistance exercise may play a role in the drastic immediate decrease in LnRMSSD observed in the current study. With the onset of exercise, activation of central command from the motor cortex and higher areas of the brain causes parasympathetic withdrawal followed by an increase in sympathetic outflow. As resistance exercise continues, metabolic by-products, such as lactate and hydrogen ions are produced (43). Chemoreceptors stimulate muscle afferents (group III/IV)

which relay sensory information to the central nervous status which regulates cardiorespiratory responses downstream through the autonomic control. Continued sympathetic adrenergic outflow occurs in conjunction with a greater release of circulating catecholamines from the adrenal medulla (43). Once the exercise stimulus has ceased, parasympathetic reactivation back to baseline levels has been shown to vary dramatically, ranging from 30 minutes to 48 hr depending on the protocol being implemented (40, 36). It can be inferred that the greater the intensity (i.e. effort or load) and set duration of resistance exercise, the greater the metabolic stress and sympathetic response leading to a longer delay in parasympathetic reactivation (24, 23). The potential relationship between Lac concentrations and RMSSD is supported by previous research that found HRV alterations to be strongly related to lactate threshold during resistance exercise and a possible noninvasive, alternative measure for gauging anaerobic thresholds during continuous exercise (44, 45)

The results of the present study, in conjunction with those of previous research, suggest that different physiological systems (e.g. autonomic, neuromuscular and metabolic) follow varying timeframes for recovery in response to resistance exercise symbolizing different aspects of fatigue needing to be accounted for by practitioners (40, 39). In the current study, pre-exercise LnRMSSD (3.89 ± 0.62) significantly dropped at Post5 (2.43 ± 1.01) and displayed an upward trend in recovery by Post30 (3.25 ± 0.65), however, remaining significantly lower than baseline. Lactate responses to resistance exercise followed a similar timeframe: pre-exercise (1.04 ± 0.44), immediately post-exercise (11.93 ± 3.85), and 30-min post-exercise (2.33 ± 1.08). Conversely, the time-course of cytokines, specifically IL-6, increases can vary greatly depending on the magnitude of the stimulus, however, continual increases have been seen anywhere from 1-hour post-exercise to 48 hr (46). In the current study, within the small subset of 16 participants, IL-6

samples were collected at 30-min post-exercise, which seemed to be an insufficient amount of time to observe a significant increase in IL-6. Finally, neuromuscular performance has been shown to take up to 48 hr to recover to baseline levels (39, 40). Neuromuscular fatigue has been associated with decreased central drive due to neurotransmitter depletion, as well as a disruption of contractility due to muscle damage and metabolite accumulation (47). However, the exact mechanisms at play vary significantly depending on the stimulus being presented and the method utilized to measure neuromuscular responses. The current study contained only one post-exercise measure following the collection of HRV, not allowing for the observation of neuromuscular performance metrics to return to pre-exercise values.

Limitations

Several limitations of the present study should be emphasized. Measurements of HRV, neuromuscular performance, and biochemical fatigue markers were only collected within a 30-min period following exercise, however, multiple recordings over subsequent days may have revealed more details concerning physiological responses. Also, different resistance exercise protocols (e.g. low volume, heavy load) produce unique physiological responses. The current study only examined one specific style of resistance exercise. Future research should compare associations between fatigue markers to different exercises and volume-load schemes. Finally, Though both males and females were recruited for this study, the female-to-male ratio was too small to run a comparative analysis. Future research should increase sample sizes, equating female-to-male ratios to investigate potential differences in recovery patterns following high volume, full-body resistance exercise.

CONCLUSION

Because fatigue is defined as a disruption of homeostasis that can acutely or chronically diminish readiness to perform, it should be understood that multiple physiological systems play a role. Significant reductions were displayed in LnRMSSD, neuromuscular performance, and biochemical fatigue markers, but strong associations were only observed between ΔLnRMSSD and ΔLac. This relationship between metabolic and cardiovascular alterations suggests the potential efficacy of LnRMSSD as an internal training load marker for resistance exercise. However, practitioners should avoid using one method to represent the responses of another. Instead, a battery of testing protocols should be implemented to gain a more accurate depiction of an individual's recovery status and readiness to perform.

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Table 3.1

Physical and Functional Characteristics of the Participants (n = 30)

Descriptives	Participants
Age (yrs)	23.77 ± 5.44
Height (cm)	171.47 ± 21.69
Weight (kg)	76.36 ± 12.84
Body fat (%)	17.98 ± 6.98
Systolic (mmHg)	104.05 ± 16.79
Diastolic (mmHg)	69.97 ± 6.78
Resting Heart Rate (bpm)	67.13 ± 11.72
Back Squat (kg)	123.33 ± 38.06
Bench Press (kg)	86.06 ± 31.05
Bent-over Rows (kg)	73.35 ± 22.23

Notes: Data displayed as means ± standard deviations

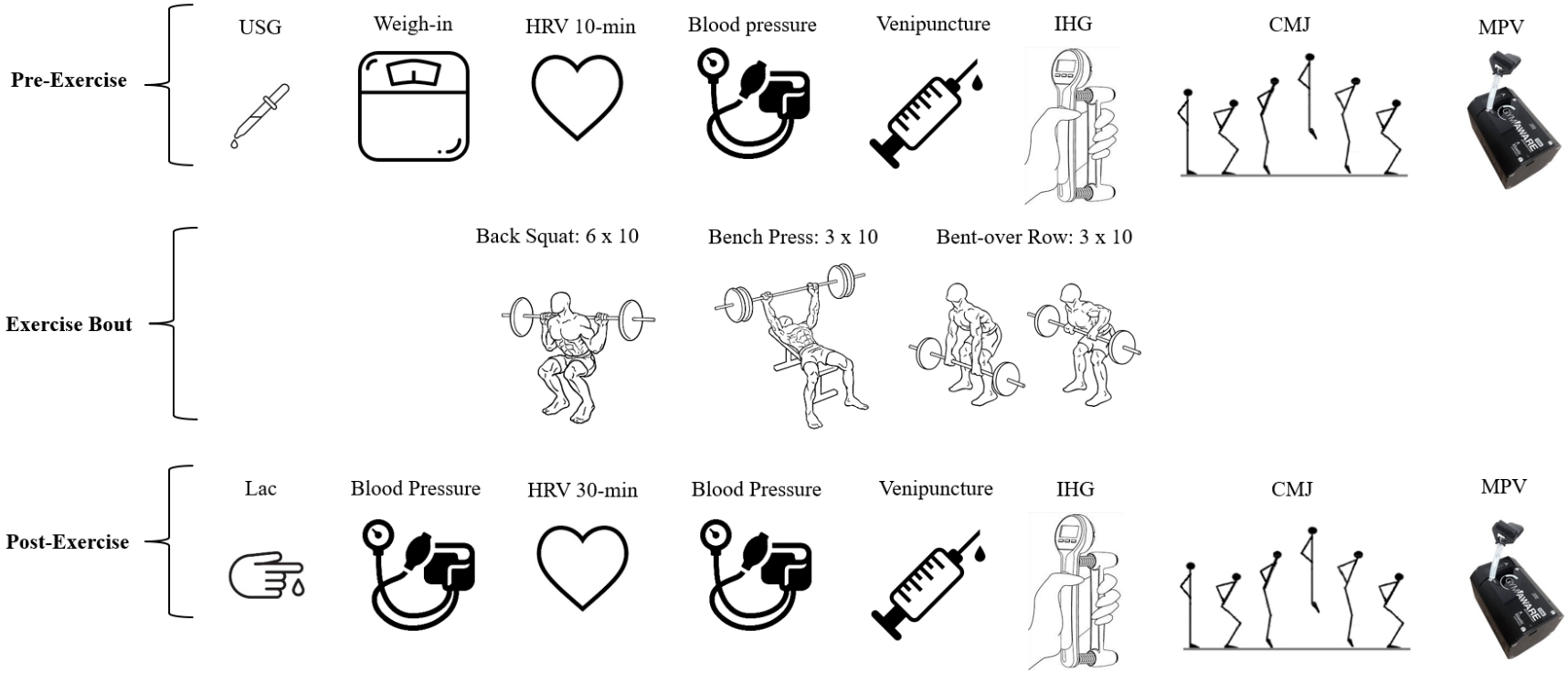

Figure 3.1. Exercise session protocol graphic summary. USG = urine specific gravity; IHG = isometric handgrip; CMJ = countermovement jump; MPV = mean propulsive velocity; BS = back squat; BP = bench press; BR = bent-over row; Lac = blood lactate

