POST-EXERCISE RECOVERY: FUNDAMENTAL AND INTERVENTIONAL PHYSIOLOGY

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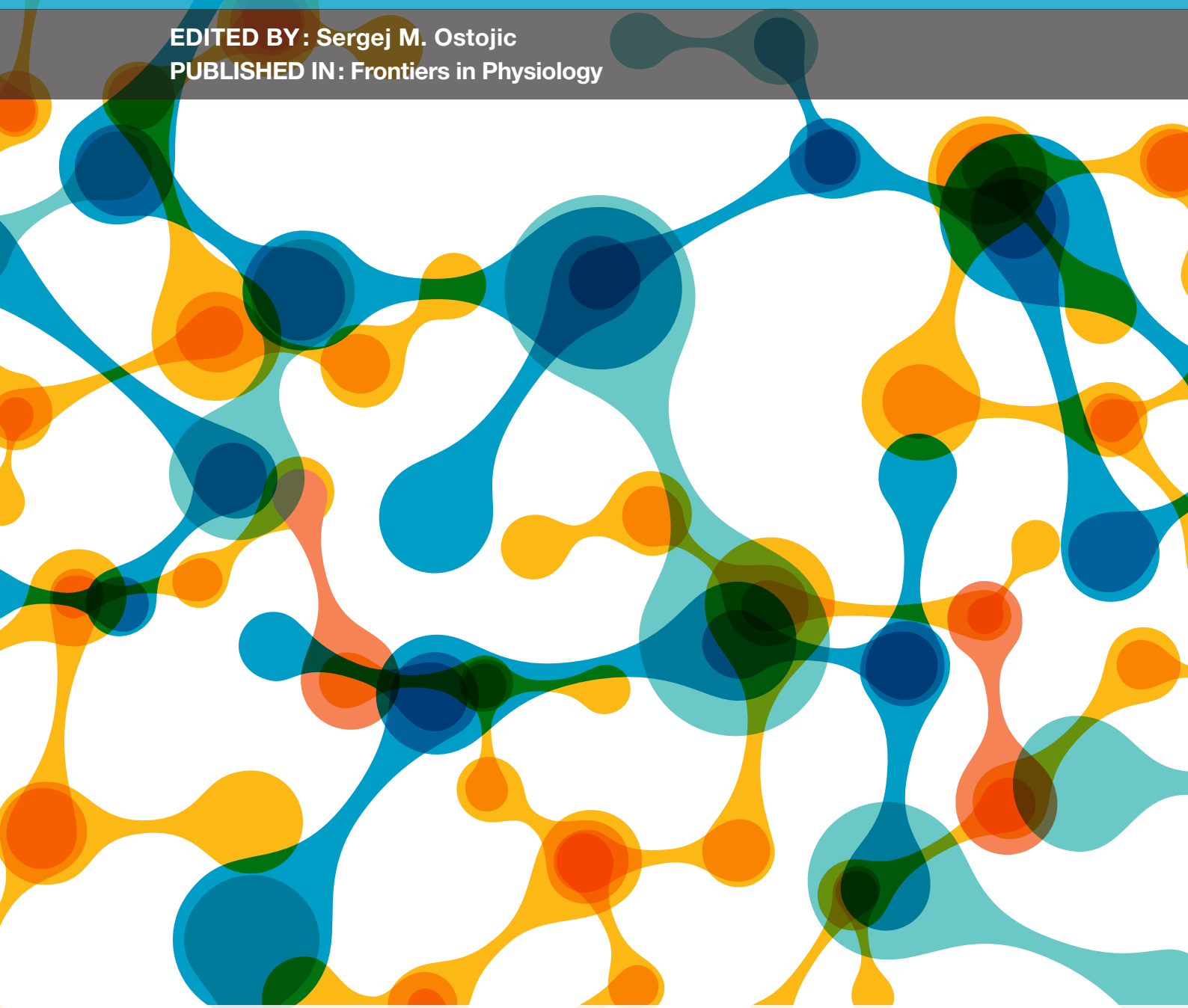

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POST-EXERCISE RECOVERY: FUNDAMENTAL AND INTERVENTIONAL PHYSIOLOGY

Topic Editor:
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Physiological responses after maximal and submaximal exercise are routinely monitored in a plethora of diseases (e.g. cardiovascular diseases, cancer, diabetes, asthma, neuromuscular disorders), and normal populations (e.g. athletes, youth, elderly), while slower or irregular post-exercise recovery usually indicates poor health and/or low fitness level. Abnormal post-exercise recovery (as assessed via blunted post-exercise heart rate dynamics) helps to predict the presence and severity of coronary artery disease, while differences in recovery outcomes in athletes might discriminate between fit and unfit individuals. Disturbances in post-exercise recovery might be due to acute or persistent changes in: (1) adaptive responses mediated by the autonomic nervous system and vasodilator substances, (2) cellular bioenergetics, and/or (3) muscular plasticity. Preliminary evidence suggests possible role of time-dependent modulation of nitric oxide synthase and adenosine receptors during post-exercise recovery, yet no molecular attributes of post-exercise recovery are revealed so far. Currently several markers of post-exercise recovery are used (e.g. heart rate measures, hormone profiles, biochemical and hematological indices); however none of them meets all criteria to make its use generally accepted as the gold standard. In addition, recent studies suggest that different pharmacological agents and dietary interventions, or manipulative actions (e.g. massage, cold-water immersion, compression garments, athletic training) administered before, during or immediately after exercise could positively affect post-exercise recovery. There is a growing interest to provide more evidence-based data concerning the effectiveness and safety of traditional and novel interventions to affect post-exercise recovery.

The goals of this research topic are to critically evaluate the current advances on mechanisms and clinical implications of post-exercise recovery, and to summarize recent experimental data from interventional studies. This knowledge may help to identify the hierarchy of key mechanisms, and recognize methods to monitor and improve post-exercise recovery in both health and disease.

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EDITORIAL
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Editorial: Post-Exercise Recovery: Fundamental and Interventional Physiology

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Keywords: skeletal muscle, recovery, exercise, nutrition, autonomic response

The Editorial on the Research Topic

Post-Exercise Recovery: Fundamental and Interventional Physiology

Post-exercise Recovery: Fundamental and Interventional Physiology is a *Frontiers in Physiology* Research Topic aimed to provide new advances in the basic and applied study regarding recovery after exercise in the context of both physiological and pathophysiological conditions. During the past decade or so, a plethora of novel findings has been provided concerning attributes of post-exercise recovery in health and disease, and different physiological, medical, and nutritional interventions targeted to manage post-exercise recovery. Since 2000, more than 1200 peer-reviewed papers have been published in the leading biomedical journals describing the biology and medicine of post-exercise recovery. In this Research Topic, we presented perspectives, opinion pieces, and original research and review articles focused to understand a complex physiology of post-exercise recovery, and to outline recent experimental data from interventional studies.

Interpreting the physiology of time period immediately after exercise as an authentic biological phenomenon recently becomes of notable importance for both clinical exercise physiologists and sports scientists. Irregular or abnormal physiological responses during this time window might indicate much more than poor health or low fitness level (Terziotti et al., 2001; Lind et al., 2009; Hausswirth and Le Meur, 2011; Rattray et al.), with the physiology of recovery period should be investigated in a more distinctive and multidisciplinary manner. On this regard, Luttrell and Halliwill present an interesting perspective paper that conceptualizes post-exercise recovery as a discrete event from exercise itself. Authors discussed that post-exercise recovery might help us to identify and monitor several clinical conditions, being a perfect time window for therapeutic interventions in both athletic and clinical environment, and/or prognostic time span providing predictive information regarding sport performance and health outcomes. This novel concept encourages multi-faceted approach to the research field of "physiology of recovery" and merits further investigation.

Another new and compelling idea explored in this Research Topic is the specificity concept for the recovery after exercise. Minett and Costello present an opinion article on the need for post-exercise recovery protocols to be suitably matched and specifically prescribed to the individual and environmental characteristics and needs. Here, authors discuss the controversy surrounding the effectiveness of post-exercise cooling for recovery, suggesting more specific approach should be emphasized for this and other post-exercise interventions in future studies. Nutrition seems to be another important player in post-exercise recovery game, requiring more specific modus operandi. Specific timing of food ingestion, food content, and quality are some distinct factors that might dictate recovery dynamics (Howe et al., 2014). Recent studies have focused on post-exercise

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protein intake to enhance metabolic adaptations and maximize training adaptations (Pasiakos et al., 2014; Beck et al., 2015). On this regard, Morton et al. present a comprehensive overview of the current knowledge of the field on nutritional manipulation to augment resistance-training induced skeletal muscle hypertrophy during the recovery period. Importantly, a variety of nutritional factors affecting balance between muscle anabolism and catabolism are highlighted, and more specific information about protein and carbohydrate co-ingestion during recovery should arise from future studies.

Important players in post-exercise recovery are the cytokines, small proteins involved in cell signaling and apoptosis (Nieman and Pedersen, 1999). Cytokines, such as tumor necrosis factor-α (TNF-α) or interleukin 6 (IL-6), are critical in the regulation of inflammation, muscular injury, and repair after exercise (Philippou et al., 2012). In this Research Topic, Townsend et al. present an original article that compared the effects of different interventional recovery protocols administered following intense resistance exercise on circulating TNF-α and TNF-α receptor expression. Authors show favorable cytokines response after specific recovery intervention, implying that the efficacy of a recovery protocol depends on both the modality of the intervention and the particular time point of its administration during recovery. However, more research is needed concerning the identification of time frames in which the recovery interventions are most beneficial to muscle function and repair.

Can recovery rates from single- and multiple-bout exercise help us to better understand "physiology of recovery"? In their opinion article, Girard et al. advance a link between neural drive, environmental stress and recovery dynamics in repeated exercise, suggesting possible role of perceptual recovery to augment athletic performance. Same group present an original paper addressing the recovery of performance during repeated sprints in manipulated hypoxia (Girard et al.), providing insight about an association between external stressors and neuro-mechanical alterations during recovery. Disturbances in autonomic neural drive during post-exercise recovery are also prominent in clinical populations (Sheppard et al., 2007; Minai et al., 2012; Florea and Cohn, 2014). Here, Trevizani et al. present an original paper that describes cardiac autonomic recovery after single resistance exercise session in hypertensive men. The concept that exercise promotes neuromuscular modulation can be a link between exercise and its performance- and health-related effects. In addition, Akazawa et al. report an original research about the effects of regular aerobic training on post-exercise reduction of blood pressure in post-menopausal women, suggesting the clinical importance of monitoring central hemodynamic responses after exercise.

In addition to all these outstanding contributions, this research topic also presents captivating original articles evaluating how low-volume high-intensity training affects tissue-specific circulating microRNAs as related to conventional biochemical and performance indices (Cui et al.); and the correlation of non-invasive recovery measures, heart rate (HR) variability and post-exercise HR recovery, with increases in physical activity and cardiorespiratory fitness in non-exercising women (Tonello et al.). Both articles support the use of novel or improved markers of post-exercise recovery that may provide new insights into the physiological perturbations that occur in the body during and after exercise.

Besides presenting contemporary knowledge and viewpoints on the issue of post-exercise recovery, hopefully this Research Topic will further advance the field of "physiology of recovery" by generating innovative ideas, multidisciplinary approaches and experimental studies.

AUTHOR CONTRIBUTIONS

Paper concept and design, drafting the paper, critical revision of the paper for intellectual content: SO.

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REFERENCES

Beck, K. L., Thomson, J. S., Swift, R. J., and von Hurst, P. R. (2015). Role of nutrition in performance enhancement and postexercise recovery. *Open Access J. Sports Med.* 6, 259–267. doi: 10.2147/OAJSM.S33605

Florea, V. G., and Cohn, J. N. (2014). The autonomic nervous system and heart failure. *Circ. Res.* 114, 1815–1826. doi: 10.1161/CIRCRESAHA.114.302589

Hausswirth, C., and Le Meur, Y. (2011). Physiological and nutritional aspects of post-exercise recovery: specific recommendations for female athletes. *Sports Med*. 41, 861–882. doi: 10.2165/11593180-000000000-00000

Howe, S. M., Hand, T. M., and Manore, M. M. (2014). Exercise-trained men and women: role of exercise and diet on appetite and energy intake. *Nutrients* 6, 4935–4960. doi: 10.3390/nu6114935

Lind, E., Welch, A. S., and Ekkekakis, P. (2009). Do 'mind over muscle' strategies work? Examining the effects of attentional association and dissociation on exertional, affective and physiological responses to exercise. *Sports Med.* 39, 743–764. doi: 10.2165/11315120-000000000-00000

Minai, O. A., Gudavalli, R., Mummadi, S., Liu, X., McCarthy, K., and Dweik, R. A. (2012). Heart rate recovery predicts clinical worsening in patients with pulmonary arterial hypertension. *Am. J. Respir. Crit. Care. Med.* 185, 400–408. doi: 10.1164/rccm.201105-0848OC

Nieman, D. C., and Pedersen, B. K. (1999). Exercise and immune function. Recent developments. *Sports Med.* 27, 73–80. doi: 10.2165/00007256-199927020-00001

Pasiakos, S. M., Lieberman, H. R., and McLellan, T. M. (2014). Effects of protein supplements on muscle damage, soreness and recovery of muscle function and physical performance: a systematic review. *Sports Med*. 44, 655–670. doi: 10.1007/s40279-013-0137-7

Philippou, A., Maridaki, M., Theos, A., and Koutsilieris, M. (2012). Cytokines in muscle damage. *Adv. Clin. Chem*. 58, 49–87. doi: 10.1016/B978-0-12-394383-5.00010-2

Sheppard, R. J., Racine, N., Roof, A., Ducharme, A., Blanchet, M., and White, M. (2007). Heart rate recovery—a potential marker of clinical outcomes in heart failure patients receiving beta-blocker therapy. *Can. J. Cardiol*. 23, 1135–1138. doi: 10.1016/S0828-282X(07)70884-X

Terziotti, P., Schena, F., Gulli, G., and Cevese, A. (2001). Post-exercise recovery of autonomic cardiovascular control: a study by spectrum and cross-spectrum analysis in humans. *Eur. J. Appl. Physiol*. 84, 187–194. doi: 10.1007/s004210170003

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ORIGINAL RESEARCH
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Correlates of Heart Rate Measures with Incidental Physical Activity and Cardiorespiratory Fitness in Overweight Female Workers

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Previous studies have suggested that physical activity (PA) levels and cardiorespiratory fitness (CRF) impact on the autonomic control of heart rate (HR). However, previous studies evaluating PA levels did not discriminate between incidental PA and regular exercise. We hypothesized that incidental PA "*per se*" would influence cardiac autonomic indices as assessed via HR variability (HRV) and HR recovery (HRR) in non-exercisers. Thus, the objective of this study was to investigate the relationships between objective PA levels, CRF, and cardiac autonomic indices in adult, regular non-exercising female workers. After familiarization with procedures and evaluation of body composition, 21 women completed a submaximal cycling test and evaluation of HRR on four different days. Resting (2-min seated and standing) and ambulatory (4-h) HRV were also recorded. Levels of PA were assessed by accelerometry over five consecutive days (i.e., Wednesday to Sunday). Maximum oxygen consumption (VO_2max) was measured as an index of CRF. As reliability was low to moderate for most HR measures, relationships between these and PA and CRF were examined using the 4-day average measures. Significant correlations were identified between post-exercise HRR in the first min with various PA indices (daily moderate PA, daily vigorous PA, and the sum of vigorous and very vigorous daily PA). Additionally, VO_2max was significantly correlated to HRV but not to HRR. The current results indicated that CRF was influential in enhancing HRV while incidental or non-exercise based PA was associated with greater autonomic reactivation in adult overweight women. Therefore, both CRF and non-exercise based PA contribute significant but diverse effects on cardiac health. The use of 4-day averages instead of single measures for evaluation of autonomic control of HR may provide a better indication of regular cardiac autonomic function that remains to be refined.