Table 3.2

Pre- and Post-Resistance Exercise LnRMSSD and Fatigue Measures

		N	*M* ± *SD*	MD	CV (%)	SWC	ES		*p*
LnRMSSD	Pre	30	3.89 ± 0.62	-	-	-	-	-	-
	$Post_{5-10}$	30	2.43 ± 1.01**	-1.45	-	-	2.36	Very Large	<.001
	$Post_{25-30}$	30	3.25 ± 0.65**	-0.64	1.45	±0.12	1.03	Moderate	<.001
IHG (kg)	Pre	30	43.77 ± 10.36	-	-	-	-	-	-
	Post	30	41.70 ± 11.29**	-2.07	6.56	±2.85	0.20	Trivial	<.001
CMJ (m)	Pre	30	0.35 ± 0.07	-	-	-	-	-	-
	Post	30	0.31 ± 0.07**	-0.04	4.63	±0.01	0.61	Moderate	<.001
MPV_{BS} (m/s)	Pre	30	0.72 ± 0.07	-	-	-	-	-	-
	Post	30	0.67 ± 0.07**	-0.05	4.46	±0.01	0.71	Moderate	<.001
MPV_{BP} (m/s)	Pre	30	0.77 ± 0.13	-	-	-	-	-	-
	Post	30	0.77 ± 0.11	-0.01	6.55	±0.03	0.04	Trivial	0.678
Lac (mmol)	Pre	23	1.04 ± 0.44	-	-	-	-	-	-
	IP	23	11.93 ± 3.85**	10.89	-	-	-25.03	Very Large	<.001
	Post30	23	3.33 ± 2.33**	2.29	12.53	±0.09	-5.27	Very Large	<.001
IL-6 (μg/ml)	Pre	16	6.80 ± 2.86	-	-	-	-	-	-
	Post	16	7.48 ± 3.12	0.68	42.09	±0.57	-0.24	Small	0.296

Notes: LnRMSSD = log-transformation of the root mean square of successive RR differences; *M* ± *SD* = mean ± standard deviation; MD = mean difference; CV = coefficient of variation; SWC = smallest worthwhile change; ES = effect size; IHG = isometric handgrip; CMJ = countermovement jump; MPV = mean propulsive velocity; BS = back squat; BP = bench press; Lac = blood lactate; IL-6 = interleukin-6; IP = immediately post-exercise; *significantly different from pre-exercise ($p \leq .05$); ** ($p \leq .001$).

Table 3.3

Correlations for Changes in LnRMSSD and Fatigue Measures

	N	ΔLnRMSSD $Post_{5-10}$		ΔLnRMSSD $Post_{25-30}$	
		r	*p*	*r*	*p*
ΔIHG	30	0.122	.520	-0.051	.791
ΔCMJ	30	0.245	.191	-0.108	.570
ΔMPV_{BS}	30	0.203	.281	-0.035	.856
ΔMPV_{BP}	30	-0.091	.632	-0.234	.213
ΔLac IP	23	-0.440*	.036	0.097	.659
ΔLac Post30	23	-0.549**	<.001	-0.306	.156
ΔIL-6	16	-0.484	.057	-0.184	.495

Notes: LnRMSSD = log-transformation of the root mean square of successive RR differences; IHG = isometric handgrip; CMJ = countermovement jump; MPV = mean propulsive velocity; BS = back squat; BP = bench press; Lac = blood lactate; IP = immediately post-exercise; IL-6 = interleukin-6; *significance $p \leq .05$; ** significance $p \leq .001$.

CHAPTER 4

VALIDITY OF SMARTPHONE HEART RATE VARIABILITY BEFORE AND POST-RESISTANCE EXERCISE

ABSTRACT

Purpose: The aim was to examine the validity of heart rate variability (HRV) measures from photoplethysmography (PPG) via smartphone application at rest and post-resistance exercise. The secondary aim was to examine the intraday and interday reliability of the smartphone PPG method. **Methods:** Thirty-one resistance-trained individuals (29% female) participated in this study. After familiarization, participants completed two simultaneous ultrashort-term electrocardiograph (ECG) and PPG measurements followed by 1-repetition maximum testing for Back Squat, Bench Press, and Bent-over Rows. During the third visit, participants performed a full-body resistance exercise protocol, where three simultaneous ultrashort-term ECG and PPG measurements of HRV were taken: two pre- and one post-exercise. The natural logarithm of the root mean square of successive RR differences (LnRMSSD) from PPG and ECG were compared with paired samples *t*-tests and Pearson product correlations. The agreement between LnRMSSD values was evaluated using Bland-Altman analysis. Intra-class correlations (ICC) were determined between PPG LnRMSSD. **Results:** Paired-samples *t*-tests showed small-moderate differences for all measurements between ECG and PPG: $Base_{Pre1}$ (p=.003), $Base_{Pre2}$ (p=.019), HV_{Pre1} (p=.036), HV_{Pre2} (p=.001), and HV_{Post} (p=<.001). Correlations between ECG and PPG ranged from moderate-very large ($Base_{Pre1}$=.59, $Base_{Pre2}$=.63, HV_{Pre1}=.626, HV_{Pre2}=.76, and HV_{Post}=.41, all p<.05). The agreement for all pre-exercise measures was

classified as “good” but the post-exercise agreement was deemed “insufficient”. The PPG LnRMSSD exhibited nearly-perfect intraday reliability (ICC=0.91) and very large interday reliability (ICC=0.88). **Conclusion:** The use of smartphone PPG seems to be an appropriate surrogate for ECG to obtain accurate and reliable HRV data at rest but deteriorates following resistance exercise.

INTRODUCTION

The monitoring of athletes’ training status has been a growing focus in exercise and sport science. The use of subjective and objective measures allows practitioners to gauge training loads, as well as an athlete’s ability to recover (1). However, accurate and practical measures of internal stress related to training, specifically in regards to resistance exercise, are uncommon. Heart rate variability (HRV) is defined as the oscillations that occur between successive heartbeats and is considered a non-invasive marker of autonomic nervous system (ANS) control of the cardiovascular system (2). Traditionally measured within clinical settings as a prognostic indicator of cardiovascular-related diseased states, the assessment of HRV has been utilized by athletes to indirectly examine ANS status in response to various training (3, 4) and has been demonstrated as a valid tool for assessing fatigue accumulation and recovery (5, 6). The majority of research involving HRV assessments occurred in controlled laboratory settings with sophisticated equipment, such as an electrocardiogram (ECG) (7). The criterion method of ECG for acquiring HRV data requires 10-min short-term measures consisting of a 5-min stabilization period followed by a 5-min recording period (2). Unfortunately, this presents a time constraint when monitoring athletes in field-conditions, especially when daily measures are recommended for determining recovery status throughout a training period (6).