Keywords: heart rate variability, heart rate recovery, cardiorespiratory fitness, incidental physical activity, females, work, allostatic load, autonomic nervous system

INTRODUCTION

Regular exercise and physical activity (PA) contribute to human health and its maintenance (Kruk, 2007; Garber et al., 2011) in similar ways for both men and women (Schumann et al., 2015). While exercise refers to structured repetitive movements that are planned for improving or maintaining physical fitness, PA refers to any body movement produced by muscular activity which results in energy expenditure above resting levels (Ainsworth et al., 2000). Undertaking regular aerobic exercise and maintaining appropriate levels of PA are simple and low-cost interventions for the improvement of cardiorespiratory fitness (CRF), a factor strongly linked to the incidence and risk of most cardiometabolic diseases (LaMonte et al., 2005; Jae et al., 2007; Jakicic et al., 2009). That is, high levels of CRF are related to lower rates of mortality and morbidity among individuals, especially those with cardiovascular disease (Lee et al., 1999, 2010; Wei et al., 1999). Further, low CRF has been reported to affect health more negatively in women compared to men (Skaug et al., 2014), therefore, gender differences may be of key importance when examining CRF and cardiovascular health.

Like the relationship between CRF and cardiovascular health, greater cardiac autonomic control, specifically enhanced parasympathetic and reduced sympathetic activity, has been associated with lower rates of mortality and morbidity in a range of chronic conditions (Nolan et al., 1998; La Rovere et al., 2001; Stein et al., 2008; Pei et al., 2015). This relationship may also be a resultant of greater CRF with improvements in cardiac autonomic activity coinciding with increases in CRF following chronic exercise training (Leicht et al., 2003; Kiviniemi et al., 2010). Previously, Davy et al. (1998) found that cardiac autonomic control and cardiac baroreflex sensitivity decline similarly with age in healthy sedentary and physically active women, however, physically active women demonstrate higher levels of cardiac autonomic control and cardiac baroreflex sensitivity compared with their sedentary peers, regardless of age. All of these factors (PA, CRF, and cardiac autonomic control) have strong effects on health (Ramsbottom et al., 2010). However, the interplay between these factors has not been fully elucidated, possibly as a result of different methods employed to evaluate cardiac autonomic control.

Heart rate variability (HRV) is a simple and widely utilized non-invasive method for evaluation of cardiac autonomic control during basal, orthostatic, and ambulatory conditions (Task Force, 1996). Additionally, post-exercise HR recovery (HRR) has been widely utilized as a simple measure of cardiac autonomic control, particularly parasympathetic reactivation (Cole et al., 1999; Boullosa et al., 2009; Gordon et al., 2011; Daanen et al., 2012). Both HRV and HRR have been extensively utilized in different settings (Gordon et al., 2011; Uusitalo et al., 2011; Boullosa et al., 2012) with a variety of methodological constraints such as reliability, body posture, duration and number of recordings, and parameters selected (Young and Leicht, 2011; Boullosa et al., 2013, 2014; Plews et al., 2014). For example, previous studies have indicated that HRV during the monitoring of training was related to CRF (Kiviniemi et al., 2007; Hautala et al., 2009) while HRR was influenced by the applied exercise load (Buchheit and Gindre, 2006; Guerra et al., 2014). More recently, studies within sport settings (Plews et al., 2012, 2014; Boullosa et al., 2013) have suggested the need for multiple HRV measures (e.g., 3–4 weekly measures; Boullosa et al., 2013; Plews et al., 2014) instead of isolated single measures for a better evaluation of autonomic adaptations. Consequently, it is possible that variable selection for cardiac autonomic control measure, along with frequency of assessment (i.e., 1 vs. >1 recording) may have a substantial impact upon its relationship with other health measures (e.g., PA or CRF). This is especially important in light of the variable reliability values reported for HRV measures during different conditions such as ambulatory (Myrtek, 1990; Ziegler et al., 1999) and following sub-maximal and maximal exercise testing (Arduini et al., 2011; Dupuy et al., 2012; Boullosa et al., 2014).

Similarly, the assessment of PA via different tools (e.g., accelerometers, questionnaire) may also influence its relationship with health indicators (Ara et al., 2015). Most studies to date have evaluated PA levels by questionnaires which are vulnerable to bias (Lindholm et al., 2012; Pavey et al., 2013; Ara et al., 2015). Consequently, objective tools (e.g., accelerometers) have been increasingly utilized to document PA levels (Buchheit et al., 2005, 2006; Hansen et al., 2011). While these devices provide a more precise indication of PA levels, particularly the intensity of the PA, very few studies have differentiated between structured exercise and incidental PA (i.e., non-purposeful PA accrued through activities of daily living; Ross and McGuire, 2011). Therefore, the positive relationships between objective PA and health indicators (e.g., CRF) may be a result of incidental PA and exercise undertaken. Recently, incidental PA was reported to influence CRF improvements during an exercise intervention with recreational athletes (Hautala et al., 2012) while the duration and intensity of incidental PA was positively correlated with CRF in obese individuals (Ross and McGuire, 2011). Therefore, examinations between objective measures of PA and other health indicators (e.g., CRF, HRV) should account for incidental PA and exercise.

Given the significant relationships noted between cardiac autonomic control, CRF and PA, and the impact of these for improved health, further examination of the interaction between these variables was required for clarification. Thus, the objective of the current study was to evaluate the relationship between different cardiac autonomic measures (HRV, HRR), and CRF and objective PA levels in overweight but healthy female adults.

MATERIAL AND METHODS

Participants

Twenty-one young, non-menopausal, overweight but healthy women, free from pathological conditions (i.e., diabetes, hypertension, cardiovascular disease, depression, etc.) and medications that could interfere with the outcome measures, volunteered for this study. All participants were full-time service workers (e.g., cleaning and administrative positions) of the Catholic University of Brasilia and were not undertaking

any structured exercise regime at the time of the study. The inclusion of non-exercising participants enabled a better isolation of the effects of incidental PA levels on health related parameters. All participants performed all procedures and adhered to similar work schedules during the day (i.e., work day beginning at 7 a.m.). The ethical committee of the Catholic University of Brasilia approved this study and all participants provided informed written consent before participation.

Study Design

This study was conducted over an 11-day period (**Figure 1**) following a familiarization session (1-week earlier). On Day 1 (i.e., Friday), participants were screened, familiarized with all procedures and assessed for body composition. During Days 4–8 (i.e., Monday–Friday), participants visited the laboratory each morning and completed an orthostatic test and a constant-load cycling exercise bout for the determination of HRV and HRR measures. Following this bout, an ambulatory 4-h R-R recording was obtained from each participant during working hours and assessed for ambulatory HRV. Participants' PA levels were recorded over 7 days (i.e., from Tuesday to Monday) of the study. On the final day (i.e., Monday, day 11), participants performed an incremental cycle ergometer test for the determination of maximum oxygen consumption (VO_2max), an index of CRF.

Anthropometry

Body mass (kg) was evaluated using a digital scale (G-Tech, 05, China®) and height (cm) assessed via a stadiometer (Sanny®, ES2040, São Bernardo do Campo, Brazil) for determination of body mass index (BMI). Waist circumference was determined at the smallest girth of the trunk using a metal anthropometric tape (Sanny, SN4010, Medical®, Brazil). Body composition (% fat) was assessed from skinfold measurements obtained using skinfold calipers (Lange Skinfold Caliper® California, USA) in accordance with Jackson and Pollock (1985). Three measurements were made at the following sites: triceps, subscapular, abdominal, axillary, thigh, chest, and suprailiac. Body fat composition (%) was calculated from the average of the three measurements at each skinfold site using the Siri equation (Siri, 1961).

Heart Rate Variability

Assessment of HRV was conducted in two stages, an orthostatic test and during normal work activities (08:00–12:00). The daily orthostatic test was performed in the laboratory (07:00–08:00) within standard environmental conditions. The orthostatic test consisted of participants sitting for 3 min followed by 4 min of standing. All analyses were conducted during the last 2 min of each position. Following the orthostatic and submaximal exercise tests (see below), participants left the laboratory and undertook their normal, morning (08:00–12:00) work activities

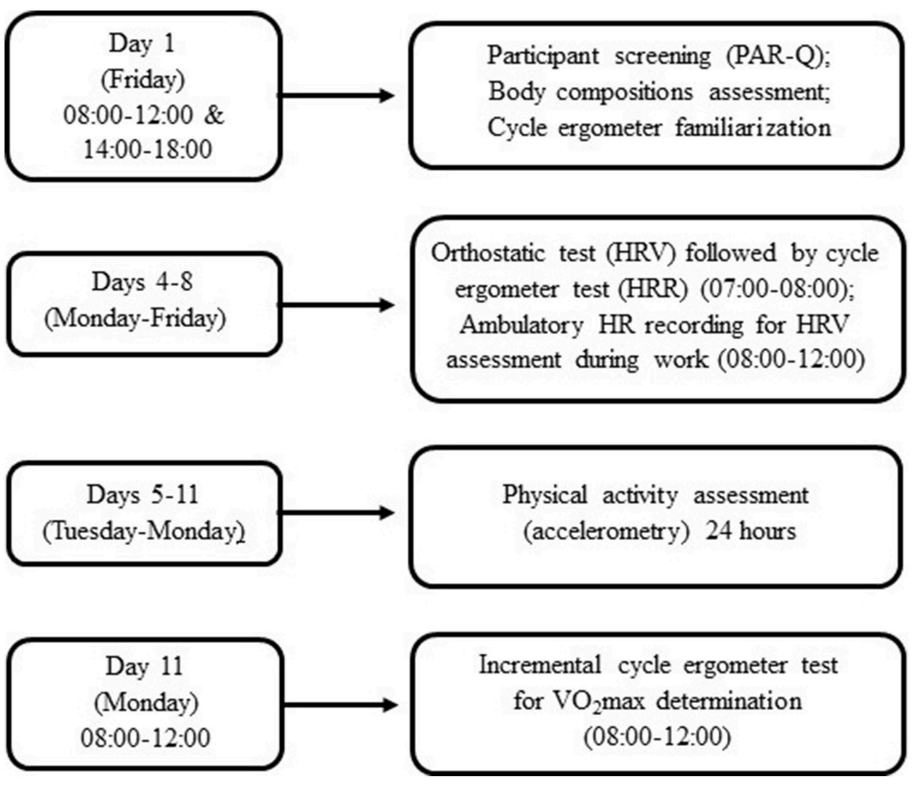

FIGURE 1 | Study design. Par-Q, Physical Activity Readiness Questionnaire; HRV, Heart Rate Variability; HRR, Heart Rate Recovery; HR, Heart Rate; VO_2max, Maximum oxygen consumption.

while wearing a telemetric HR monitor (see below). Assessment of daily HRV during work was determined from the entire 4-h HR recording.

All HR recordings were obtained using a HR monitor (RS800CX, Polar Electro Oy, Finland) reported to provide valid recordings for HRV assessment (Wallén et al., 2012) at a sampling rate of 1000 Hz. Recordings were uploaded into a computer and filtered using the manufacturers' software (Polar ProTrainer® version 5.0, POLAR Electro Oy, Kuopio, Finland) followed by exportation to a dedicated program (Kubios HRV v2.0, Kuopio University, Finland) for the HRV analyses. The HRV variables examined included those previously examined in other studies of exercise and HRV (Leicht et al., 2011; Boullosa et al., 2014): time domain (SDNN and RMSSD), frequency domain (LF and HF in both absolute and normalized units), and non-linear measures (SD1, SD2, Sample Entropy, and α1) (Task Force, 1996; Acharya et al., 2004; Boullosa et al., 2014). Based on previous studies (Boullosa et al., 2013; Plews et al., 2014), daily HRV measures for each of the 4 days (from Tuesday to Friday) as well as the average of the 4 days were used for further analyses.

Heart Rate Recovery

A submaximal, square-wave, exercise bout was undertaken by participants each day over 4 consecutive days and utilized for the determination of HRR. Participants exercised on a cycle ergometer (Monark model 8348, Monark, Sweden) at 60 rpm for 6 min (Arduini et al., 2011) with the workload increased every 30 s for the first 3 min to achieve a target HR and thereafter remained constant for the exercise bout. The set target was a workload that induced a HR of ∼86% of age-predicted maximum HR (HR_{max}, Tanaka et al., 2001) with the target HR and protocol determined during the familiarization session to ensure a consistent exercise response. This target HR was suggested to provide the most reliable HRR measures (Lamberts et al., 2011).

The change (Δ) in HR during the first 1, 2, 3, and 5 min of recovery were evaluated as HRR measures (Arduini et al., 2011). Additionally, the HRR index, as proposed by Imai et al. (1994), was calculated via semi-logarithmic regression. Briefly, the natural logarithm of the instantaneous HR during the initial rapid HR decrease (from the 10th to the 40th s) was plotted against the elapsed time of recovery and a linear regression analysis was applied. The time constant of the short, post-exercise HR decay (T30) was thus determined as the negative reciprocal of the slope of the regression line. Following the same rationale for the HRV measures (Boullosa et al., 2013), the daily HRR measures (Imai et al., 1994; Arduini et al., 2011), as well as the average HRR for the 4 monitoring days were utilized for analyses.

Physical Activity Assessment

PA was objectively measured by an accelerometer (GT1M, Actigraph, USA) for 7 consecutive days. The devices were used on the right hip of participants and recorded continuously from Tuesday to the next Monday, except during the bathing, sleeping and the cycle ergometer evaluations.

The start and end days of the accelerometer recording (i.e., data from Tuesday and Monday) were excluded with the final analysis of PA comprising 5 full days (i.e., Wednesday to Sunday only). Three to five days of PA assessment have been suggested to be sufficient to accurately reflect weekly PA patterns of adults (Trost et al., 2005). Technical details of this accelerometry device and its measurement of PA intensity have been published elsewhere (John and Freedson, 2012). Briefly, these devices measure accelerations in the vertical plane at a sampling frequency (epoch) of 5 Hz, which is preferable over longer epochs (example 60 s) (Orme et al., 2014). The unit of measurement of the accelerometer was counts per minute with higher counts per minute indicating greater accelerations and intensity of the activity. In the present study, the well-established (Freedson et al., 1998) cut-off limits were chosen to determine moderate (1952 counts/min), vigorous (5725 counts/min), and very vigorous (>9499 counts/min) PA categories. These cut-off limits represent PA intensities of 3, 6, and >8.99 METS, respectively (Freedson et al., 1998). The accumulated time spent (minutes per day) in physical activities for each of these PA categories was calculated.

In addition to PA intensity, step count was also included in the analysis. The GT1M includes a step counting mode which records the number of positive accelerations followed immediately by a negative acceleration (i.e., steps and steps/day) undertaken by the user. This measure was included as an easily interpretable indicator of overall volume of PA (Tudor-Locke et al., 2011).

Cardiorespiratory Fitness

Cardiorespiratory fitness was assessed as the VO_2max during a maximal incremental test on a cycle ergometer (Lode Excalibur, Lode, Netherlands; or Monark model 8348, Monark, Sweden). The test started with a load of 0 W and thereafter the load was increased at a rate of 20 $W \cdot min^{-1}$, maintaining a constant cadence of 60 rpm. Throughout the graded exercise test, HR was recorded using a telemetric monitor (POLAR Electro Oy, Finland) while ventilatory parameters (e.g., oxygen consumption, VO_2) were assessed breath-by-breath via a metabolic cart (Metalyzer 3B, Cortex, Leipzig, Germany). All participants were verbally encouraged to exercise until voluntary exhaustion with HR similar to or greater than age-predicted HR_{max} (Tanaka et al., 2001) and respiratory exchange ratio greater than 1.1 (Howley et al., 1995) defining VO_2max.