Technological advances have allowed HRV to be collected with mobile devices. A recent meta-analysis consisting of twenty-three studies concluded that portable devices yielded small but acceptable ranges of error in comparison to ECG (effect size=0.23, 95% CI: 0.05, 0.42) (7). Photoplethysmography (PPG) has been proposed as a surrogate to traditional ECG for the measurement of HRV (8). The PPG method is a noninvasive optical technique for monitoring beat-to-beat relative blood volume changes in the microvasculature of peripheral tissues. The pulse rate variability (PRV) of the PPG signal has been highly correlated to both time and frequency-domain metrics from ECG-derived HRV indices (9-12). Several PPG-based HRV smartphone applications have been validated in the literature (7, 13-15) for acquiring the parasympathetically-derived marker of the root mean square of successive R-R interval differences (RMSSD) (13, 16, 17). Previous research has suggested that RMSSD is the preferred HRV metric for field recordings, primarily because it can be accurately measured with an ultra-shortened recording time of only 1 minute (16) following a 1-min stabilization period (18).

Examining the effects of training on HRV has primarily been performed using single day analyses in pre-to-post study designs. However, isolated measures do not account for the fluctuations in HRV that occurs daily (4). Because of this, it is recommended to measure HRV on a near-daily basis, with at least three days per week as a minimum requirement (19, 4). The daily measurement of HRV has recently become more feasible because of the emerging smartphone PPG technology mentioned above. However, several concerns related to the agreement between mobile PPG smartphone applications and ECG for HRV determination exist; hence further research is necessary. For instance, physical and mental stimuli have been suggested to decrease the agreement for HRV assessment between PPG and ECG (12). Furthermore, only a few studies have validated smartphone PPG technology for measuring HRV,

and there is no existing research to determine the reliability of such an approach across a period of days or following physical exercise. This is a significant gap in the literature, considering the recommendation of daily HRV assessment in athletes and the stressors received from frequent training. Therefore the present study aimed to examine the validity of HRV measures from a photoplethysmography (PPG) smartphone application under resting and post-full-body, hypertrophic-style resistance exercise conditions. The secondary aim was to examine the intraday and interday reliability of the PPG method. It was hypothesized that PPG and ECG measures of HRV would display strong relationships and no significant differences being found pre- or post-exercise recording times. Additionally, it was hypothesized that the PPG application would demonstrate intraclass correlations of "near-perfect" levels for intra- and inter-day reliability.

METHODS

Participants

Thirty-one healthy, resistance-trained males (n = 22) and females (n = 9) participated in this study. Descriptive statistics of physical and functional characteristics can be seen in Table 4.1. Participants were classified as "advanced" resistance-trained from having at least one year of resistance training experience consisting of at 2-3 sessions per week (20). Participants were free from the presence of symptoms of cardiovascular, metabolic, musculoskeletal and renal disorders or diseases (21). This project was approved by the University's Institutional Review Board and conformed to the Declaration of Helsinki.

Experimental Design

The study required participants to visit the laboratory three times over approximately two weeks. All testing sessions began between 6 and 11 a.m. to control diurnal variations (22) and participants reported to the laboratory within the same 2 hour period for every session. During the initial visit, participants provided written informed consent, completed screening questionnaires, and were familiarized with all testing procedures. During the second visit, anthropometrics and skinfold measurements were collected. Next, two ultrashort-term (i.e. 5-min stabilization and 1-min recording) ECG and PPG measurements of HRV were taken simultaneously measured. Blood pressure assessment occurred between the two HRV recordings. After HRV assessment, 1-repetition maximum (1RM) testing for the Back Squat (BS), Bench Press (BP), and Bent-over Row (BR) exercises were performed. During the third visit, participants performed a high-volume, full-body resistance exercise protocol that involved the three resistance exercises. Three simultaneous ultrashort-term HRV recordings were performed with ECG and smartphone PPG as follows: two recordings occurred pre-exercise and another occurred five minutes post-exercise. A summary graphic illustration of the experimental design can be seen in Figure 4.1.

Baseline Session

Upon arrival at the laboratory, anthropometrics and body composition measures were taken during the second visit. Participants were asked to come to the laboratory having refrained from eating any heavy meals (≤ 300 calories) or drinking beverages other than water (≤ 500 mL) 2 hr before arrival. Nude body mass was measured (to the nearest 0.1 kg) with a calibrated digital scale (Tanita BWB-800, Tanita©, Arlington Heights, IL) and standing height was measured (to the nearest 0.1 cm) with a stadiometer (SECA 213, Seca Ltd., Hamburg, Germany). Two measurements (within 2 mm of each other) of skinfold (SKF) thickness were taken using

calibrated skinfold calipers (Lange Skinfold Caliper, Seko, USA) across 7 standard sites on the right side of the body. Percent body fat (%BF) from SKF was calculated using the Brozek equation (%BF = [(4.57/Db) – 4.142] x 100) (23).

Following the completion of SKF testing, HRV and blood pressure assessments were performed. The HRV data collection procedures were based on standardized recommendations with ECG (2) and validated alternative (i.e., smartphone PPG) methods reported in previous studies involving groups of collegiate athletes (18, 24). Heart rate variability was assessed in the seated position using both criterion ECG methods and a previously validated (15) smartphone application, HRV4Training (see: https://www.hrv4training.com/). The HRV4Training application utilizes a configuration of PPG referred to as "transmission mode", where the blood perfused tissue (i.e. fingertip) is placed over the source of light (i.e. flash) and the detector (i.e.camera) simultaneously (12). The camera records small variations in light absorption as beat-by-beat capillary blood volume fluctuates between successive systolic and diastolic cardiac cycles (25). The ECG signals were collected with an electronic signal acquisition system (BIOPAC MP150 Physiograph) connected to a Dell PC. Acknowledge software (v 4.4, BIOPAC, Goletta, CA, USA) was used to collect real-time ECG. ECG assessment was performed with a modified lead II configuration where three surface electrodes (BIOPAC EL504 disposable Ag-AgCl) placed at the following anatomical locations: 1) negative polarity over the right midclavicular notch, 2) positive polarity over the fifth intercostal space along the midaxillary line, and 3) ground placement over iliac crest of the left hip along the midclavicular line. During all HRV procedures, recordings took place in a quiet room maintained at a temperature of 20°-23° C. The seated position was assumed in order to reduce the possibility of parasympathetic saturation that may be observed in individuals with low resting heart rate. Heart rate variability

measurements consisted of a 1-min recording timeframe following a 5-min stabilization period with spontaneous breathing patterns throughout.

The procedures for determining reliability among the HRV measures were adapted from previous research (26). The reliability of PPG measures was assessed via two components: intraday, comparing measures collected within the same day (i.e. baseline session), and interday, comparing measures collected on two separate days (i.e. baseline and exercise session). Reliability was only assessed between measures taken under resting conditions to avoid measurement discrepancies due to vast differences in physiological states during pre- and post-resistance exercise. The recordings took place at the following time points: twice during the baseline line visit ($Base_{Pre1}$ and $Base_{Pre2}$); twice before (HV_{Pre1} and HV_{Pre2}) and once 5 minutes after (HV_{Post}) the high volume resistance exercise session. Successive recordings during a specific period ($Base_{Pre1}$ and $Base_{Pre2}$, HV_{Pre1} and HV_{Pre2}) were separated by 10 minutes of quiet rest (see Figure 4.1). Each recording commenced when the tip of the right index finger was position to cover the camera and flash of the smartphone (iPhone 6, Apple Inc., Foxconn, Pegatron). A technician manually marked the ECG within the Acknowledge software at the precise commencement and completion of the 1-minute PPG recording (15, 13). For consistency of the measure, the same smartphone device was used for all measurements. Blood pressure changes have been shown to affect PRV, thus leading to discrepancies in HRV measurements (27, 28). For this reason, additional blood pressure assessments, outside of screening purposes, were done to aide in explaining any possible differences seen in HRV between ECG and PPG. Assessments were conducted with the BPM-100 automated blood pressure monitor (BpTRU Medical Devices; Coquitlam, Canada) at least 3 times, 2 minutes apart in the dominant arm (29, 30). Resting blood pressure was determined by averaging 3 readings that agree within 5 mmHg

for both systolic and diastolic blood pressure according to AHA/ACC protocols (30, 29). If systolic and/or diastolic blood pressure did not agree within 5 mmHg across the first 3 readings, additional blood pressure measurements were performed until an agreement was reached.