Statistical Analysis

Statistical analysis was performed with a statistical package (SPSS, v 20.0, IBM). Descriptive statistics were used to present means, standard deviations (±SD) and 90% confidence interval (90% CI). Normality was assessed by Shapiro-Wilk test. Variables with non-normal distribution were log-transformed (Ln) for analysis but presented in original units. As varying degrees of reliability have been reported for HR measures during various conditions (Arduini et al., 2011; Young and Leicht, 2011; Dupuy et al., 2012; Boullosa et al., 2014), reliability for HRV and HRR measures were assessed via typical error of measurement (TEM) expressed as the coefficient of variation (CV, %) for absolute reliability and intra-class correlation coefficient (ICC) for relative reliability (i.e., ratio of variance due to differences between subjects to the total variability in the data) (Hopkins, 2000a).

Reliability measures were calculated using a reliability spreadsheet (Hopkins, 2000b) with known thresholds for varying levels of reliability. For the HRR kinetics, data were modeled with a monoexponential fit (Sigmaplot 12; SPSS Science, Chicago, IL) as previously described (Boullosa et al., 2014) with the time constant (τ) used for further analysis. Pearson product correlation coefficients (r) with 90% confidence intervals (90% CI) were calculated to assess the relationships between selected parameters. The level of significance was set at $p < 0.05$.

RESULTS

Demographic characteristics and PA levels of participants are shown in **Table 1**. Briefly, all females were overweight (BMI>25), with low CRF, undertook an average of >10,000 steps/day and were engaged mainly in moderate levels of PA.

Seated and standing HRV measures during the orthostatic test, ambulatory HRV measures, and HRR measures on the 4 different days are presented in **Tables 2A,B**, **3**, **4**, respectively. Average reliability for the HRV measures during seated rest (see **Table 2A**) was moderate while low to moderate during orthostatic stress (see **Table 2B**). Similarly, the HRV ambulatory measures exhibited variable reliability from poor to excellent (see **Table 3**). For the HRR measures, reliability was low to moderate (see **Table 4**). As reliability was low to moderate for most HR measures, relationships between these and other variables were examined using the 4-day average measures.

Relationships between Autonomic Indices and PA

When considering the 4-day average ambulatory HRV measures, correlations were observed between VPA and: RMSSD ($r = -0.449$, $p = 0.041$), HF ($r = -0.520$, $p = 0.016$), and SD1 ($r = -0.463$, $p = 0.035$); and between VPA+VVPA and: RMSSD ($r = -0.453$, $p = 0.039$), HF ($r = -0.526$, $p = 0.014$), and SD1 ($r = -0.473$, $p = 0.030$). Additionally, 4-day average HRR1 was correlated to MPA, VPA, and VPA+VVPA (see **Figure 2**) but not with Step·day^{1} ($r = 0.376$, $p = 0.093$). No correlations were found between HRV measures in the orthostatic test and measures of PA.

TABLE 1 | Demographic characteristics and physical activity levels of participants ($n = 21$).

Parameters	Mean (*SD*)
Age (years)	34.5 (6.4)
Height (m)	1.60 (0.06)
Weight (kg)	67.0 (11.37)
BMI (kg/m^2)	26.3 (4.1)
% Fat	37.0 (4.7)
WC (cm)	79.7 (9.7)
VO_2max (ml·kg^{-1}·min^{-1})	24.6 (5.3)
Steps·day^{-1}	10424 (3047)
MPA (min/day)	57.22 (18.23)
VPA (min/day)	1.16 (0.93)
VVPA (min/day)	0.08 (0.11)
VPA + VVPA (min/day)	1.24 (0.96)

SD, Standard Deviation; BMI, body mass index; WC, waist circumference; VO_2max, Maximum oxygen consumption. PA variables are presented as a daily mean for the 5 days of data collection; Steps·day^{-1}, mean daily steps; MPA, mean daily moderate physical activity; VPA, mean daily vigorous physical activity; VVPA, mean daily very vigorous physical activity; VPA + VVPA, sum of vigorous physical activity and very vigorous physical activity.

Relationships between Autonomic Indices and CRF

Significant relationships were identified between CRF and 4-day average HRV measures with VO_2max correlated to standing HF ($r = 0.523$, $p = 0.015$), standing LF ($r = 0.550$, $p = 0.01$), standing RMSSD ($r = 0.641$, $p = 0.002$), standing SDNN ($r =$

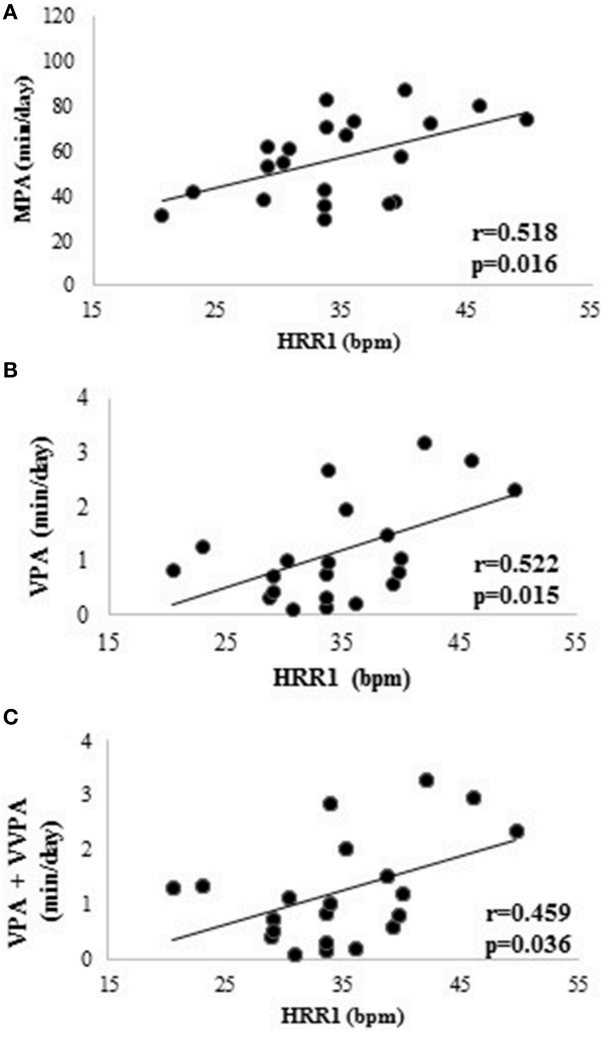

FIGURE 2 | Relationships between heart rate recovery in the first min and physical activity measures. (A) Relationship between heart rate recovery in the first min and mean daily moderate physical activity. **(B)** Relationship between heart rate recovery in the first min and mean daily vigorous physical activity. **(C)** Relationship between heart rate recovery in the first min and sum of vigorous physical activity and very vigorous physical activity. HRR1, 4-day average heart rate recovery within the first minute; VPA, mean daily vigorous physical activity; VPA+VVPA, sum of vigorous physical activity and very vigorous physical activity.

Table 2A | Heart rate variability measures seated during the orthostatic test and their corresponding reliability measures.

	Tuesday	Wednesday	Thursday	Friday	Average	CV, %	ICC
SDNN (ms)	38.9 ± 13.0 (34.2–43.5)	42.1 ± 12.0 (37.8–46.4)	42.7 ± 12.0 (38.4–47.0)	42.6 ± 17.6 (36.3–48.9)	41.5 ± 11.3 (37.5–45.6)	8.6 (7.4–10.5)	0.63 (0.45–0.79)
RMSSD (ms)	26.1 ± 13.2 (21.3–30.8)	27.0 ± 12.7 (22.4–31.5)	24.5 ± 9.2 (21.1–27.8)	29.8 ± 17.7 (23.4–30.3)	26.8 ± 9.8 (23.3–30.3)	10.4 (9.0–12.7)	0.43 (0.22–0.64)
LF $(ms)^2$	520 ± 398 (377–663)	540 ± 463 (373–706)	611 ± 580 (403–820)	777 ± 671 (553–926)	615 ± 456 (451–779)	310 (267–378)	0.69 (0.52–0.82)
LF (n.u.)	65.48 ± 23.79 (56.94–74.02)	64.37 ± 23.15 (56.06–72.68)	68.39 ± 20.14 (61.16–75.62)	69.71 ± 23.11 (61.41–78.00)	66.99 ± 18.83 (60.23–73.75)	14.42 (12.45–17.60)	0.32 (0.12–0.55)
HF $(ms)^2$	324 ± 322 (208–439)	291 ± 278 (191–391)	267 ± 214 (190–344)	451 ± 663 (213–689)	333 ± 289 (229–437)	281 (243–343)	0.55 (0.35–0.73)
HF (n.u.)	34.51 ± 20.42 (27.18–41.85)	35.63 ± 19.99 (28.45–42.80)	31.60 ± 15.18 (26.15–37.05)	30.28 ± 18.78 (23.54–37.03)	33.00 ± 19.93 (28.00–38.01)	14.42 (12.45–17.60)	0.32 (0.12–0.55)
SD1 (ms)	19.7 ± 8.9 (16.4–22.8)	20.0 ± 10.3 (16.3–23.7)	16.5 ± 7.3 (13.8–19.1)	20.8 ± 12.6 (16.3–25.3)	19.2 ± 13.0 (16.6–21.8)	7.6 (6.5–9.2)	0.43 (0.23–0.64)
SD2 (ms)	53.4 ± 15.7 (47.8–59.1)	57.8 ± 16.8 (51.8–63.8)	54.0 ± 16.8 (47.9–60.0)	58.7 ± 24.2 (50.0–67.3)	56.0 ± 15.6 (50.3–61.5)	11.7 (10.1–14.2)	0.63 (0.54–0.76)
α1	1.13 ± 0.30 (1.02–1.24)	1.24 ± 0.28 (1.14–1.34)	1.21 ± 0.17 (1.15–1.28)	1.25 ± 0.27 (1.16–1.36)	1.21 ± 0.27 (1.14–1.28)	20.3 (17.5–24.8)	0.43 (0.22–0.64)
SampEn	1.42 ± 0.35 (1.29–1.55)	1.25 ± 0.40 (0.72–2.26)	1.34 ± 0.35 (1.25–1.50)	1.47 ± 0.30 (1.36–1.57)	1.38 ± 0.35 (1.23–1.90)	0.98 (0.84–1.19)	0.05 (−0.12–0.28)

Values are mean ± SD (confidence intervals, CI 90%); standard deviation of all R-R intervals (SDNN), root mean square of successive differences between normal sinus R-R intervals (RMSSD), low-frequency (LF), high-frequency (HF), Very-low frequency (VLF), Total power, short-term beat-to-beat R-R variability from the Poincaré plot (SD1), long-term beat-to-beat variability from the Poincaré plot (SD2), and detrended fluctuations of short fractal scaling (α1), sample entropy (SampEn), TEM expressed as the coefficient of variation (CV%), intra-class correlation coefficient (ICC).

Table 2B | Heart rate variability measures standing during the orthostatic test and their corresponding reliability measures.

	Tuesday	Wednesday	Thursday	Friday	Average	CV, %	ICC
SDNN (ms)	34.1 ± 9.6 (30.7–37.6)	33.1 ± 12.0 (28.8–37.4)	33.1 ± 10.6 (29.2–36.9)	37.5 ± 14.1 (32.4–42.5)	34.4 ± 9.9 (30.9–38.0)	6.85 (5.91–8.36)	0.68 (0.51–0.82)
RMSSD (ms)	16.0 ± 4.8 (14.3–17.7)	17.3 ± 8.9 (14.0–20.4)	16.9 ± 7.3 (14.3–19.5)	19.1 ± 8.9 (15.9–22.3)	17.3 ± 6.4 (15.0–19.6)	4.47 (3.85–5.45)	0.68 (0.51–0.82)
LF $(ms)^2$	598 ± 638 (306–818)	524 ± 548 (327–721)	469 ± 305 (359–578)	577 ± 694 (327–826)	540 ± 505 (358–721)	324 (280–395)	0.69 (0.53–0.83)
LF (n.u.)	82.13 ± 20.48 (74.30–89.48)	78.91 ± 20.65 (71.50–86.33)	79.26 ± 21.09 (71.69–86.83)	78.13 ± 21.94 (70.25–86.00)	79.61 ± 19.19 (72.72–86.49)	10.45 (9.02–12.75)	0.25 (0.05–0.49)
HF $(ms)^2$	106 ± 60 (85–128)	129 ± 124 (84–173)	120 ± 86 (89–151)	140 ± 125 (95–184)	124 ± 71 (98–149)	83 (72–101)	0.36 (0.15–0.58)
HF (n.u)	17.87 ± 10.34 (14.16–21.58)	21.08 ± 12.37 (16.64–25.52)	20.73 ± 12.94 (16.09–25.38)	21.86 ± 14.63 (16.61–27.12)	20.39 ± 9.25 (17.06–23.71)	10.45 (9.02–12.75)	0.25 (0.05–0.49)
SD1 (ms)	12.5 ± 6.1 (10.3–14.7)	13.6 ± 7.3 (10.9–16.2)	12.5 ± 5.6 (10.5–14.5)	13.4 ± 6.7 (11.0–15.8)	13.0 ± 5.4 (11.0–14.9)	3.98 (3.44–4.86)	0.43 (0.23–0.64)
SD2 (ms)	45.7 ± 21.1 (38.1–53.2)	46.2 ± 15.6 (40.5–51.8)	51.4 ± 19.5 (44.3–58.3)	49.7 ± 21.2 (42.0–57.2)	48.2 ± 15.5 (42.6–53.8)	13.23 (11.42–16.15)	0.56 (0.36–0.74)
α1	1.40 ± 0.24 (1.31–1.49)	1.33 ± 0.19 (1.31–1.45)	1.46 ± 0.20 (1.40–1.54)	1.46 ± 0.18 (1.40–1.53)	1.42 ± 0.17 (1.39–1.47)	19.2 (16.5–23.4)	0.14 (−0.05–0.38)
SampEn	1.30 ± 0.39 (1.16–1.44)	1.19 ± 0.36 (1.06–1.32)	1.14 ± 0.28 (1.04–1.24)	1.20 ± 0.39 (1.06–1.34)	1.21 ± 0.36 (1.13–1.29)	0.95 (0.82–1.16)	0.10 (−0.08–0.34)

Values are mean ± SD (confidence intervals, CI 90%); standard deviation of all R-R intervals (SDNN), root mean square of successive differences between normal sinus R-R intervals (RMSSD), low-frequency (LF), high-frequency (HF), Very-low frequency (VLF), Total power, short-term beat-to-beat R-R variability from the Poincaré plot (SD1), long-term beat-to-beat variability from the Poincaré plot (SD2), and detrended fluctuations of short fractal scaling (α1), sample entropy (SampEn), TEM expressed as the coefficient of variation (CV%), intra-class correlation coefficient (ICC).

0.445, $p = 0.043$), seated SDNN ($r = 0.475$, $p = 0.030$), seated SD2 ($r = 0.491$, $p = 0.024$), and ambulatory LF ($r = 0.554$, $p = 0.009$). Average 4-day HRR measures were not correlated to CRF.

Relationships Among Autonomic Indices

Significant relationships were determined between the 4-day average values for HRV measures during the orthostatic test and the 4-h ambulatory period (see **Table 5**). Most of these

Table 3 | Heart rate variability ambulatory measures (4 h) and their corresponding reliability measures.

	Tuesday	Wednesday	Thursday	Friday	Average	CV, %	ICC
SDNN (ms)	95.0 ± 29.3 (82.2–107.7)	94.9 ± 20.0 (84.8–104.9)	102.3 ± 36.7 (87.1–117.4)	101.9 ± 29.6 (88.2–108.8)	98.5 ± 20.2 (88.2–114.8)	25.7 (22.2–31.4)	0.25 (0.05–0.49)
RMSSD (ms)	26.4 ± 10.0 (22.6–30.2)	24.3 ± 10.2 (20.3–28.1)	24.7 ± 8.9 (21.0–28.3)	26.2 ± 9.8 (22.2–28.3)	25.3 ± 8.6 (21.8–28.8)	5.0 (4.3–6.1)	0.75 (0.60–0.86)
LF $(ms)^2$	905 ± 413 (740–1067)	887 ± 468 (708–1071)	876 ± 412 (709–1037)	954 ± 529 (753–1160)	905 ± 428 (736–1075)	191 (165–234)	0.84 (0.73–0.91)
LF (n.u.)	80.16 ± 19.11 (73.30–87.02)	80.24 ± 19.37 (73.29–87.19)	80.62 ± 19.57 (73.60–87.65)	79.94 ± 19.33 (73.00–86.88)	80.24 ± 19.00 (73.42–87.06)	4.42 (3.81–5.39)	0.76 (0.62–0.87)
HF $(ms)^2$	239 ± 172 (189–286)	234 ± 186 (168–295)	232 ± 174 (166–288)	256 ± 199 (191–338)	240 ± 168 (183–298)	81 (70–99)	0.82 (0.70–0.90)
HF (n.u.)	19.84 ± 8.30 (16.86–22.82)	19.75 ± 8.48 (16.58–22.93)	19.37 ± 9.22 (16.06–22.68)	20.05 ± 9.06 (16.80–23.30)	19.75 ± 8.09 (16.85–22.66)	4.42 (3.81–5.39)	0.76 (0.62–0.87)
SD1 (ms)	18.2 ± 7.3 (15.4–20.9)	17.5 ± 6.9 (14.7–20.1)	17.3 ± 6.2 (14.8–19.9)	18.7 ± 6.9 (15.8–21.5)	17.9 ± 6.1 (15.4–20.4)	3.5 (3.1–4.3)	0.75 (0.60–0.86)
SD2 (ms)	141.4 ± 59.5 (117.4–165.3)	131.9 ± 28.2 (117.8–146.0)	142.4 ± 51.7 (121.1–163.7)	142.9 ± 41.5 (124.7–161.0)	139.6 ± 31.5 (124.2–155.0)	39.4 (34.0–48.1)	0.30 (0.10–0.53)
α1	1.48 ± 0.12 (1.33–1.57)	1.45 ± 0.14 (1.33–1.57)	1.46 ± 0.12 (1.34–1.58)	1.44 ± 0.12 (1.32–1.56)	1.45 ± 0.11 (1.33–1.57)	0.07 (0.06–0.09)	0.69 (0.53–0.83)
SampEn	0.92 ± 0.23 (0.82–1.03)	1.01 ± 0.67 (0.76–1.27)	0.84 ± 0.19 (0.74–0.93)	0.90 ± 0.19 (0.81–1.00)	0.92 ± 0.38 (0.80–1.04)	0.95 (0.82–1.16)	0.11 (-0.07–0.35)

Values are mean ± SD (confidence intervals, CI 90%); standard deviation of all R-R intervals (SDNN), root mean square of successive differences between normal sinus R-R intervals (RMSSD), low-frequency (LF), high-frequency (HF), Very-low frequency (VLF), Total power, short-term beat-to-beat R-R variability from the Poincaré plot (SD1), long-term beat-to-beat variability from the Poincaré plot (SD2), and detrended fluctuations of short fractal scaling (α1), sample entropy (SampEn), TEM expressed as the coefficient of variation (CV%), intra-class correlation coefficient (ICC).

Table 4 | Heart rate recovery over 5 min during 4 days and their corresponding reliability measures.

	Tuesday	Wednesday	Thursday	Friday	Average	CV, %	ICC
HRend	155 ± 6 (152–157)	153 ± 4 (152–155)	155 ± 4 (153–157)	155 ± 3 (153–156)	154 ± 5 (153–156)	2.67 (2.31–3.26)	0.74 (0.59–0.85)
HR RECOVERY							
Δ 1′ (bpm)	36 ± 7 (33–39)	34 ± 7 (31–37)	33 ± 8 (29–35)	34 ± 11 (30–38)	34 ± 7 (32–37)	6.33 (5.46–7.72)	0.51 (0.31–0.70)
Δ 2′ (bpm)	49 ± 6 (46–51)	44 ± 9 (40–47)	46 ± 8 (43–48)	45 ± 8 (42–48)	46 ± 5 (44–48)	6.50 (5.61–7.93)	0.39 (0.18–0.61)
Δ 3′ (bpm)	51 ± 8 (48–54)	50 ± 13 (45–55)	50 ± 7 (47–52)	51 ± 9 (47–54)	50 ± 7 (48–53)	8.32 (7.18–10.15)	0.50 (0.29–0.69)
Δ 5′ (bpm)	58 ± 10 (54–61)	53 ± 8 (50–56)	56 ± 6 (54–58)	55 ± 8 (52–58)	55 ± 5 (53–57)	6.90 (5.95–8.42)	0.37 (0.16–0.59)
T30	253 ± 100 (217–288)	266 ± 100 (230–301)	226 ± 69 (201–251)	238 ± 57 (217–258)	246 ± 84 (216–275)	75.29 (64.98–91.86)	0.20 (0.01–0.44)
HRR τ (s)	67 ± 27 (54–80)	65 ± 14 (57–72)	74 ± 31 (58–89)	60 ± 18 (51–69)	67 ± 23 (56–78)	31.3 (26.3–39.7)	0.36 (0.14–0.59)

Values are mean ± SD (confidence intervals, CI 90%); end heart rate in the test (HRend), negative reciprocal of the slope of the regression line of heart beats during the initial rapid HR decrease (from the 10th to the 40th s) (T30); heart rate change in first minute (Δ 1′), heart rate change in two first minute (Δ 2′), heart rate change in three first minute (Δ 3′), heart rate change in five first minute (Δ 5′), heart rate recovery in time constant (HRR τ). TEM expressed as the coefficient of variation (CV%), intra-class correlation coefficient (ICC).

correlations were moderate. No correlations were found between HRR and HRV measures.

No correlations were identified between CRF and levels of PA.

DISCUSSION

The current study has confirmed that cardiac autonomic indices were associated with PA and CRF in a group of healthy, overweight women who did not exercise with different relationships identified based upon the HRV condition (i.e., seated, standing, or ambulatory). Specifically, greater measures of HRV were associated with greater CRF. Similarly greater HRR1 was associated with greater PA including MPA, VPA, and VVPA. Therefore, CRF may be a more important influence than PA in enhancing HRV while PA may be integral for enhancing parasympathetic reactivation. Given the low-to-moderate levels of reliability exhibited by these HR measures, the use of average weekly recordings in further studies is

Table 5 | Matrix of correlations between 4-day average values for HRV measures during the orthostatic test and 4-h ambulatory recordings.

		Ambulatory HRV							
		RMSSD *r (p)*	**SDNN** *r (p)*	**LF** *r (p)*	**HF** *r (p)*	**SD1** *r (p)*	**SD2** *r (p)*	**SampEn** *r (p)*	α 1 *r (p)*
RMSSD	Standing	0.602 (0.00)	0.316 (0.16)	0.651 (0.00)	0.493 (0.02)	0.602 (0.00)	0.311 (0.17)	0.522 (0.01)	−0.208 (0.20)
	Seated	0.640 (0.00)	0.203 (0.37)	0.586 (0.00)	0.524 (0.01)	0.672 (0.00)	0.199 (38)	0.297 (0.19)	−0.234 (0.07)
SDNN	Standing	0.407 (0.06)	0.262 (0.25)	0.517 (0.01)	0.701 (0.00)	0.406 (0.06)	0.257 (0.26)	0.442 (0.04)	−0.246 (0.28)
	Seated	0.598 (0.00)	0.718 (0.00)	0.733 (0.00)	0.924 (0.00)	0.596 (0.00)	0.710 (0.00)	0.385 (0.08)	−0.654 (0.00)
LF	Standing	0.651 (0.00)	0.456 (0.03)	0.570 (0.00)	0.634 (0.00)	0.719 (0.00)	0.455 (0.03)	0.372 (0.09)	−0.294 (0.10)
	Seated	0.594 (0.00)	0.386 (0.08)	0.618 (0.00)	0.338 (0.15)	0.406 (0.06)	0.383 (0.08)	0.568 (0.00)	−0.007 (0.92)
HF	Standing	0.535 (0.01)	0.308 (0.18)	0.548 (0.01)	0.547 (0.01)	0.510 (0.01)	0.299 (0.18)	0.455 (0.04)	−0.508 (0.01)
	Seated	0.541 (0.00)	0.231 (0.31)	0.388 (0.07)	0.632 (0.00)	0.577 (0.00)	0.226 (0.32)	0.348 (0.12)	−0.422 (0.05)
SD1	Standing	0.602 (0.00)	0.386 (0.08)	0.751 (0.00)	0.480 (0.02)	0.596 (0.00)	0.383 (0.08)	0.471 (0.03)	−0.222 (0.33)
	Seated	0.641 (0.00)	0.634 (0.00)	0.615 (0.00)	0.597 (0.00)	0.926 (0.00)	0.626 (0.00)	0.481 (0.02)	−0.753 (0.00)
SD2	Standing	0.733 (0.00)	0.570 (0.00)	0.569 (0.00)	0.615 (0.00)	0.773 (0.00)	0.564 (0.00)	0.276 (0.22)	−0.081 (0.72)
	Seated	0.460 (0.03)	0.456 (0.04)	0.680 (0.00)	0.405 (0.07)	0.461 (0.04)	0.455 (0.04)	0.389 (0.08)	−0.055 (0.81)
SampEn	Standing	0.357 (0.11)	−0.047 (0.84)	0.409 (0.06)	0.309 (0.17)	0.361 (0.10)	−0.060 (0.79)	0.314 (0.16)	−0.175 (0.44)
	Seated	0.585 (0.00)	0.313 (0.16)	0.404 (0.07)	0.511 (0.01)	0.557 (0.00)	0.327 (0.14)	0.527 (0.01)	−0.583 (0.00)
α 1	Standing	0.562 (0.00)	0.303 (0.18)	0.643 (0.00)	0.480 (0.03)	0.562 (0.00)	0.299 (0.19)	−0.211 (0.36)	−0.236 (0.30)
	Seated	0.672 (0.00)	0.250 (0.27)	0.570 (0.00)	0.597 (0.00)	0.673 (0.00)	0.245 (0.28)	−0.079 (0.73)	−0.466 (0.03)

Root mean square of successive differences between normal sinus R-R intervals (RMSSD), Standard deviation of all R-R intervals (SDNN), absolute low-frequency (LF), absolute high-frequency (HF), short-term beat-to-beat R-R variability from the Poincaré plot (SD1), long-term beat-to-beat variability from the Poincaré plot (SD2), sample entropy (SampEn), and detrended fluctuations of short fractal scaling (α1).

recommended for a more precise evaluation of autonomic control of HR.

The most novel finding of the current study was the moderate relationships between PA measures and the 4-day average of HRR1. To our knowledge, this is the first time a relationship between objectively measured PA levels and parasympathetic reactivation has been reported and reinforces the important role that incidental PA has on the autonomic control of HR and potential cardiovascular health (Cole et al., 1999). This relationship was identified in a unique sample of overweight women who did not exercise and clearly highlights the impact of incidental PA on HR control unlike previous studies evaluating the relationship between PA levels and autonomic indices (Buchheit et al., 2005, 2006; Buchheit and Gindre, 2006). Previous studies failed to differentiate between incidental and exercise based PA (Buchheit et al., 2005, 2006) and employed questionnaires for the identification of training load (Buchheit and Gindre, 2006). These factors may have masked potential associations between PA and HR control with further studies warranted to verify this relationship in other populations of different gender and age.

In line with a previous report (Hautala et al., 2010), we observed negative relationships between short-term, vagally related HRV measures (e.g., RMSSD, HF, and SD1) during ambulatory conditions and objectively measured PA levels. This was an expected finding that reinforces the robustness of long-term HRV measures (i.e., SDNN and SD2) for autonomic evaluations in ambulatory conditions as these measures were unaffected by PA levels. Further, these results may indirectly reflect the relationship between CRF, PA, and HRV with those adults undertaking more PA likely to exhibit greater CRF and subsequently higher HRV. While we did not find any relationship between PA and CRF, maybe because of the low levels of VPA and VVPA recorded, further longitudinal interventions may elucidate the possible influence of greater levels of incidental PA on CRF in different populations.

In our study, a greater CRF (i.e., VO_2max) was positively related to greater weekly average HRV levels during a range of conditions (e.g., postures, ambulation). These moderate relationships were in line with previous studies that reported a greater autonomic control of HR in those individuals with greater CRF or running capacity (Buchheit and Gindre, 2006; Kiviniemi et al., 2007; Hautala et al., 2009; Boullosa et al., 2013). However, and in contrast to previous studies (Boullosa et al., 2009; Daanen et al., 2012), no relationship was observed between CRF and HRR measures. Although speculative, this lack of significant correlation could reflect the homogeneous sample of women that were not engaged in regular exercise (i.e., low VO_2max). In addition, the absence of correlations between CRF and HRR measures could reflect a differential regulation of HRR in females with gender differences in post-exercise, autonomic control previously reported (Mendonca et al., 2010; Barak et al., 2014). Further, the females undertook incidental PA of varying intensities that may have impacted HRR in a similar way that training load impacted on HRR in previous studies with regular exercisers (Buchheit and Gindre, 2006; Guerra et al., 2014). This consideration is important as previous studies were conducted within sport settings and participants with high CRF. In contrast, the current study has been the first to our knowledge, to report these relationships within a work environment (i.e., non-athletes). Overall, these findings highlight the various contributors (i.e., exercise and incidental

PA) to different cardiac autonomic indices and reinforce the value of considering both HRV and HRR when evaluating the autonomic control of HR and its relationship with CRF in different populations. The exact contributions of incidental PA and exercise-based PA to cardiac autonomic control in physically active individuals remains to be differentiated in future studies.

The relationships between average HRV measures during the orthostatic test and the averaged ambulatory HRV measures (**Table 5**) were primarily moderate with long-term HRV measures unrelated to ambulatory measures. Previously, long-term HRV indices were reported to be more robust during ambulatory conditions (Hautala et al., 2010). Within the current study, short-term (i.e., RMSSD, SD1, and α1) and frequency domain (i.e., HF and LF) HRV measures exhibited better absolute and relative reliability during ambulatory conditions (i.e., 4 h) when compared to shorter recordings (i.e., 2 min) in seated and standing postures. The moderate reliability for a range of HRV measures reinforces the variable nature of HRV over a normal week with special attention to the reliability of HRV parameters in different conditions encouraged for better assessments of cardiac autonomic adaptations. Finally, the absence of a relationship between HRR and HRV was in agreement with a previous study of healthy individuals (Esco et al., 2010), and reinforces the specific and unique contributors to these different autonomic measures.

The cross-sectional design of the current study suggests some caution and the necessity of further longitudinal studies for verifying the direction and strength of the relationships between these health related parameters. Additionally, our findings were limited to adult female workers with further studies of other populations needed for elucidating the possible role of age on these relationships. Of note, recent evidence (Triggiani et al., 2015) suggests that HRV seems to be reduced in overweight healthy adult women therefore further studies should verify if the current results would be different in males and females with different percentages of body fatness. Finally, different protocols for HRV and HRR evaluations were conducted with further studies encouraged to employ other recent protocols such as recording HR measures during walking at a fixed speed (Boullosa et al., 2014) and ultra-short term HR measures (Ostojic et al., 2010; Nakamura et al., 2015).

In conclusion, the current study has defined the relationships between cardiac autonomic indices and PA and CRF in a group of healthy, non-exercising overweight women. Greater HRV was associated with greater CRF that highlighted the merit of improving CRF as a means to enhance cardiac autonomic control. In contrast, greater post-exercise HRR was associated with greater PA that reflects the unique pertinence of PA for enhancing parasympathetic reactivation. Finally, relationships between cardiac autonomic indices and other health indicators (PA and CRF) were influenced by the type and frequency of measure utilized with further studies recommended for an enhanced understanding of the contributors to autonomic control of HR for health.

AUTHOR CONTRIBUTIONS

LT, study design, data collection, data analysis and interpretation of the results, writing of the manuscript. FR, study design, data analysis and interpretation of the results, writing of the manuscript. IO, data analysis and interpretation of the results, writing of the manuscript. SD, data analysis and interpretation of the results, writing of the manuscript. AL, data analysis and interpretation of the results, writing of the manuscript. DB, study design, data analysis and interpretation of the results, writing of the manuscript.