The RMSSD metric was solely investigated due to its previous validation with ultrashort-term measures (16) and with the current smartphone application (15). All ECG segments were visually inspected using the Acknowledge software for any potential motion-related artifact noted during collection not indicative of true ectopic/non-sinus beats before being transformed into a tachogram and exported into Kubios HRV Standard 3.3.0 software (Biosignal Analysis Medical Imaging Group at the Department of Applied Physics, University of Kuopio, Kuopio Finland). Occasional artifact noise was automatically replaced with the interpolated adjacent RR interval values (threshold = 0.45 s or "very low"), amounting to ≤ 3% of error correction. The analysis process was carried out by the same researcher to ensure consistency (31, 26, 32, 33). For PPG measures, erroneous data was discarded from any recording. The data-capturing application was designed to inform the user whether data were of sufficient quality or not. The automated and proprietary algorithm of the application identifies periods of high noise by analyzing the percentage of discarded RR intervals over a given time (15). High noise periods were based on when timing differences were outside of expected or normal values typically due to underlying noise or ectopic beats (15). In cases where the RMSSD data attained was inappropriate due to participant error (e.g. movement of the finger over the camera), the participant was informed and ECG data was discarded. A total of up to three measurements were taken to achieve a signal quality of "good" to "optimal", otherwise, data from the third measurement was saved for analysis. Measurements were taken consecutively without additional stabilization periods and participants were given instructions to improve signal quality as

provided by the HRVTraining website (see: https://www.hrv4training.com/). Traditionally used short-term recordings of HRV consist of a 5-min recording period. The researchers of the current study limited attempts at three since additional measurements with smartphone PPG would eclipse this time period, thus eliminating the practicality of ultrashort-term measures.

After the collection of HRV and blood pressure, participants completed 1RM testing for BS, BP, and BR using procedures adapted from previously reported protocols (20). For BS, participants were instructed to get to a bottom position where either the femur was parallel with the ground or the peak of the anterior portion of the thigh was perpendicular to the top of the patella. For BP, participants maintained five points of contact and touched the bar to the chest before locking out the repetition with arms fully extended. For BR, participants unracked the bar and proceeded to flex at the hip until the torso was approximately parallel with the ground. For each repetition, the participants were then instructed to touch the bar to the torso, between the umbilicus and the xiphoid process and end with arms fully extended. Each repetition began and ended with the arms fully extended and the bar elevated off the ground. The warm-up consisted of 5 minutes of cycling, ~5 minutes of self-selected dynamic stretches (i.e. arm circles, lunges, walking hamstring stretches, etc.), 10 repetitions with the unloaded Olympic barbell, 5 repetitions at 50%, 3 repetitions at 70%, and 1 repetition at 85% of 1RM. The participants then attempted their self-reported 1RM (20). Upon a successful attempt, the load was increased by 2-10 kg per attempt until technical or volitional failure is reached. A minimum of 180 s of rest was be given between each attempt to ensure adequate recovery (34, 35, 20, 36).

Resistance Exercise Session

At least 72 hr after the 1RM testing was completed, participants performed a full-body bout of resistance exercise. Participants were asked to come to the laboratory having refrained

from the ingestion of alcohol for 24 hr, caffeine 12 hr, and any heavy meals 2 hr before the arrival (37). The following procedures done during the baseline session were repeated before exercise: hydration status assessment, nude body mass, simultaneous ECG and PPG recording, and blood pressure measurement. The warm-up consisted of 5 minutes on a cycle ergometer, ~5 minutes of self-selected dynamic stretches, and 10, 5, and 3 repetitions of BS and BP with the unloaded bar, 30% and 50% of 1RM, respectively. Upon completion of the warm-up, participants completed the resistance exercise protocol consisting of 6 sets of 10 repetitions of BS, 3 sets of 10 repetitions of BP, and 3 sets of 10 repetitions of BR. The relative load was 70% of 1RM for all exercises with 120 s of rest between each set and 180 s of rest between each exercise (38-40). If a participant reached failure during a set before completing the prescribed 10 repetitions, 30-60 s of rest was given before continuing with the set. This process was repeated until 10 repetitions were completed.

Statistical Analysis

All data were analyzed with IBM SPSS version 25.0 for Windows (Somers, NY) and Microsoft Excel 2016 for Windows (Microsoft Corporation, Redmond, WA). Non-normal (i.e., skewed) distributions are common among collected HRV group data (19, 16). To prevent this and ensure a normal distribution of collected data, the natural logarithmic transformation of RMSSD (LnRMSSD) was applied (41, 42). Mean values between the PPG and criterion ECG LnRMSSD measures were compared with paired-samples *t*-tests. The magnitudes of the mean differences were quantified using Cohen's *d* effect size (ES) and classified as trivial (0.0-0.2), small (0.2-0.6), moderate (0.6-1.2), large (1.2-2.0), and very large (>2.0) (43). Pearson product-moment correlation coefficients (*r*) were calculated to assess the association between the ECG and PPG-derived LnRMSSD values. Agreement between the ultra-short-term LnRMSSD values

was evaluated using Bland-Altman analysis. The agreement was qualified as the calculated ratio of half the 95% confidence interval (CI) and the mean of the average values where a "good" agreement was considered if the ratio was less than 0.1, "moderate" agreement was considered if the ratio was 0.1-0.2, and "insufficient" agreement if the ratio was >0.2 (17, 12). Validation statistics also involved calculating the standard error of the estimate (SEE) for the PPG values against ECG.

Intraday reliability was determined by comparing the PPG measurements from $Base_{Pre1}$ and $Base_{Pre2}$. Interday reliability was determined by comparing the PPG measurements from $Base_{Pre1}$, $Base_{Pre2}$, HV_{Pre1}, and HV_{Pre2}. Reliability statistics were done using Friedman chi-square (χ^2) and intra-class correlations (ICC) were determined between the LnRMSSD parameters. Correlation values between 0 to 0.30 were considered small, 0.31 to 0.49 was moderate, 0.50 to 0.69 was large, 0.70 to 0.89 was very large, and 0.90 to 1.00 was near perfect (43). Repeated-measures analysis of variance (ANOVA) with Bonferroni correction was done to assess differences in pre- and post-exercise heart rate (HR) and blood pressure. Unless otherwise stated, all data were presented as mean ± standard deviation ($M \pm SD$) and statistical significance was accepted at $p < .05$.

RESULTS

Validity of Photoplethysmography

The $M \pm SD$ for the LnRMSSD values of both PPG and ECG as well as validity statistics are displayed in Table 4.2. Frequencies of measures within each signal quality category are displayed in Table 4.3. Paired-samples *t*-tests showed statistically significant differences (p>.05) for all resting comparison measurements of ECG and PPG under resting conditions. However, all Cohen's *d* effect sizes were quantitatively classified as small. Significant correlations ranging

from large to very large were also observed for pre-exercise measurements. The CE and SEE values were consistent with all resting measures with CE values falling within the upper and lower ranges of limits of agreement. The quality of the agreement for all pre-exercise measures, according to the Bland-Altman ratio was classified as "good". Plots can be seen in Figure 4.2. A moderate statistically significant mean difference was observed between ECG and PGG measures post-exercise. However, a moderate correlation was found between post-exercise recordings, relatively weaker than under resting conditions. The quality of agreement was determined to be "insufficient" with CE and SEE values increasing from pre-exercise recordings. Statistically significant differences were observed between pre- and post-exercise diastolic blood pressure ($p = .003$) and HR measures ($p < .001$), however, no significant differences were observed in pre-post systolic blood pressure (see Figure 4.3).

Reliability of Photoplethysmography

All intraday and interday reliability statistics are displayed in Table 4.4. The PPG-derived LnRMSSD baseline measurements for intraday reliability displayed non-significant Friedman chi-square values ($\chi^2 = 2.08$, $p = .149$) while ICC values were "nearly perfect" (ICC = 0.91). For interday reliability, four measurements were compared: $Base_{Pre1}$, $Base_{Pre2}$, HV_{Pre1}, and HV_{Pre2}. The chi-square was also not statistically significant ($\chi^2 = 5.02$, $p = .171$) with an ICC of "very large" (ICC = 0.88).

DISCUSSION

The present study aimed to examine the validity of HRV measures from a PPG smartphone application under resting and post-resistance exercise conditions. The secondary aim was to examine the intraday and interday reliability of ultrashort-term measures from PPG. We hypothesized there would be good agreement and large correlations between ECG and PPG

measures at all points of measure and that smartphone PPG would demonstrate “near perfect” levels of reliability. Under resting conditions, significant differences were found between all simultaneous ECG and PPG measures, yet the effect sizes were considered small with strong correlations and good agreement between methods. Conversely, post-exercise simultaneous HRV measures were significantly different with moderate effect size, the lowest correlation, and insufficient agreement. Finally, strong reliability was displayed by smartphone PPG measures within-day and between days. These findings demonstrate that smartphone PPG is a valid and reliable surrogate under resting conditions that can be utilized daily, but significantly overestimates LnRMSSD following resistance exercise.

Pulse rate variability via the PPG recording method has been considered an accurate reflection of beat-to-beat cardiac intervals (i.e. HRV), where the time of each pulse is dictated by the heartbeat, the amplitude of circulating blood volume and the path length of light traveling through the arteries (44, 12). Fingertip PPG assessment has been highly correlated to both time and frequency-domain metrics from ECG-derived HRV indices (12). Although the current study produced significant correlations between ECG- and PPG-derived LnRMSSD values, the relationship fluctuated between recording periods and all resting comparisons produced “small”, yet significant mean differences. The differences in strength of PPG and ECG relationships found between the findings of previous research and the present study may be methodological. Various studies have investigated the efficacy of PPG to derive HRV, however, the equipment used for PPG differed. Plews et al. (15) investigated the validity of the same smartphone application as the current study. Even though the application was the same, several differences exist in the procedures. First, Plews et al. used a standard 12-lead ECG set-up with a Cosmed Quark T12x system while the current study utilized a modified lead II configuration with a

Biopac MP150 system. Next, ECG data analysis procedures can heavily influence the resulting HRV values. Plews et al. used custom software along with manual editing while the current study relied on the algorithms of Kubios HRV Standard software. Additionally, the smartphone application implements its unique algorithm to determine RR intervals, which may differ from those used in ECG-derived HRV analysis (15). Peng et al. (25) investigated the extraction of HRV from smartphone PPG signals from five different algorithms and compared them to ECG-derived metrics in 30 healthy participants in the supine position. The obtained RMSSD measures demonstrated large-to-very large correlations (r = 0.60-0.78), however, all time-domain parameters displayed insufficient agreement using the Bland-Altman ratio (25). Based on the aforementioned results, it can be inferred that the specific PPG method of measure (e.g. flash/camera or finger sensor) and the RR interval detection algorithm can significantly impact resulting HRV metrics even under resting conditions.

Though smartphone PPG shows much promise as an alternative method to ECG for determining HRV (8), some controversy towards its efficacy still exists which may be linked to the physiological differences between PRV and HRV. Rauh et al. (2004) found highly significant ($p < .001$) correlations ($r \geq 0.9$) between HRV (from ECG) and of PRV (from PPG), however, the PRV parameters were higher than HRV showing only moderate or insufficient (> 20%) agreement for all metrics (45). The findings of Rauh et al. fall in line with observations of the current results where mean values of LnRMSSD from PPG were significantly higher at all measurement periods. In a review by Schafer and Vagedes (12), it was found that PRV seems to overestimate RMSSD compared to HRV, which was attributed to the difference being due to pulse transit time. Resulting PRV is going to be dictated by pulse transit time, or the time needed for a pulse pressure wave to travel to the periphery, commonly the fingers or toes (28). Because

of pulse transit time, PRV is heavily influenced by HR, blood pressure changes and the compliance of the arteries. An increase in HR leads to a temporary increase in blood pressure, reducing arterial compliance, which culminates in delayed pulse transit time (28, 27). A significant increase would cause longer interval periods in PRV which may have caused the trend in the overestimation of LnRMSSD observed during all measurement periods of the current study, especially following exercise.

Our findings reflect the results of previous research with smartphone PPG measures of LnRMSSD being higher than ECG-derived HRV on average. Though the exact mechanisms behind possible differences seen in some of the literature between ECG- and PPG-derived HRV metrics have not been fully agreed upon, it has been suggested that different positions (i.e. supine, seated, and standing) may cause variations in the accuracy in PRV and thus the estimation of HRV (12). Research investigating the effects of position on the agreement of ECG and PPG has shown slight deteriorations occur during upright posture in comparison to supine measures (17). It has been speculated that the slight deterioration in mean accuracy in seated and standing positions may have been due to an orthostatic stress-induced increase in sympathetic activation leading to heightened arterial stiffness and decreased pulse wave velocity (17). While investigating the simultaneous measures of PPG and ECG, Lu et al. (2009) found good agreement between PRV and HRV during supine and upright positions but saw slight deteriorations during the upright posture (46). In the present study, participants were instructed to remain motionless during recordings and all measurements were taken in the seated position in an attempt to reduce all possible parasympathetic saturation, a common observation among highly fit individuals with low resting HR (47, 2, 44). Though practical for athletes outside of laboratory conditions, the seated position may result in delayed pulse transit time and

subsequently worse PRV compared to supine measurements due to slight increases in parasympathetic withdrawal and/or sympathetic activity. A recent meta-analysis found a non-significant difference in error among different positions, however, it was concluded that positions are not interchangeable and should be kept consistent for longitudinal monitoring (7).

The present findings saw that post-exercise simultaneous measures demonstrated significant differences, the weakest correlation, and insufficient agreement. These results fall in line with previous research that found physical activity and mental stimuli are determinates of the inaccuracy of PPG assessment (12, 8, 16). This overestimation of HRV from PPG may be linked to increased sympathetic activity commonly seen during resistance exercise and the physiological effects that follow. Charlot et al. (48) observed that agreement with ECG progressively worsened along with the increase in sympathetic stimulation from being in an upright position to performing an exhaustive bout of aerobic exercise. It was speculated that the increased differences could be related to changes in the mechanical properties of the arteries, such as an increase in arterial stiffness due to exercise which leads to a decrease of pulse wave velocity and decreased PRV (48). High volume resistance exercise using moderately heavy loads has been associated with increased HR, breathing rate and spikes in blood pressure. Respiration-induced changes in intrathoracic pressure caused by the Valsalva maneuver utilized during resistance exercise are known to cause blood flow variations in the venous as well as in the arterial circulatory system (12). Additionally, resistance exercise has been shown to transiently increase arterial stiffness for up to 30 minutes post (49, 50). In the current study, multiple compound, barbell-based exercises were utilized and the Valsalva maneuver was not controlled for. It can be inferred that this combination resulted in greater degrees of the aforementioned sympathetic responses in comparison to previous studies that implemented exercise protocols

that isolated muscle groups through less intense machine-based exercises (49, 50). Our results showed significant elevations in HR and reductions in diastolic blood pressure post-exercise, however, no significant changes in systolic pre-post were observed. The combination of these physiological responses to the bout of resistance exercise may have caused a greater lag in pulse transit time and inaccurate pulse cycle detection due to artifacts and noise leading to the weaker correlation and insufficient agreement displayed in post-exercise PPG-derived LnRMSSD in the current study. However, the appropriate monitoring of hemodynamics during and following resistance exercise was beyond the scope of the current study so no definitive conclusions can be made.