REFERENCES

Acharya, U. R., Kannathal, N., Sing, O. W., Ping, L. Y., and Chua, T. (2004). Heart rate analysis in normal subjects of various age groups. *Biomed. Eng. Online* 3:24. doi: 10.1186/1475-925X-3-24

Ainsworth, B. E., Haskell, W. L., Whitt, M. C., Irwin, M. L., Swartz, A. M., Strath, S. J., et al. (2000). Compendium of physical activities: an update of activity codes and MET intensities. *Med. Sci. Sports Exerc*. 32, 498–504.

Ara, I., Aparicio-Ugarriza, R., Morales-Barco, D., Nascimento de Souza, W., Mata, E., and Gonzáles-Gross, M. (2015). Physical activity assessment in the general population validated self-report methods. *Nutr Hosp*. 31(Suppl. 3), 211–218. doi: 10.3305/nh.2015.31.sup3.8768

Arduini, A., Gomez-Cabrera, M. C., and Romagnoli, M. (2011). Reliability of different models to assess heart rate recovery after submaximal bicycle exercise. *J. Sci. Med. Sport*. 14, 352–357. doi: 10.1016/j.jsams.2011.02.012

Barak, O. F., Klasnja, A., Gacesa, J. P., Ovcin, Z. B., and Grujic, N. G. (2014). Gender differences in parasympathetic reactivation during recovery from Wingate anaerobic test. *Period. Biol.* 116, 53–58.

Boullosa, D. A., Abreu, L., Nakamura, F. Y., Muñoz, V. E., Domínguez, E., and Leicht, A. S. (2013). Cardiac autonomic adaptations in elite Spanish soccer players during preseason. *Int. J. Sports Physiol. Perform.* 8, 400–409.

Boullosa, D. A., Abreu, L., Tuimil, J. L., and Leicht, A. S. (2012). Impact of a soccer match on the cardiac autonomic control of soccer referees. *Eur. J. Appl. Physiol.* 112, 2233–2242. doi: 10.1007/s00421-011-2202-y

Boullosa, D. A., Barros, E. S., Del Rosso, S., Nakamura, F. Y., and Leicht, A. S. (2014). Reliability of heart rate measures during walking before and after running maximal efforts. *Int. J. Sports Med.* 35, 999–1005. doi: 10.1055/s-0034-1372637

Boullosa, D. A., Tuimil, J. L., Leicht, A. S., and Crespo-Salgado, J. J. (2009). Parasympathetic modulation and running performance in distance runners. *J. Strength Cond. Res.* 23, 626–631. doi: 10.1519/JSC.0b013e31818dc44e

Buchheit, M., and Gindre, C. (2006). Cardiac parasympathetic regulation: respective associations with cardiorespiratory fitness and training load. *Am. J. Physiol. Heart Circ. Physiol.* 1, H451–H458. doi: 10.1152/ajpheart.00008.2006

Buchheit, M., Simon, C., Charloux, A., Doutreleau, S., Piquard, F., and Brandenberger, G. (2005). Heart rate variability and intensity of habitual physical activity in middle-aged persons. *Med. Sci. Sports Exerc*. 37, 1530–1534. doi: 10.1249/01.mss.0000177556.05081.77

Buchheit, M., Simon, C., Charloux, A., Doutreleau, S., Piquard, F., and Brandenberger, G. (2006). Relationship between very high physical activity energy expenditure, heart rate variability and self-estimate of health status in middle-aged individuals. *Int. J. Sports Med.* 27, 697–701. doi: 10.1055/s-2005-872929

Cole, C. R., Blackstone, E. H., Pashkow, F. J., Snader, C. E., and Lauer, M. S. (1999). Heart-rate recovery immediately after exercise as a predictor of mortality. *N. Engl. J. Med*. 341, 1351–1357. doi: 10.1056/NEJM199910283411804

Daanen, H. A., Lamberts, R. P., Kallen, V. L., Jin, A., and Van Meeteren, N. L. (2012). Systematic review on heart rate recovery to monitor changes in training status in athletes. *Int. J. Sports Physiol. Perform*. 7, 251–260.

Davy, K. P., DeSouza, C. A., Jones, P. P., and Seals, D. R. (1998). Elevated heart rate variability in physical active young and older adult women. *Clin. Sci*. 94, 579–584. doi: 10.1042/cs0940579

Dupuy, O., Mekary, S., Berryman, N., Bherer, L., Audiffren, M., and Bosquet, L. (2012). Reliability of heart rate measures used to assess post-exercise parasympathetic reactivation. *Clin. Physiol. Funct. Imaging*. 32, 296–304. doi: 10.1111/j.1475-097X.2012.01125.x

Esco, M. R., Olson, M. S., Williford, H. N., Blessing, D. L., Shannon, D., and Grandjean, P. (2010). The relationship between resting heart rate variability and heart rate recovery. *Clin. Auton. Res*. 20, 33–38. doi: 10.1007/s10286-009-0033-2

Freedson, P. S., Melanson, E., and Sirard, J. (1998). Calibration of the Computer Science and Applications, Inc. accelerometer. *Med. Sci. Sports Exerc.* 30, 777–781. doi: 10.1097/00005768-199805000-00021

Garber, C. E., Blissmer, B., Deschenes, M. R., Frankliin, B. A., Lamonte, M. J., Lee, I.-M., et al. (2011). American college of sports medicine position stand. quantity and quality of exercise for developing and maintaining cardiorespiratory, musculoskeletal, and neuromotor fitness in apparently healthy adults: guidance for prescribing exercise. *Med. Sci. Sports Exerc.* 43, 1334–1359. doi: 10.1249/MSS.0b013e318213fefb

Gordon, J. L., Ditto, B., Lavoie, K. L., Pelletier, R., Campbell, T. S., Arsenault, A., et al. (2011). The effect of major depression on post exercise cardiovascular recovery. *Psychophysiology* 48, 1605–1610. doi: 10.1111/j.1469-8986.2011.01232.x

Guerra, Z. F., Peçanha, T., Moreira, D. N., Silva, L. P., Laterza, M. C., Nakamura, F. Y., et al. (2014). Effects of load and type of physical training on resting and postexercise cardiac autonomic control. *Clin. Physiol. Funct. Imaging* 34, 114–120. doi: 10.1111/cpf.12072

Hansen, B. H., Kolle, E., Dyrstad, S. M., Holme, I., and Anderssen, S. A. (2011). Accelerometer-determined physical activity in adults and older people. *Med. Sci. Sports Exerc.* 44, 266–272. doi: 10.1249/MSS.0b013e31822cb354

Hautala, A. J., Karjalainen, J., Kiviniemi, A. M., Kinnunen, H., Mäkikallio, T. H., Huikuri, H. V., et al. (2010). Physical activity and heart rate variability measured simultaneously during waking hours. *Am. J. Physiol. Heart Circ. Physiol.* 298, 874–880. doi: 10.1152/ajpheart.00856.2009

Hautala, A. J., Kiviniemi, A. M., and Tulppo, M. P. (2009). Individual responses to aerobic exercise: the role of the autonomic nervous system *Neurosci. Biobehav. Rev*. 33, 107–115. doi: 10.1016/j.neubiorev.2008.04.009

Hautala, A., Martinmaki, K., Kiviniemi, A., Kinnunen, H., Virtanen, P., Jaatinen, J., et al. (2012). Effects of habitual physical activity on response to endurance training. *J. Sports Sci*. 30, 563–569. doi: 10.1080/02640414.2012.658080

Hopkins, W. G. (2000a). Measures of reliability in sports medicine and science. *Sports Med.* 30, 1–15. doi: 10.2165/00007256-200030010-00001

Hopkins, W. G. (2000b). "Reliability from consecutive pairs of trials (Excel spreadsheet)," in *Sportscience*. Available online at: http://sportsci.org/

Howley, E. T., Bassett Junior, D. R., and Welch, H. G. (1995). Criteria for maximal oxygen uptake: review and commentary. *Med. Sci. Sports Exerc.* 27, 1292–1301.

Imai, K., Sato, H., Hori, M., Kusuoka, H., Ozaki, H., Yokoyama, H., et al. (1994). Vagally mediated heart rate recovery after exercise is accelerated in athletes but blunted in patients with chronic heart failure. *J. Am. Coll. Cardiol.* 24, 1529–1535. doi: 10.1016/0735-1097(94)90150-3

Jackson, A. S., and Pollock, M. L. (1985). Practical assessment of body composition. *Physician Sport Med*. 13, 76–90.

Jae, S. Y., Carnethon, M. R., Heffernan, K. S., Choi, Y. H., Lee, M. K., and Fernhall, B. (2007). Association between cardiorespiratory fitness and prevalence of carotid atherosclerosis among men with hypertension. *Am. Heart J*. 153, 1001–1005. doi: 10.1016/j.ahj.2007.02.035

Jakicic, J. M., Jaramillo, S. A., Balasubramanyam, A., Bancroft, B., Curtis, J. M., Mathews, A., et al. (2009). Effect of a lifestyle intervention on change in cardiorespiratory fitness in adults with type 2 diabetes: results from the Look AHEAD Study. *Int. J. Obes. (Lond)*. 33, 305–316. doi: 10.1038/ijo.2008.280

John, D., and Freedson, P. (2012). Actigraph and actical physical activity monitors: a peek under the hood. *Med. Sci. Sports Exerc.* 44, S86–S89. doi: 10.1249/MSS.0b013e3182399f5e

Kiviniemi, A. M., Hautala, A. J., Kinnunen, H., Nissilä, J., Virtanen, P., Karjalainen, J., et al. (2010). Daily exercise prescription on the basis of HR variability among men and women. *Med. Sci. Sports Exerc.* 42, 1355–1363. doi: 10.1249/MSS.0b013e3181cd5f39

Kiviniemi, A. M., Hautala, A. J., Kinnunen, H., and Tulppo, M. P. (2007). Endurance training guided individually by daily heart rate variability measurements. *Eur. J. Appl. Physiol.* 101, 743–751. doi: 10.1007/s00421-007-0552-2

Kruk, J. (2007). Physical activity in the prevention of the most frequent chronic diseases: an analysis of the recent evidence. *Asian Pac. J. Cancer Prev.* 8, 325–338.

Lamberts, R. P., Maskell, S., Borresen, J., and Lambert, M. I. (2011). Adapting workload improves the measurement of heart rate recovery. *Int. J. Sports Med.* 32, 698–702. doi: 10.1055/s-0031-1275357

LaMonte, M. J., Ainsworth, B. E., and Durstine, J. L. (2005). Influence of cardiorespiratory fitness on the association between C-reactive protein and metabolic syndrome prevalence in racially diverse women. *J. Womens. Health (Larchmt)*. 14, 233–239. doi: 10.1089/jwh.2005.14.233

La Rovere, M. T., Pinna, G. D., Hohnloser, S. H., Marcus, F. I., Mortara, A., Nohara, R., et al. (2001). Baroreflex sensitivity and heart rate variability in the identification of patients at risk for life-threatening Arrhythmias: implications for clinical trials. *Circulation* 103, 2072–2077.

Lee, C. D., Blair, S. N., and Jackson, A. S. (1999). Cardiorespiratory fitness, body composition, and all cause and cardiovascular disease mortality in men. *Am. J. Clin. Nutr*. 69, 373–380.

Lee, D. C., Artero, E. G., Sui, X., and Blair, S. N. (2010). Mortality trends in the general population: the importance of cardiorespiratory fitness. *J. Psychopharmacol. (Oxf)*. 24, 27–35. doi: 10.1177/1359786810382057

Leicht, A. S., Allen, G. D., and Hoey, A. J. (2003). Influence of intensive cycling training on heart rate variability during rest and exercise. *Can. J. Appl. Physiol.* 28, 898–909. doi: 10.1139/h03-064

Leicht, A. S., Crowther, R. G., and Golledge, J. (2011). Influence of peripheral arterial disease and supervised walking on heart rate variability. *J. Vasc. Surg.* 54, 1352–1359. doi: 10.1016/j.jvs.2011.05.027

Lindholm, H., Sinisalo, J., Ahlberg, J., Hirvonen, A., Hublin, C., Partinen, M., et al. (2012). Attenuation of vagal recovery during sleep and reduction of cortisol/melatonin ratio in late afternoon associate with prolonged daytime sleepiness among media workers with irregular shift work. *Am. J. Ind. Med.* 55, 643–649. doi: 10.1002/ajim.22042

Mendonca, G. V., Heffernan, K. S., Rossow, L., Guerra, M., Pereira, F. D., and Fernhall, B. (2010). Sex differences in linear and nonlinear heart rate variability during early recovery from supramaximal exercise. *Appl. Physiol. Nutr. Metab.* 35, 439–446. doi: 10.1139/H10-028

Myrtek, M. (1990). Covariation and reliability of ECG parameters during 24-hour monitoring. *Int. J. Psychophysiol.* 10, 117–123. doi: 10.1016/0167-8760(90)90026-A

N3akamura, F. Y., Flatt, A. A., Pereira, L. A., Ramirez-Campillo, R., Loturco, I., and Esco, M. R. (2015). Ultra-short-term heart rate variability is sensitive to training effects in team sports players. *J. Sports Sci. Med.* 14, 602–605.

Nolan, J., Batin, P. D., Andrews, R., Lindsay, S. J., Brooksby, P., Mullen, M., et al. (1998). Prospective study of heart rate variability and mortality in chronic heart failure: results of the United Kingdom heart failure evaluation and assessment of risk trial (UK-heart). *Circulation* 98, 1510–1516. doi: 10.1161/01.CIR.98.15.1510

Orme, M., Wijndaele, K., Sharp, S. J., Westgate, K., Ekelund, U., and Brage, S. (2014). Combined influence of epoch length, cut-point and bout duration on accelerometry-derived physical activity. *Int. J. Behav. Nutr. Phys. Act.* 11, 11–34 doi: 10.1186/1479-5868-11-34

Ostojic, S. M., Markovic, G., Calleja-Gonzalez, J., Jakovljevic, D. G., Vucetic, V., and Stojanovic, M. D. (2010). Ultra short-term heart rate recovery after maximal exercise in continuous versus intermittent endurance athletes. *Eur. J. Appl. Physiol.* 108, 1055–1059. doi: 10.1007/s00421-009-1313-1

Pavey, T. G., Peeters, G., Bauman, A. E., and Brown, W. J. (2013). Does vigorous physical activity provide additional benefits beyond those of moderate? *Med. Sci. Sports Exerc*. 45, 1948–1955. doi: 10.1249/MSS.0b013e3182940b91

Pei, J., Tang, W., Li, L. X., Su, C. Y., and Wang, T. (2015). Heart rate variability predicts mortality in peritoneal dialysis patients. *Ren. Fail.* 30, 1–6. doi: 10.3109/0886022x.2015.1061729

Plews, D. J., Laursen, P. B., Kilding, A. E., and Buchheit, M. (2012). Heart rate variability in elite triathletes, is variation in variability the key to effective training? A case comparison. *Eur. J. Appl. Physiol*. 112, 3729–3741. doi: 10.1007/s00421-012-2354-4

Plews, D. J., Laursen, P. B., Le Meur, Y., Hausswirth, C., Kilding, A. E., and Buchheit, M. (2014). Monitoring training with heart rate-variability: how much compliance is needed for valid assessment? *Int. J. Sports Physiol. Perform.* 9, 783–790. doi: 10.1123/IJSPP.2013-0455

Ramsbottom, R., Currie, J., and Gilder, M. (2010). Relationships between components of physical activity, cardiorespiratory fitness, cardiac autonomic health, and brain-derived neurotrophic factor. *J. Sports Sci.* 28, 843–849. doi: 10.1080/02640411003702686

Ross, R., and McGuire, K. A. (2011). Incidental physical activity is positively associated with cardiorespiratory fitness. *Med. Sci. Sports Exerc.* 43, 2189–2194. doi: 10.1249/MSS.0b013e31821e4ff2

Schumann, M., Yli-Peltola, K., Abbiss, C. R., and Häkkinen, K. (2015). Cardiorespiratory adaptations during concurrent aerobic and strength training in men and women. *PLoS ONE* 10:e0139279. doi: 10.1371/journal.pone.0139279

Siri, W. (1961). "Body composition from fluid paces and density: analysis of methods," in *Techniques for Measuring Body Composition*, eds J. Brozek and A. Henschel (Washington, DC: National Academy of Science), 223–244.

Skaug, E. A., Madssen, E., Aspenes, S. T., and Wisløff, U., Øyvind, E. (2014). Cardiovascular risk factors have larger impact on endothelial function in self-reported healthy women than men in the HUNT3 fitness study. *PLoS ONE* 9:e101371. doi: 10.1371/journal.pone.0101371

Stein, P. K., Barzilay, J. I., Chaves, P. H., Mistretta, S. Q., Domitrovich, P. P., Gottdiener, J. S., et al. (2008). Novel measures of heart rate variability predict cardiovascular mortality in older adults independent of traditional cardiovascular risk factors: the Cardiovascular Health Study (CHS). *J. Cardiovasc. Electrophysiol.* 19, 1169–1174. doi: 10.1111/j.1540-8167.2008.01232.x

Tanaka, H., Monahan, K. D., and Seals, D. R. (2001). Age-predictes maximal heart rate revisited. *J. Am. Coll. Cardiol.* 37, 153–156. doi: 10.1016/S0735-1097(00)01054-8

Task Force (1996). Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology: Heart rate variability. Standards of measurement, physiological interpretation, and clinical use. *Circulation* 93, 1043–1065. doi: 10.1161/01.CIR.93.5.1043

Triggiani, A. I., Valenzano, A., Ciliberti, M. A., Moscatelli, F., Villani, S., Monda, M., et al. (2015). Heart rate variability is reduced in underweight and overweight healthy adult women. *Clin. Physiol. Func. Imaging*. doi: 10.1111/cpf.12281. [Epub ahead of print].

Trost, S. G., Mclver, K. L., and Pate, R. R. (2005). Conducting accelerometer-based activity assessments in field-based research. *Med. Sci. Sports Exerc.* 37, S531–S543. doi: 10.1249/01.mss.0000185657.86065.98

Tudor-Locke, C., Craig, C. L., Brown, W. J., Clemes, S. A., De Cocker, K., Giles-Corti, B., et al. (2011). How many steps/day are enough? for adults. *Int. J. Behav. Nutr. Phys. Act.* 8:79. doi: 10.1186/1479-5868-8-79

Uusitalo, M. T., Martinmäki, K., Mauno, S., Kinnunen, U., and Rusko, H. (2011). Heart rate variability related to effort at work. *Appl. Ergon.* 42, 830–838. doi: 10.1016/j.apergo.2011.01.005

Wallén, M. B., Hasson, D., Theorell, T., Canlon, B., and Osika, W. (2012). Possibilities and limitations of the Polar RS800 in measuring heart rate variability at rest. *Eur. J. Appl. Physiol.* 112, 1153–1165. doi: 10.1007/s00421-011-2079-9

Wei, M., Kampert, J. B., Barlow, E. C., Nichaman, M. Z., Gibbons, L., Paffenbarger Junior, R. P., et al. (1999). Relationship between low cardiorespiratory fitness and mortality in Normal-Weight, Overweight, and Obese Men. *JAMA* 282, 1547–1553. doi: 10.1001/jama.282.16.1547

Young, F. L., and Leicht, A. S. (2011). Short-term stability of resting heart rate variability: influence of position and gender. *Appl. Physiol. Nutr. Metab.* 36, 210–218. doi: 10.1139/h10-103

Ziegler, D., Piolot, R., Strassburger, K., Lambeck, H., and Dannehl, K. (1999). Normal ranges and reproducibility of statistical, geometric, frequency domain, and non-linear measures of 24-hour heart rate variability. *Horm. Metab. Res.* 31, 672–679. doi: 10.1055/s-2007-978819

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Acute responses of circulating microRNAs to low-volume sprint interval cycling

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Low-volume high-intensity interval training is an efficient and practical method of inducing physiological responses in various tissues to develop physical fitness and may also change the expression of circulating microRNAs (miRNAs). The purpose of the present study was to examine whether miRNAs for muscle, heart, somatic tissue and metabolism were affected by 30-s intervals of intensive sprint cycling. We also examined the relationship of these miRNAs to conventional biochemical and performance indices. Eighteen healthy young males performed sprint interval cycling. Circulating miRNAs in plasma were detected using TaqMan-based quantitative PCR and normalized to Let-7d/g/i. In addition, we determined the levels of insulin-like growth factor-I, testosterone and cortisol, and anaerobic capacity. Compared to plasma levels before exercise muscle-specific miR-1 (0.12 ± 0.02 vs. 0.09 ± 0.02), miR-133a (0.46 ± 0.10 vs. 0.31 ± 0.06), and miR-133b (0.19 ± 0.02 vs. 0.10 ± 0.01) decreased (all $P < 0.05$), while miR-206 and miR-499 remained unchanged. The levels of metabolism related miR-122 (0.62 ± 0.07 vs. 0.34 ± 0.03) and somatic tissues related miR-16 (1.74 ± 0.27 vs. 0.94 ± 0.12) also decreased (both $P < 0.05$). The post-exercise IGF-1 and cortisol concentrations were significantly increased, while testosterone concentrations did not. Plasma levels of miR-133b correlated to peak power ($r = 0.712$, $P = 0.001$) and miR-122 correlated to peak power ratio ($r = 0.665$, $P = 0.003$). In conclusion sprint exercise provokes genetic changes for RNA related to specific muscle or metabolism related miRNAs suggesting that miR-133b and miR-122 may be potential useful biomarkers for actual physiological strain or anaerobic capacity. Together, our findings on the circulating miRNAs may provide new insight into the physiological responses that are being performed during exercise and delineate mechanisms by which exercise confers distinct phenotypes and improves performance.

Keywords: plasma microRNAs, biomarkers, blood lactate, blood hormones, anaerobic capacity

INTRODUCTION

Sprint interval training, interspersed with sufficient recovery periods (2–4 min), can produce the best possible average sprint performance over a series of sprints (<45 s; Weston et al., 2014). Therefore, sprint interval training is a commonly used intervention to maintain skeletal muscle health and to improve exercise performance, especially in individuals who require a high contribution of glycolytic energy (e.g., track-and-field sprint athletes and some team sport athletes; Buchheit and Laursen, 2013). Similarly, sprint interval cycling (SIC), which is modeled on the Wingate Anaerobic test, is extremely demanding and involves all-out 30-s sprints interspersed with 4 min active recovery periods with no resistance (Burgomaster et al., 2005). The high-intensity nature of SIC is thought to recruit all types of muscle fibers (Weston et al., 2014). However, repeated all-out 30-s bouts of exercise separated by 4 min of rest increasingly depend on aerobic metabolism (Burgomaster et al., 2005). Exercise intensity places mechanical and/or metabolic stress on contracting muscles (Laursen, 2010). The anabolic (e.g., growth hormone, testosterone, and insulin-like growth factor-1) and catabolic (e.g., cortisol) processes of tissue remodeling following exercise loading are typically reflected by acute changes in hormonal concentrations (Schoenfeld, 2012).

The microRNAs (miRNAs) are typically small, ∼19–22 nucleotides long, non-coding RNA molecules that post-transcriptionally regulate gene expression by base-pairing with the 3′ untranslated region of complementary messenger RNA targets (Bartel, 2004). Recently, some miRNA species (particularly muscle-specific miRNAs and inflammatory-related miRNAs) have been found to change in human serum/plasma after acute prolonged endurance training, eccentric exercise, and strength exercise (Baggish et al., 2011, 2014; Aoi et al., 2013; Banzet et al., 2013; Gomes et al., 2014; Mooren et al., 2014; Uhlemann et al., 2014). Studies have suggested that they can be used as potential biomarkers for aerobic capacity and as markers or mediators of physiological adaptations (Baggish et al., 2011, 2014; Aoi et al., 2013; Banzet et al., 2013; Mooren et al., 2014; de Gonzalo-Calvo et al., 2015).

Exhaustive exercise has a deep effect on cellular, humoral, and metabolic processes of the body (Spencer et al., 2005; Meckel et al., 2011; Gibala et al., 2012). We hypothesized that a single session of low-volume sprint interval cycling can change the circulating miRNAs (c-miRNAs) profile. Accordingly, the levels of the myomiR family (miR-1, miR-133a, miR-133b, and miR-206) and other muscle-specific miRNA (miR-499), metabolism-related miRNA (miR-122), and somatic tissues-related miRNA (miR-16) were evaluated in response to extreme SIC. In addition, to provide insight into the potential roles of these c-miRNAs, we also determined whether alterations in the levels of these miRNAs in response to extreme SIC are correlated with changes in conventional anabolic-catabolic hormonal biomarkers or anaerobic capacity.

MATERIALS AND METHODS

Ethical Approval and Participants

Eighteen healthy young male participants (age, 20.23 ± 0.97 years; height, 1.75 ± 0.06 m; body mass, 68.90 ± 8.83 kg; and BMI, 22.39 ± 2.06 $kg \cdot m^{-2}$) who were habituated to a regular exercise regimen were recruited to participate in this study. University cadets were contacted to volunteer for this study who led similar lives and who had the same dietary habits for 2 years prior to the study. None of the subjects had any current or prior chronic disease, a history of smoking or current use of any medications. Written informed consent was obtained from all of the participants. Ethical approval for this study conformed to the standards of the Declaration of Helsinki, and the protocol was approved by the Institutional Review Board of Nanjing University.

Study Design

Before the SIC session, the participants refrained from exercise for at least 72 h. Sprint interval cycling was performed on a mechanically braked stationary cycle ergometer (Monark Ergomedic 839E, Monark, Sweden) against a pre-determined force load of approximately 7.5% of the subject's body weight in kilograms. Sprint interval cycling involved two 30-s all-out sprints (Sprint 1 and Sprint 2) with 4 min of active recovery between them (Burgomaster et al., 2005). The tests were conducted between 10:00 am and 11:30 am.

Anaerobic Capacity

The first 30 s of Wingate data for each participant were used to assess anaerobic power. Fatigue index was calculated as ([Peak Power − Minimum Power]/Peak Power) × 100%. The change in Wingate data for Sprint 1 and Sprint 2 were used to assess the ability to maintain anaerobic power.

Plasma Sampling

Venous blood was collected at two different time points during the acute exercise test. Five milliliters of blood was collected in standard anticoagulant (EDTA)-treated Vacutainer tubes prior to acute exercise testing (Pre) and within 1 min of exercise testing completion (Post). All of the blood samples were centrifuged at 1500 × g for 10 min to pellet cellular elements immediately after each blood draw and were then centrifuged at 10,000 × g for 5 min at 4°C to completely remove cell debris. To minimize freeze–thaw degradation, the supernatant plasma was then aliquoted and immediately frozen at −80°C.

Blood Lactate and Hormones

The blood lactate (LA) concentration was measured with an automatic lactate analyzer (EKF Diagnostic, Germany). Blood samples were analyzed for conventional physiological markers, including lactate, insulin-like growth factor-1 (IGF-1), testosterone, and cortisol. The IGF-1 level was measured with an IMMULITE 2000 Analyzer (EuroDPC Med Limited, Llanberis, UK). The assays were conducted using the solid-phase,

enzyme-labeled, chemiluminescent immunometric method in accordance with the manufacturer's instructions. The testosterone and cortisol levels in the plasma were determined by a chemiluminescence immunoassay (UniCel DxI 800, Access Immunoassay System, Beckmann Coulter GmbH, Krefeld, Germany). The IGF-1, testosterone and cortisol levels were considered normal according to the reference ranges for ages provided by the kit manufacturer.

miRNA Isolation and RT-qPCR

Total RNA, including miRNAs, was isolated from the plasma samples using a 1-step phenol/chloroform purification protocol as previously described (Liu et al., 2011). A panel of miRNAs was investigated that are related to either skeletal/heart muscle (miR-1, miR-133a, miR-133b, miR-206, and miR-499) or to metabolism and somatic tissue (miR-122 and miR-16; Aoi et al., 2013; Boettger et al., 2014; Bandiera et al., 2015). To quantify the abundance of mature miRNAs, real time quantitative polymerase chain reaction (RT-qPCR) was performed. RT-qPCR was performed using a TaqMan PCR Kit and an Applied Biosystems 7300 Sequence Detection System as previously described (Wu et al., 2014). The cycle threshold (Ct) data were determined using default threshold settings, and the mean Ct was determined from triplicate PCRs. In addition, we calculated the Ct values of Let-7d, Let-7g, and Let-7i because the use of this combination of reference genes (Chen et al., 2013) in human serum for normalization has been demonstrated to be superior to that of the other commonly used single reference genes. The relative levels of miRNAs were normalized to a Let-7d/g/i trio and were calculated using the $2^{-\Delta\Delta Ct}$ method. ΔCt was calculated by subtracting the Ct values of Let-7d/g/i from the average Ct values of the target miRNAs. $\triangle$Ct values were then compared ($\Delta\Delta$Ct) with each participant's own resting baseline at the Pre time point (normalized to fold change of 1).

Statistical Analyses

GraphPad Prism 5 and SigmaPlot 10.0 packages were used. Subject characteristics, exercise testing data and blood parameters were reported as the mean ± standard deviation, and c-miRNA data were presented as the mean ± standard error of the mean (SEM). Paired variables were compared in Student's *t*-test or a Wilcoxon's matched pairs test as appropriate for the data distribution. Correlation analyses were performed using the Spearman or Pearson's method as appropriate for the data distribution. Values of $P < 0.05$ were considered significant.

RESULTS

Wingate Performance

After the SIC, Wingate peak power was significantly decreased by 11% following two 30-s periods of all-out SIC (659.48 ± 217.64 W vs. 562.78 ± 146.38 W, $P = 0.006$). The average power was also significantly decreased by 16%. The average power of Sprint 1 was 6.69 ± 0.78 W·kg^{-1} and that of Sprint 2 was 5.58 ± 0.80 W·kg^{-1} ($P < 0.001$). There was no significant difference in the fatigue index between the first and second 30-s sprint (62.87 ± 11.22% vs. 60.26 ± 12.44%, $P = 0.820$). In addition, there was no significant difference in maximum speed between the first and second sprint (151.96 ± 16.83 rpm vs. 150.57 ± 20.04 rpm, $P = 0.740$).

Blood Lactate

After exercise, the LA concentration significantly increased (1.56 ± 1.90 mmol·L^{-1} vs. 11.27 ± 1.90 mmol·L^{-1}, $P < 0.001$).

Plasma Hormones

Compared to plasma levels before exercise, the post-exercise IGF-1 concentration was significantly increased by 13% following two 30-s periods of all-out SIC (298.94 ± 50.13 ng·mL^{-1} vs. 332.72 ± 57.76 ng·mL^{-1}, $P < 0.001$). Testosterone concentrations were similar between pre- and post-exercise (40.59 ± 8.45 nmol·L^{-1} vs. 41.74 ± 11.57 nmol·L^{-1}, $P = 0.608$). The cortisol concentration increased by 45% (216 ± 87 nmol·L^{-1} vs. 278 ± 91 nmol·L^{-1}, $P = 0.028$) and a decrease in testosterone/cortisol ratio tended to be statistical significant.

The Plasma miRNA Levels in Response to an Acute Bout of Sprint Interval Cycling

The Ct values of Let-7d/g/i at pre- and post-SIC show low variability (**Figure 1**; $P = 0.280$). Acute sprint interval cycling significantly decreased the levels of miR-1, miR-133a, miR-133b, miR-122, and miR-16 (**Figure 2**). Levels of miR-206 and miR-499 were not significantly changed by acute sprint interval cycling (**Figure 3**).