The present study also examined the intraday and interday reliability of smartphone PPG measures under resting conditions. Plews et al. (19) established that multiple HRV measures, averaged from week-to-week were superior to isolated collections taken in a pre-post fashion (19). With this change in methodology came a greater need for valid mobile devices to collect near-daily HRV. Though many studies have analyzed the accuracy of various new devices (13-15), fewer have observed reliability through consecutive recordings. Nakamura et al. (26) found high ICC values in ultra-short-term LnRMSSD measures using a portable HR monitor for both intraday (ICC = 0.96) and interday (ICC = 0.90) reliability in rugby athletes. Our results support these previous findings. The near-perfect intraday reliability can be attributed to the uniformity of the environment in which the recordings took place. Though consecutive measures were taken with a 10-min gap between them, the participants were not heavily stimulated during this time and the environmental conditions (e.g. temperature, humidity, etc.) remained constant. Interday reliability was found to be very large, displaying a slight decrease in comparison to intraday reliability. This may be due to external factors outside the control of laboratory conditions

affecting the participants prior to the follow-up session. However, based on these results, in addition to recommendations given by previous research (3, 6), practitioners can expect valid HRV measures on a near-daily basis as long as conditions during the time of recording remain relatively constant.

Limitations

Several limitations of the present study should be emphasized. The breathing frequency and tidal volume were not controlled; however, this was done to simulate field conditions. It has also been shown that breathing rate does not appear to influence RMSSD, unlike spectral indexes (51, 52, 13). Another limitation was that all measurements were performed in a well-controlled laboratory setting with researchers overseeing all procedures to ensure measurement quality, which is not truly indicative of field settings done with athletes. Finally, though both males and females were recruited for this study, the female-to-male ratio was too small with only 29% consisting of females.

CONCLUSION

The monitoring of daily HRV in athletes is a valuable and reliable tool in gauging autonomic modulation. Smartphone PPG offers a noninvasive, cost-effective alternative to the traditional ECG method. The LnRMSSD measures from PPG tend to be higher than ECG recordings producing small, yet significant mean differences. However, strong correlations and good agreement were seen across LnRMSSD comparisons during resting conditions, which falls in line with previous research. Post-exercise PPG values showed a significant mean difference with moderate effect size, along with a slightly weaker correlation and insufficient agreement compared to ECG. Additionally, near-perfect intraday and interday reliability were displayed

with smartphone PPG measures at rest, which is seems extremely beneficial due to the recommendation of multiple measures per week for a more accurate depiction of HRV. Based on the validity, time-efficiency and straightforward nature of smartphone-derived PPG LnRMSSD values taken under resting conditions, sports coaches, exercise physiologists, and other practitioners should consider the incorporation of this tool into daily monitoring of athlete. Caution should be taken to decrease stress-induced sympathetic activation, which may reduce the accuracy of PPG measures of HRV.

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Table 4.1

Physical and Functional Characteristics of the Participants (*n* = 31)

Descriptives	Participants
Age (yrs.)	23.9 ± 5.4
Height (cm)	171.0 ± 21.5
Body Mass (kg)	75.9 ± 12.9
Body Fat (%)	17.6 ± 7.2
Resting Heart Rate (bpm)	67.9 ± 13.2
Systole (mmHg)	104.5 ± 16.7
Diastole (mmHg)	70.1 ± 6.7
Training Experience (yrs.)	3.95 ± 1.18

Notes: Data displayed as means ± standard deviations

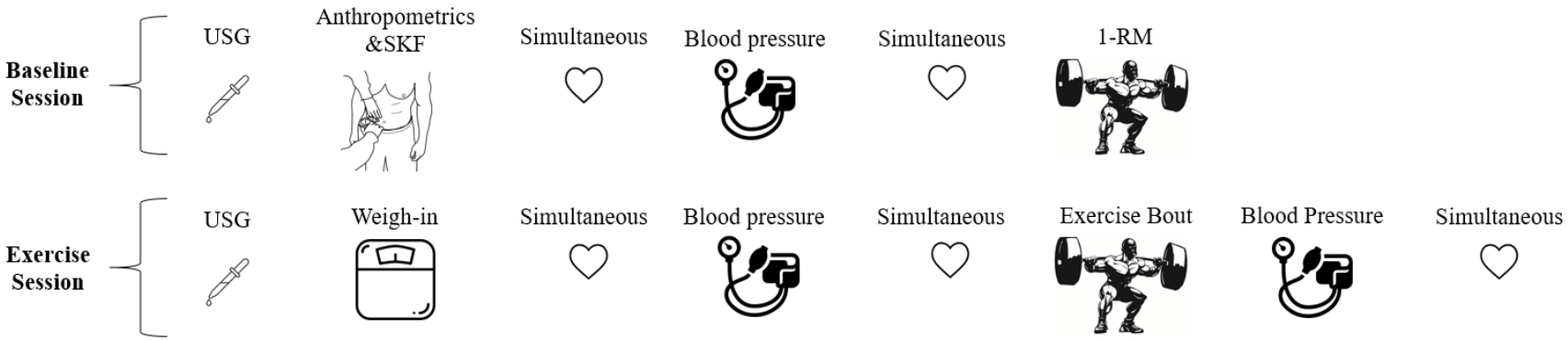

Figure 4.1. Experimental design graphic. USG = urine specific gravity; SKF = skinfold measurements; Simultaneous = simultaneous ECG and PPG measurement of HRV; 1-RM = one-repetition maximum testing; Exercise Bout = 6 sets of 10 repetitions back squat, 3 sets of 10 repetitions bench press, and 3 sets of 10 repetitions bent-over row.

Table 4.2

Comparison of ECG and PPG-derived LnRMSSD

		N	***M* ± *SD***	***p***	**Effect Size**		***r***	**SEE**	**Ratio**	**CE ± 1.96*SD**	**Upper**	**Lower**	**Trend (*r*)**
Baseline 1st	ECG	31	4.05 ± 0.65										
Measure	PPG	31	4.38 ± 0.61*	.003	0.42	Small	0.59	0.54	0.08	0.34 ± 1.12	1.5	-0.8	-0.09
Baseline 2nd	ECG	31	4.06 ± 0.63										
Measure	PPG	31	4.29 ± 0.59*	.019	0.30	Small	0.63	0.50	0.05	0.23 ± 1.03	1.3	-0.8	-0.08
High Volume 1st	ECG	31	4.05 ± 0.59										
Measure	PPG	31	4.25 ± 0.53*	.041	0.26	Small	0.63	0.47	0.05	0.19 ± 0.95	1.1	-0.8	-0.13
High Volume	ECG	29	3.88 ± 0.62										
2nd Measure	PPG	29	4.15 ± 0.58*	.001	0.36	Small	0.76	0.42	0.07	0.28 ± 0.83	1.1	-0.6	-0.10
High Volume 3rd	ECG	31	2.44 ± 1.00										
Measure	PPG	31	3.50 ± 0.72*	<.001	1.14	Mod	0.41	0.92	0.34	0.98 ± 0.96	2.9	-0.9	-0.33

Notes: ECG = electrocardiogram; PPG = photoplethysmography; LnRMSSD = log-transformation of the root mean square of successive RR differences; *N* = number; *M* ± *SD* = mean ± standard deviation; SEE = standard error of estimate; CE = constant error ; *significance level at .05.