Correlations between Blood, Anaerobic Parameters and c-miRNA Levels

To explore the feasibility of using c-miRNAs as biomarkers of an acute exercise response, the association of specific changes

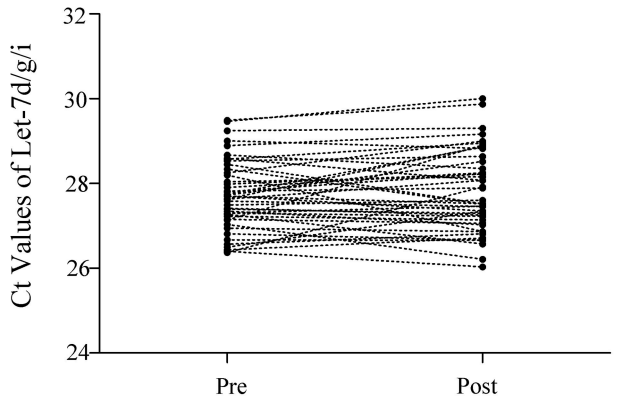

FIGURE 1 | The Ct values of Let-7d/g/i in plasma samples before and after the sprint interval cycling. The total amount of Let-7d/g/i trio was simultaneously measured in a same RT-qPCR reaction. Let-7d, Let-7g, and Let-7i were reverse-transcribed in a single reaction using a mixture of stem-loop primers of Let-7d, Let-7g, and Let-7i (in the ratio of 1:1:1). Accordingly, real-time PCR was performed using a TaqMan miRNA probe pool of Let-7d, Let-7g and Let-7i (in the ratio of 1:1:1). Ct values of every individual's Let-7d/g/i before (Pre) and after (Post) the exercise almost remained unchanged.

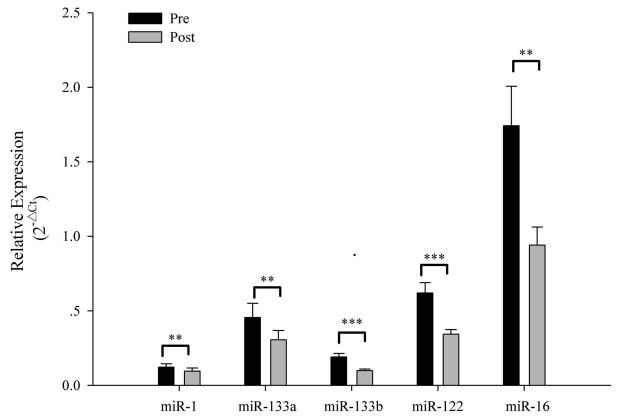

FIGURE 2 | The plasma miRNA levels in response to sprint interval cycling. Plasma levels of miR-1, miR-133a, miR-133b, miR-122, and miR-16 decreased significantly after an acute bout of sprint interval cycling. Values represent the mean ± SEM obtained from 18 subjects. $^{**}P < 0.01$, $^{***}P < 0.001$.

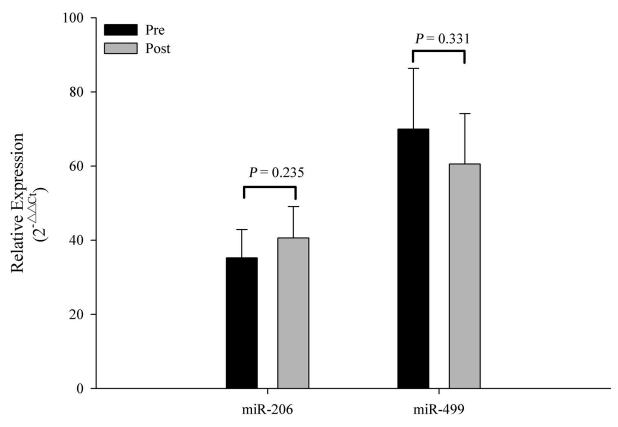

FIGURE 3 | Changes in circulating miR-206 and miR-499 in response to acute exercise. Plasma levels of miR-206 and miR-499 showed no significant difference after an acute bout of sprint interval cycling. Values represent the mean ± SEM obtained from 18 subjects.

in c-miRNA was examined in relation to the changes in blood hormonal and anaerobic parameters. No correlations were found between the changes in the plasma IGF-1, testosterone, and cortisol levels, or the testosterone/cortisol ratio and the changes in the miR-1, miR-133a, miR-133b, miR-122, and miR-16 levels (**Table 1**).

There were not correlations among the levels of peak power, average power and fatigue index and levels of plasma miR-133a, miR-1, and miR-16 levels (**Table 1**). However, there was a significant correlation between the levels of peak power of Sprint 1 and levels of plasma miR-133b (**Figure 4A**). These results suggest a potential role for plasma miR-133b as a marker of anaerobic capacity. Furthermore, there was a significant correlation between the levels of the peak power ratio of Sprint 1/Sprint 2 and plasma miR-122 level (**Figure 4B**), suggesting a potential role of plasma miR-122 as a restriction marker of anaerobic capacity.

DISCUSSION

Sprint interval cycling is one of the most frequently used training methods in anaerobic sports. Our data indicated that several miRNA species in plasma responded to sprint interval cycling. These acute responses may be more critical to tissue growth and remodeling than chronic changes in resting concentrations (Kraemer and Ratamess, 2005). Exercise rapidly and transiently regulates several miRNA species in circulation, suggesting that they could be used to precisely monitor physiological acute responses to exercise.

Sprint interval exercise involves very high intensity, and the myosin heavy chain (MHC) IIa and IIx fibers are highly responsive to intense exercise at the transcriptional level for genes involved with muscle growth and remodeling (Trappe et al., 2015). miR-1 and miR-133a are expressed in both skeletal and cardiac muscles (Boettger et al., 2014). miR-133b and miR-206 are specific to skeletal muscle and preferentially detected in slow myofibers (Boettger et al., 2014). Pervious study has shown that circulating plasma miR-1 levels were significantly decreased in patients with supraventricular tachycardia, while miR-133 significantly increased in patients with ventricular tachycardia (Sun et al., 2015). The interactions of miRNAs (such as miR-1 and miR-133) with ion channel-encoding genes and calmodulin regulate cardiac contractility, rhythm and excitement (Terentyev et al., 2009). However, cardiac contraction during exercise is the physiological process most sensitive to calmodulin integrity, which can be affected by acute exercise (Sondergaard et al., 2015). Moreover, extracellular miRNAs are dynamic indices of pathophysiological processes in skeletal muscle (Roberts et al., 2013). Endurance also increased the miR-1 and miR-133a levels both in plasma and skeletal muscle (Nielsen et al., 2010; Baggish et al., 2014; Gomes et al., 2014; Mooren et al., 2014). During high intensity exercise the heart rate can greatly increase. In the present study, miR-1 and miR-133a levels in plasma decreased, and miR-1 and miR-133a expression in adult mice decreased during skeletal muscle hypertrophy (Mccarthy and Esser, 2007). These results indicate that these miRNAs may partly reflect the cardiac or skeletal muscle responses induced by exercise or a temporal regulation controlled by miRNAs during exercise.

Furthermore, miR-133b, miR-206, and miR-499 levels in plasma decreased or remained unchanged in the current study but significantly increased after acute endurance exercise in a previous study (Mooren et al., 2014). Training with high intensity exercise induces expression of fast-twitch fibers. Therefore, no change of circulating miR-206 and miR-499 observed in the present work, associated with slow myofibers or oxidative red fibers (Gan et al., 2013; Boettger et al., 2014), might reflect a non-predominance of slow-twitch fibers in SIC. However, at present, specific miRNAs related to fast-twitch fibers in human, such as MHC IIa and MHC IIx muscle fibers, have not been found.

Two miRNAs not restricted to muscle in origin (miR-122 and miR-16) also deceased in plasma following acute high intensity exercise. A previous study has shown that miR-122 is a key factor

TABLE 1 | Correlations between changes in exercise-related blood parameters and anaerobic parameters and changes in plasma levels of miRNAs ($n = 18$).

	IGF-1		Testosterone		Cortisol		Testosterone/ Cortisol		Peak Power of Sprint 1		Average Power of Sprint 1		Peak Power Ratio of Sprint1/Sprint2		Fatigue Index	
	r	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*
miR-1	−0.101	ns	−0.060	ns	−0.111	ns	0.165	ns	0.410	ns	−0.018	ns	−0.057	ns	0.201	ns
miR-133a	−0.379	ns	0.078	ns	−0.155	ns	0.060	ns	0.441	ns	−0.145	ns	−0.078	ns	−0.087	ns
miR-133b	−0.179	ns	0.086	ns	0.284	ns	−0.118	ns	0.712	0.001	0.076	ns	−0.053	ns	−0.059	ns
miR-122	−0.045	ns	0.023	ns	0.037	ns	0.009	ns	0.257	ns	−0.132	ns	0.655	0.003	0.012	ns
miR-16	0.066	ns	−0.137	ns	−0.143	ns	0.102	ns	0.189	ns	0.050	ns	−0.104	ns	0.096	ns

ns, not significant.

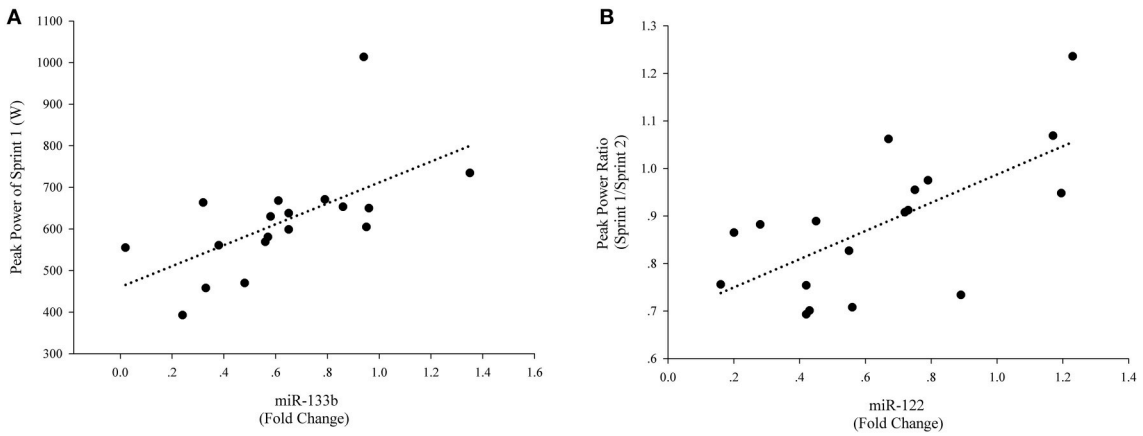

FIGURE 4 | Correlations of plasma miRNAs and exercise-related anaerobic parameters. For each participant, baseline c-miRNAs levels before the exercise were assigned a fold change of 1, to which measurements obtained after the exercise were compared. A direct correlation was observed between levels of peak power of Sprint 1 and levels of plasma miR-133b (Post exercise) **(A)** A direct correlation was also observed between the peak power ratio of Sprint 1/Sprint 2 and levels of miR-122 (Post exercise) **(B)**.

in liver development, differentiation, homeostasis, and metabolic function (Bandiera et al., 2015). The peroxisome proliferator-activated receptor (PPAR) γ coactivator 1-α (PGC-1α) plays a pivotal role in the regulation of the expression of mitochondrial proteins cytochrome c and cytochrome oxidase subunit I in the liver in response to a single exercise bout (Haase et al., 2011). Exercise induced beneficial alterations in the liver mitochondrial morphology and increased mitochondrial biogenesis (PGC-1α and mitochondrial transcription factor A) (Santos-Alves et al., 2015), and liver AMP-activated protein kinase (AMPK) activity increased during heavy exercise (Carlson and Winder, 1999). The putative effectors of miR-122-mediated metabolic control in the liver may be involved in both circadian metabolic regulators of the PPAR family and AMPK (Bandiera et al., 2015). Thus, the currently observed decrease of circulating miR-122 might reflect a liver cellular temporal regulation controlled by miRNAs during exercise. In addition, circulating miR-16 level also decreased, suggesting that non-muscle tissue is also needed to cope with this stress.

Exercise is a potent stimulus for the release of many hormones in response to the specific demands of the particular exercise type (Stokes et al., 2013). Changes in the anabolic-catabolic hormonal balance were found following brief sprint interval exercise training, may be used to gauge training adaptation to different anaerobic exercises (Kraemer and Ratamess, 2005; Meckel et al., 2011). But in the present study, there was no significant correlation between the change in these plasma hormone levels and the change in the c-miRNAs levels. Given their different physiological roles, it is likely that the c-miRNAs would show different expression patterns induced by SIC. However, in our study a negative correlation was observed between the reduced magnitudes in miR-133b and peak power and between the reduced magnitudes in miR-122 and the ability to maintain anaerobic power. In this case, the reduction in miR-133b and miR-122 in circulation may be considered a biomarker that reflects internal physiological stress caused by SIC.

At present, the mechanism(s) and clinical significance of an exercise-induced decrease in specific c-miRNAs remains poorly understood. An exercise-induced miRNA uptake by certain recipient cells has been postulated (Aoi et al., 2013; Aoi and Sakuma, 2014). Given the transportability of vesicles, the role

of miRNAs in exosomes is gaining increasing attention. In the present study, high-intensity exercise may destroy exosomes, leading to degradation of miRNAs by RNases. However, the miR-206 and miR-499 in circulation remained unchanged, suggesting that significant degradation of exosomes did not occur as a result of SIC (Aoi et al., 2013; Aoi and Sakuma, 2014). The miRNAs in the exosomes are selectively packaged rather than included indiscriminately (Etheridge et al., 2013; Zhang et al., 2015). Moreover, the exosomal miRNA expression levels are altered under different physiological conditions (Etheridge et al., 2013; Zhang et al., 2015). It is reasonable to consider that acute high-intensity exercise may promote the uptake of some c-miRNAs into certain recipient cells (Aoi et al., 2013; Aoi and Sakuma, 2014). Further investigation is required to validate this hypothesis.

In conclusion, our data demonstrate that extreme sprint interval cycling affects the expression patterns of plasma miRNAs. Selective c-miRNAs, such as miR-133b and miR-122, may be potentially suitable for use as novel biomarkers to monitor training-induced acute changes within diverse tissues in response to low-volume sprint interval cycling.

AUTHOR CONTRIBUTIONS

SC designed the study, performed the experiments, collected and analyzed the data, and revised the final version of the manuscript. WL and JN recruited participants, collected samples and performed the experiments. CZ and XC designed the study and critically revised the final version of the manuscript. JM designed the study, recruited participants, analyzed the data and wrote the final version of the manuscript. All authors read and approved the final version of the manuscript.