Table 4.3

Frequency of PPG Signal Quality Categories (n = 31)

	N	Not Optimal (%)	Good (%)	Optimal (%)
$Base_{Pre1}$	39	33.3	17.9	48.7
$Base_{Pre2}$	37	21.6	24.3	54.1
HV_{Pre1}	36	19.4	19.4	61.1
HV_{Pre2}	36	22.2	13.9	63.9
HV_{Post}	36	30.6	16.7	52.8

Notes: PPG = photoplethysmography; $Base_{Pre}$ = first pre-exercise baseline measure; HV_{Pre} = high volume pre-exercise measure; HV_{Post} = high volume post-exercise measure; n = total number of measures taken

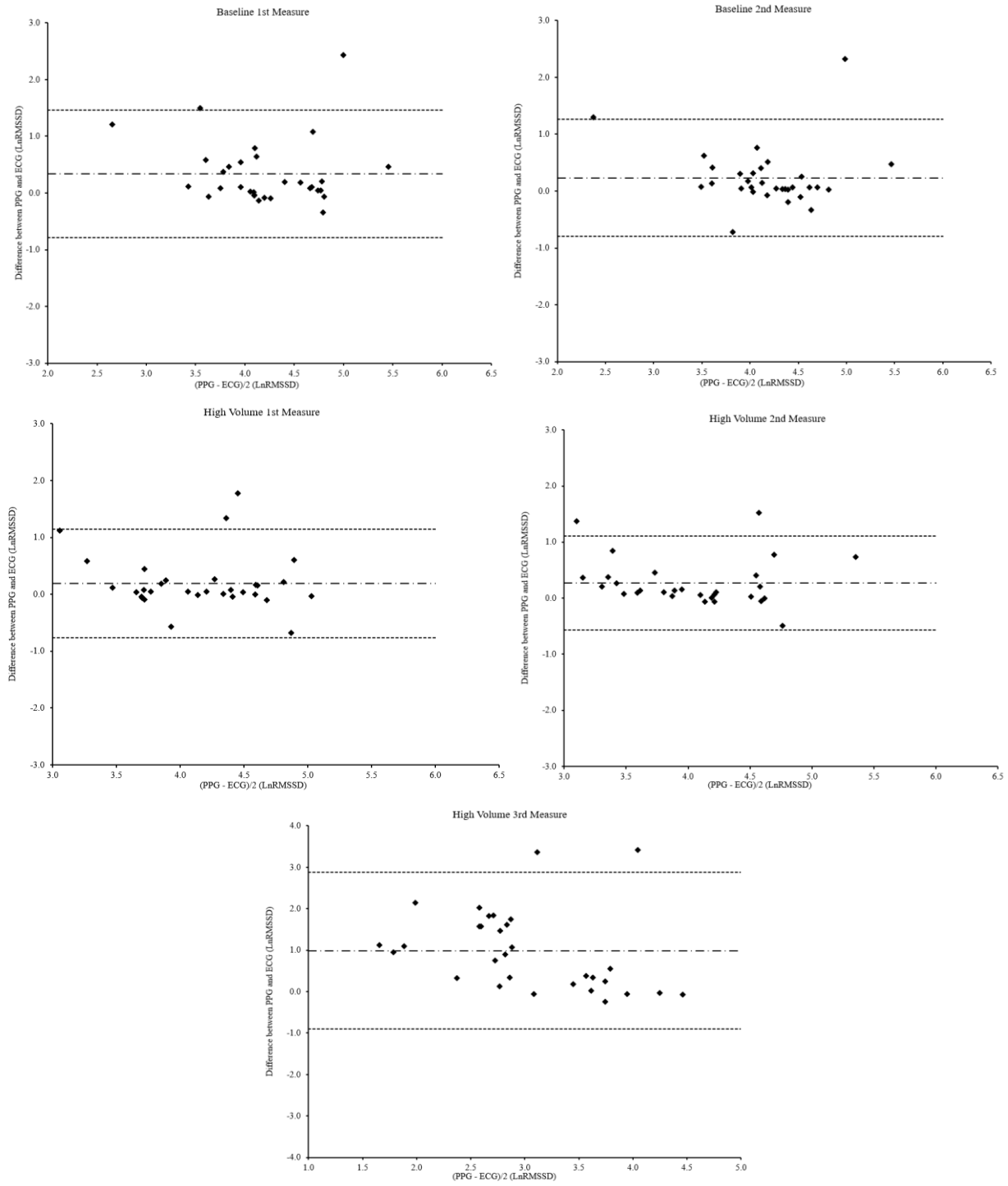

Figure 4.2. Bland-Altman plots comparing the log transformation of the root mean square of successive RR differences (LnRMSSD) values from the smartphone application photoplethysmography (PPG) with the criterion electrocardiogram (ECG). The solid lines represent the mean bias, whereas the outside dashed lines represent 95% limits of agreement.

Table 4.4

Intraday and Interday Reliability of PPG-derived LnRMSSD Measures

		M* ± *SD	***α***	***df***	χ^2	**Sig.**	**ICC**	**95% CI**		***F***	***p***
								Lower	**Upper**		
LnRMSSD	Intraday	4.34 ± 0.20	0.912	30	2.083	.149	0.91*	.812	.956	11.319	<.001
	Interday	4.28 ± 0.29	0.886	28	5.016	.171	0.88*	.795	.940	8.745	<.001

Notes: PPG = photoplethysmography; LnRMSSD = log-transformation of the root mean square of successive RR differences; *M* ± *SD* = mean ± standard deviation; *α* = Cronbach's alpha; *df* = degrees of freedom; ICC = intraclass correlations; CI = confidence intervals ;*significance level at .05.

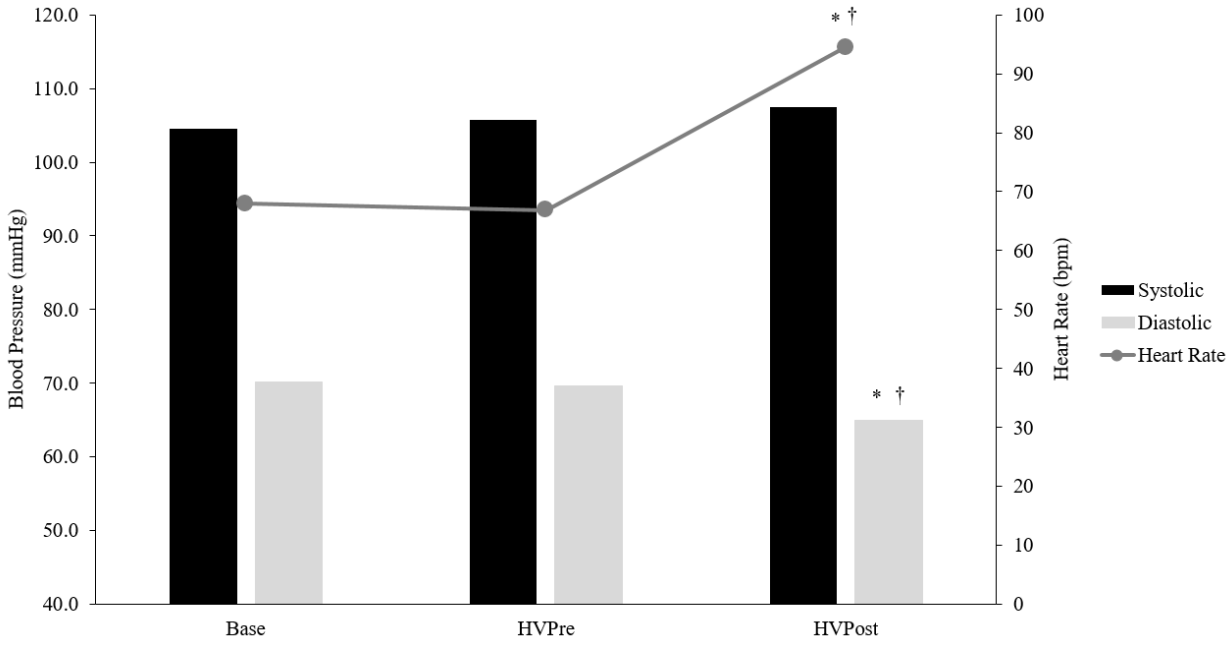

Figure 4.3. Blood pressure and heart rate measurements under resting and post-exercise conditions. *Significantly different from baseline resting conditions. †Significantly different from high volume pre-exercise measure ($p < .05$).

CHAPTER 5

CONCLUSION

For most athletes at all levels, frequent resistance exercise is a fundamental part of any annual training program. Resistance exercise has been shown to expose the body to various types of stress and elicit acute responses and chronic adaptations through the manipulation of different training variables (1). In conjunction with the possible improvements in sports performance, resistance exercise can cause varying degrees of fatigue depending on the magnitude of the stimulus (2-4). Because fatigue can diminish an athlete's readiness to perform and general fitness, proper steps must be taken to allow adequate recovery before engaging in another overloading bout of resistance exercise (5). A multitude of subjective and objective tools exist today to help sports coaches, strength and conditioning specialists, and exercise physiologists quantify training loads and monitor recovery. Heart rate variability (HRV) has emerged as a promising indicator of internal training loads through the measurement of the autonomic nervous system control of the cardiovascular system (6-8). Though the utility of HRV has been demonstrated for monitoring stress responses to aerobic exercise (9-13), its responses to different resistance exercise protocols are not yet fully understood.

To better understand the effects of resistance exercise on HRV, we conducted three studies. In study 1, the aim was to compare the effects of low, moderate, and high set volumes in acute resistance exercise sessions on post-exercise parasympathetic reactivation measured using the root mean square of successive RR differences (RMSSD). We hypothesized that exercise sessions with higher volumes would result in significantly greater decreases in RMSSD value

from pre- to immediately post-exercise. We also hypothesized greater delays in the recovery time of parasympathetic activity would be observed following sessions of higher volume. A 3 (session) x 6 (time) repeated-measures ANOVA was conducted to evaluate the effects of three different volumes of resistance exercise on HRV. The findings of the current study supported this hypothesis with statistically significant differences being observed across sessions, recording times, and the session × time interaction for the log-transformation of RMSSD (LnRMSSD). Mean LnRMSSD values obtained from the low volume session were significantly different from both the moderate and high volume sessions but the moderate and high volume sessions were not different from one another. We also hypothesized greater delays in the recovery time of parasympathetic activity would be observed following sessions of higher set volume. The results found this to be partially true. When comparing post-exercise LnRMSSD measures to pre-exercise, significant differences were found at all time segments across all sessions indicating delays in recovery. When investigating the LnRMSSD values between sessions, the low volume session resulted in significantly higher post-exercise values. However, no significant differences were found between the moderate and high volumes sessions. Also, after calculating the $\Delta LnRMSSD_{post5\text{-}30}$ for each exercise session, no statistically significant differences were observed between the varying volume sessions.

In study 2, the purpose was to determine the relationship between pre-post changes in HRV and neuromuscular performance and biochemical fatigue markers in response to resistance exercise. Heart rate variability changes (via LnRMSSD were compared to neuromuscular performance (IHG, CMJ, and MPV), metabolic stress (lactate), and inflammatory (interleukin-6) responses to resistance exercise. We hypothesized that LnRMSSD would display significant associations with all fatigue markers. Lactate concentrations were only obtained from 23

participants and plasma IL-6 concentrations were only obtained from 16 participants. Statistically, significant mean differences were found between all pre- and post-exercise variables, except for IL-6 (p = .296) and MPV_{BP} (p = .678). Statistically significant correlations were observed with ΔLnRMSSD $Post_{5\text{-}10}$ and ΔLactate immediately post-exercise (r = -0.440, p = .036), and ΔLnRMSSD $Post_{5\text{-}10}$ and ΔLactate $Post_{30}$ (r = -0.549, p <.001).

In study 3, the aim was to examine the agreement of HRV measures from electrocardiography (ECG) signals and photoplethysmography (PPG) via smartphone application under resting and post-resistance exercise conditions. The secondary aim was to examine the intraday and interday reliability of ultrashort-term measures from PPG. We hypothesized there would be good agreement and large correlations between ECG and PPG measures at all points of measure and the app would demonstrate "near perfect" levels of reliability. The LnRMSSD measures from PPG tend to be higher than ECG recordings producing small, yet significant mean differences. One-sample *t*-tests showed significant differences for all measurement periods, however, effect sizes for all LnRMSSD comparisons were considered "small" (0.2-0.6), except for HV_{Post} which was "moderate" (1.14). Statistically significant, moderate-to-very large correlations were found for all LnRMSSD comparison measures ($p \leq 0.05$). The quality of the Bland-Altman agreement for all pre-exercise measures was classified as "good" but the post-exercise agreement was deemed "insufficient". Additionally, near-perfect intraday (R = 0.909) and very large (R = 0.883) interday reliability were displayed with smartphone PPG measures at rest, which seems extremely beneficial due to the recommendation of multiple measures per week for a more accurate depiction of HRV.

Fatigue is defined as a disruption of homeostasis that can acutely or chronically diminish readiness to perform. Based on the results of the presented studies, it should be understood that

multiple physiological systems play a role in the fatigue generated from resistance exercise. The monitoring of daily HRV in athletes is a valuable and reliable tool in gauging autonomic modulation and the effects of stress. Smartphone PPG offers a noninvasive, cost-effective, and valid alternative to the traditional ECG method to allow for daily assessments of HRV outside the confines of a laboratory. From a functional perspective, acute bouts of full-body resistance exercise can cause reductions in HRV and can delay parasympathetic reactivation for at least 30 minutes following exercise. Additionally, high volume resistance exercise may cause reductions in neuromuscular performance and biochemical fatigue markers in conjunction with decreases in HRV. However, HRV may not be sensitive enough to measure or display all clear differences between varying volumes and has shown no strong associations with other fatigue metrics. Though HRV is a useful measure of autonomic modulation in response to resistance exercise, practitioners should look to incorporate multiple methods of monitoring fatigue and recovery to assess an athlete's readiness to perform more effectively.

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APPENDIX

THE UNIVERSITY OF ALABAMA | *Office of the Vice President for* Research & Economic Development
Office for Research Compliance

October 3, 2019

Michael Esco, Ph.D.
Assistant Professor
Department of Kinesiology
College of Education
The University of Alabama
Box 870312

Re: IRB Protocol # 19-015-ME
"Comparison of Various Measures of Training Load Pre and Post Resistance Exercise"

Dr. Esco:

The University of Alabama Medical Institutional Review Board has granted approval for your proposed research. You have also been granted the requested waiver of documentation of informed consent. Your application has been given full board approval according to 45 CFR part 46.

The approval for your application will lapse on September 4, 2020. If your research will continue beyond this date, please submit a continuing review to the IRB as required by University policy before the lapse. Please note, any modifications made in research design, methodology, or procedures must be submitted to and approved by the IRB before implementation. Please submit a final report form when the study is complete.

Please use reproductions of the IRB approved stamped consent form to obtain consent from your participants.

Good luck with your research.

Sincerely [redacted]

Medical IRB Chair

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THE UNIVERSITY OF ALABAMA | Office of the Vice President for Research & Economic Development
Office for Research Compliance

October 7, 2019

Michael Esco, Ph.D.
Assistant Professor
Department of Kinesiology
College of Education
The University of Alabama
Box 870312

Re: IRB Protocol # 19-014-ME
"Comparison of Heart Rate Variability Responses to Varying Resistance Exercise Volume-Loads"

Dr. Esco:

The University of Alabama Medical Institutional Review Board has granted approval for your proposed research. You have also been granted the requested waiver of documentation of informed consent. Your application has been given full board approval according to 45 CFR part 46.

The approval for your application will lapse on September 4, 2020. If your research will continue beyond this date, please submit a continuing review to the IRB as required by University policy before the lapse. Please note, any modifications made in research design, methodology, or procedures must be submitted to and approved by the IRB before implementation. Please submit a final report form when the study is complete.

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Good luck with your research.

Sincerely,

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