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REFERENCES

Aoi, W., Ichikawa, H., Mune, K., Tanimura, Y., Mizushima, K., Naito, Y., et al. (2013). Muscle-enriched microRNA miR-486 decreases in circulation in response to exercise in young men. *Front. Physiol.* 4:80. doi: 10.3389/fphys.2013.00080

Aoi, W., and Sakuma, K. (2014). Does regulation of skeletal muscle function involve circulating microRNAs? *Front. Physiol.* 5:39. doi: 10.3389/fphys.2014.00039

Baggish, A. L., Hale, A., Weiner, R. B., Lewis, G. D., Systrom, D., Wang, F., et al. (2011). Dynamic regulation of circulating microRNA during acute exhaustive exercise and sustained aerobic exercise training. *J. Physiol.* 589, 3983–3994. doi: 10.1113/jphysiol.2011.213363

Baggish, A. L., Park, J., Min, P. K., Isaacs, S., Parker, B. A., Thompson, P. D., et al. (2014). Rapid upregulation and clearance of distinct circulating microRNAs after prolonged aerobic exercise. *J. Appl. Physiol. (1985)* 116, 522–531. doi: 10.1152/japplphysiol.01141.2013

Bandiera, S., Pfeffer, S., Baumert, T. F., and Zeisel, M. B. (2015). miR-122–a key factor and therapeutic target in liver disease. *J. Hepatol.* 62, 448–457. doi: 10.1016/j.jhep.2014.10.004

Banzet, S., Chennaoui, M., Girard, O., Racinais, S., Drogou, C., Chalabi, H., et al. (2013). Changes in circulating microRNAs levels with exercise modality. *J. Appl. Physiol. (1985)* 115, 1237–1244. doi: 10.1152/japplphysiol.00075.2013

Bartel, D. P. (2004). MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell* 116, 281–297. doi: 10.1016/S0092-8674(04)00045-5

Boettger, T., Wüst, S., Nolte, H., and Braun, T. (2014). The miR-206/133b cluster is dispensable for development, survival and regeneration of skeletal muscle. *Skelet. Muscle* 4, 23. doi: 10.1186/s13395-014-0023-5

Buchheit, M., and Laursen, P. B. (2013). High-intensity interval training, solutions to the programming puzzle. Part II: anaerobic energy, neuromuscular load and practical applications. *Sports Med.* 43, 927–954. doi: 10.1007/s40279-013-0066-5

Burgomaster, K. A., Hughes, S. C., Heigenhauser, G. J., Bradwell, S. N., and Gibala, M. J. (2005). Six sessions of sprint interval training increases muscle oxidative potential and cycle endurance capacity in humans. *J. Appl. Physiol. (1985)* 98, 1985–1990. doi: 10.1152/japplphysiol.01095.2004

Carlson, C. L., and Winder, W. W. (1999). Liver AMP-activated protein kinase and acetyl-CoA carboxylase during and after exercise. *J. Appl. Physiol. (1985)* 86, 669–674.

Chen, X., Liang, H., Guan, D., Wang, C., Hu, X., Cui, L., et al. (2013). A combination of Let-7d, Let-7g and Let-7i serves as a stable reference for normalization of serum microRNAs. *PLoS ONE* 8:e79652. doi: 10.1371/journal.pone.0079652

de Gonzalo-Calvo, D., Dávalos, A., Montero, A., García-González, Á., Tyshkovska, I., Gonzalez-Medina, A., et al. (2015). Circulating inflammatory miRNA signature in response to different doses of aerobic exercise. *J. Appl. Physiol. (1985)* 119, 124–134. doi: 10.1152/japplphysiol.00077.2015

Etheridge, A., Gomes, C. P., Pereira, R. W., Galas, D., and Wang, K. (2013). The complexity, function and applications of RNA in circulation. *Front. Genet.* 4:115. doi: 10.3389/fgene.2013.00115

Gan, Z., Rumsey, J., Hazen, B. C., Lai, L., Leone, T. C., Vega, R. B., et al. (2013). Nuclear receptor/microRNA circuitry links muscle fiber type to energy metabolism. *J. Clin. Invest.* 123, 2564–2575. doi: 10.1172/JCI67652

Gibala, M. J., Little, J. P., Macdonald, M. J., and Hawley, J. A. (2012). Physiological adaptations to low-volume, high-intensity interval training in health and disease. *J. Physiol.* 590, 1077–1084. doi: 10.1113/jphysiol.2011.224725

Gomes, C. P., Oliveira-Jr, G. P., Madrid, B., Almeida, J. A., Franco, O. L., and Pereira, R. W. (2014). Circulating miR-1, miR-133a, and miR-206 levels are increased after a half-marathon run. *Biomarkers* 19, 585–589. doi: 10.3109/1354750X.2014.952663

Haase, T. N., Ringholm, S., Leick, L., Biensø, R. S., Kiilerich, K., Johansen, S., et al. (2011). Role of PGC-1alpha in exercise and fasting-induced adaptations in mouse liver. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 301, R1501–R1509. doi: 10.1152/ajpregu.00775.2010

Kraemer, W. J., and Ratamess, N. A. (2005). Hormonal responses and adaptations to resistance exercise and training. *Sports Med.* 35, 339–361. doi: 10.2165/00007256-200535040-00004

Laursen, P. B. (2010). Training for intense exercise performance: high-intensity or high-volume training? *Scand. J. Med. Sci. Sports* 20(Suppl. 2), 1–10. doi: 10.1111/j.1600-0838.2010.01184.x

Liu, R., Zhang, C., Hu, Z., Li, G., Wang, C., Yang, C., et al. (2011). A five-microRNA signature identified from genome-wide serum microRNA expression profiling

serves as a fingerprint for gastric cancer diagnosis. *Eur. J. Cancer* 47, 784–791. doi: 10.1016/j.ejca.2010.10.025

McCarthy, J. J., and Esser, K. A. (2007). MicroRNA-1 and microRNA-133a expression are decreased during skeletal muscle hypertrophy. *J. Appl. Physiol. (1985)* 102, 306–313. doi: 10.1152/japplphysiol.00932.2006

Meckel, Y., Nemet, D., Bar-Sela, S., Radom-Aizik, S., Cooper, D. M., Sagiv, M., et al. (2011). Hormonal and inflammatory responses to different types of sprint interval training. *J. Strength Cond. Res.* 25, 2161–2169. doi: 10.1519/JSC.0b013e3181dc4571

Mooren, F. C., Viereck, J., Krüger, K., and Thum, T. (2014). Circulating microRNAs as potential biomarkers of aerobic exercise capacity. *Am. J. Physiol. Heart Circ. Physiol.* 306, H557–H563. doi: 10.1152/ajpheart.00711.2013

Nielsen, S., Scheele, C., Yfanti, C., Akerström, T., Nielsen, A. R., Pedersen, B. K., et al. (2010). Muscle specific microRNAs are regulated by endurance exercise in human skeletal muscle. *J. Physiol.* 588, 4029–4037. doi: 10.1113/jphysiol.2010.189860

Roberts, T. C., Godfrey, C., McClorey, G., Vader, P., Briggs, D., Gardiner, C., et al. (2013). Extracellular microRNAs are dynamic non-vesicular biomarkers of muscle turnover. *Nucleic Acids Res.* 41, 9500–9513. doi: 10.1093/nar/gkt724

Santos-Alves, E., Marques-Aleixo, I., Rizo-Roca, D., Torrella, J. R., Oliveira, P. J., Magalhães, J., et al. (2015). Exercise modulates liver cellular and mitochondrial proteins related to quality control signaling. *Life Sci.* 135, 124–130. doi: 10.1016/j.lfs.2015.06.007

Schoenfeld, B. J. (2012). Does exercise-induced muscle damage play a role in skeletal muscle hypertrophy? *J. Strength Cond. Res.* 26, 1441–1453. doi: 10.1519/JSC.0b013e31824f207e

Søndergaard, M. T., Sorensen, A. B., Skov, L. L., Kjaer-Sorensen, K., Bauer, M. C., Nyegaard, M., et al. (2015). Calmodulin mutations causing catecholaminergic polymorphic ventricular tachycardia confer opposing functional and biophysical molecular changes. *FEBS J.* 282, 803–816. doi: 10.1111/febs.13184

Spencer, M., Bishop, D., Dawson, B., and Goodman, C. (2005). Physiological and metabolic responses of repeated-sprint activities:specific to field-based team sports. *Sports Med.* 35, 1025–1044. doi: 10.2165/00007256-200535120-00003

Stokes, K. A., Gilbert, K. L., Hall, G. M., Andrews, R. C., and Thompson, D. (2013). Different responses of selected hormones to three types of exercise in young men. *Eur. J. Appl. Physiol.* 113, 775–783. doi: 10.1007/s00421-012-2487-5

Sun, L., Sun, S., Zeng, S., Li, Y., Pan, W., and Zhang, Z. (2015). Expression of circulating microRNA-1 and microRNA-133 in pediatric patients with tachycardia. *Mol. Med. Rep.* 11, 4039–4046. doi: 10.3892/mmr.2015.3246

Terentyev, D., Belevych, A. E., Terentyeva, R., Martin, M. M., Malana, G. E., Kuhn, D. E., et al. (2009). miR-1 overexpression enhances Ca(2+) release and promotes cardiac arrhythmogenesis by targeting PP2A regulatory subunit B56alpha and causing CaMKII-dependent hyperphosphorylation of RyR2. *Circ. Res.* 104, 514–521. doi: 10.1161/CIRCRESAHA.108.181651

Trappe, S., Luden, N., Minchev, K., Raue, U., Jemiolo, B., and Trappe, T. A. (2015). Skeletal muscle signature of a champion sprint runner. *J. Appl. Physiol. (1985)* 118, 1460–1466. doi: 10.1152/japplphysiol.00037.2015

Uhlemann, M., Möbius-Winkler, S., Fikenzer, S., Adam, J., Redlich, M., Möhlenkamp, S., et al. (2014). Circulating microRNA-126 increases after different forms of endurance exercise in healthy adults. *Eur. J. Prev. Cardiol.* 21, 484–491. doi: 10.1177/2047487312467902

Weston, K. S., Wisløff, U., and Coombes, J. S. (2014). High-intensity interval training in patients with lifestyle-induced cardiometabolic disease: a systematic review and meta-analysis. *Br. J. Sports Med.* 48, 1227–1234. doi: 10.1136/bjsports-2013-092576

Wu, C., Wang, C., Guan, X., Liu, Y., Li, D., Zhou, X., et al. (2014). Diagnostic and prognostic implications of a serum miRNA panel in oesophageal squamous cell carcinoma. *PLoS ONE* 9:e92292. doi: 10.1371/journal.pone.0092292

Zhang, J., Li, S., Li, L., Li, M., Guo, C., Yao, J., et al. (2015). Exosome and exosomal microRNA: trafficking, sorting, and function. *Genomics Proteomics Bioinformatics* 13, 17–24. doi: 10.1016/j.gpb.2015.02.001

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Influence of aerobic exercise training on post-exercise responses of aortic pulse pressure and augmentation pressure in postmenopausal women

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Central arterial blood pressure (BP) is more predictive of future cardiovascular events than is brachial BP because it reflects the BP load imposed on the left ventricle with greater accuracy. However, little is known about the effects of exercise training on central hemodynamic response to acute exercise. The purpose of the present study was to determine the influence of an aerobic exercise regimen on the response of aortic BP after a single aerobic exercise in postmenopausal women. Nine healthy postmenopausal women (age: 61 ± 2 years) participated in a 12-week aerobic exercise training regimen. Before and after the training, each subjects performed a single bout of cycling at ventilatory thresholds for 30 min. We evaluated the post-exercise aortic BP response, which was estimated via the general transfer function from applanation tonometry. After the initial pre-training aerobic exercise session, aortic BP did not change significantly: however, aortic pulse pressure and augmentation pressure were significantly attenuated after the single aerobic exercise session following the 12-week training regimen. The present study demonstrated that a regular aerobic exercise training regimen induced the post-exercise reduction of aortic pulse pressure and augmentation pressure. Regular aerobic exercise training may enhance post-exercise reduction in aortic BP.

Keywords: augmentation pressure, aortic blood pressure, aerobic exercise, aerobic training, postmenopausal women

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Introduction

Aging can increase both blood pressure (BP) and arterial stiffness, both of which are major risk factors for cardiovascular disease (Blacher et al., 1999; O'Rourke, 1999). Central BP, including aortic or carotid pressures, reportedly has a greater influence on cardiovascular disease than dose peripheral BP (Roman et al., 2007). In addition, the pulsatile component of central arterial pressure (e.g., pulse pressure) is strongly correlated with cardiovascular events and outcome than is systolic pressure (Benetos et al., 1998; Safar et al., 2002; Roman et al., 2007). Central aortic pulse pressure can be subdivided into amplitude of the first systolic peak and augmentation pressure. The augmentation pressure is influenced by aging, body height, and arterial stiffness (Nichols and Edwards, 2001; Nichols and O'Rourke, 2005). In women, the increase in arterial stiffness with age accelerates around menopause (Tomiyama et al., 2003). In addition,

the increase in central pulse pressure in women is much greater than that in men (McEniery et al., 2005). Therefore, the management of central BP, especially pulse pressure and argumentation pressure, may be of great pathophysiological importance in postmenopausal women.

Exercise is beneficial for the treatment of BP and vascular aging. A single bout of aerobic exercise can cause a transient reduction in peripheral BP (Kaufman et al., 1987; MacDonald, 2002). Kingwell et al. (1997) reported that arterial stiffness decreases after acute aerobic exercise in young men. Recently, we demonstrated that a single bout of aerobic exercise decreased not only arterial stiffness but also the central pulse pressure in young men (Sugawara et al., 2015). Alternatively, a previous study by our group reported that arterial stiffness in older women did not change after acute aerobic exercise: however, after a period of regular exercise training, arterial stiffness was significantly decreased after acute aerobic exercise (Maeda et al., 2008). This study implied that long-term exercise training may enhance the cardiovascular response to acute exercise. However, little is known about the effect of exercise training on the post-exercise response of central hemodynamics in older women.

The present study aimed to determine the effect of exercise training on the central BP response to an acute single bout of aerobic cycling. We hypothesize that persistent exercise training can enhance the aortic BP response to a single bout of aerobic exercise. To test our hypothesis, we measured aortic pulse pressure and augmentation pressure during recovery from a single bout of exercise before and after 12 weeks of aerobic exercise training.

Methods

Subjects

Nine sedentary postmenopausal women (52-66 years old) participated in the study. The subjects were nonsmokers, non-obese, and free of cardiovascular disease, as assessed by medical history. None of the subjects were taking medications affecting the cardiovascular system or hormone replacement therapy. All potential risks associated with the study were explained to the subjects, and they gave written informed consent for participation in the study. All procedures were reviewed and approved by the ethical committee of the University of Tsukuba.

Experimental Protocol

All 9 subjects completed an aerobic exercise training regimen. Before and after aerobic exercise training, each subject performed an acute exercise test that consisted of a 30-min aerobic cycling exercise at the intensity of the individual's ventilatory threshould (VT). All participants were at least 3 h postprandial, and did not consume caffeine and alcohol for 12-h and strenuous exercise for 24 h. We measured brachial BP, central BP and heart rate (HR) before and 30 and 60 min after the single bout of exercise. At least 2 days before the acute exercise test, VT and blood chemistries were measured after overnight fast. All measurements were performed at a constant room temperature (24-26°C).

Measurement

Pressure waveforms were obtained simultaneously in the common carotid artery using an applanation tonometry sensor (FormPWV/ABI, Colin Medical Technology, Komaki, Japan). Carotid arterial pressure waveforms were sampled at 1000 Hz for off line analysis and resampled at 128 Hz with data analysis software (AcqKnowledge, BIOPAC system Santa Barbara, CA) (Sugawara et al., 2010). And then, pressure waveform transferred into aortic pressure waveforms with an arterial waveform analysis software involving a validated generalized transfer function (SphygmoCor software, AtCor Medical, Sydney, Australia) (Karamanoglu et al., 1993). Pressure waveforms were calibrated to brachial mean arterial pressure and diastolic BP. To qualify the magnitude of wave reflection from the periphery to the heart, the first and second systolic peak, defined as P1 and P2, respectively, of the aortic pressure waveforms were analyzed. Aortic systolic arterial pressure, diastolic arterial pressure, and augmentation pressure (peak pressure—pressure at the inflection point at systolic shoulder) was computed from synthesized aortic pressure waveforms. Pulse pressure was calculated systolic blood pressure—diastolic blood pressure. The day-to-day coefficient of variation for systolic blood pressure, diastolic blood pressure, pulse pressure and augmentation pressure was 4 ± 1, 5 ± 1, 6 ± 2, and 11 ± 3%, respectively.

Carotid-femoral pulse wave velocity (cfPWV) was measured as arterial stiffness by a semi-automated vascular testing system (Sugawara et al., 2015). Briefly, carotid and femoral pressure waveforms were obtained by two applanation tonometry sensors incorporating an array of 15 transducers (Form PWV/ABI, Colin Medical Technology, Komaki, Japan). The distance between the left common carotid and left common femoral arterial recording sites divided by the transit time resulted in the calculation of cfPWV.

VT was measured during the incremental cycle ergometer exercise by using online computer-assisted circuit spirometry (AE300S; Minato Medical Science, Osaka, Japan). All subjects performed a symptom-limited cycling exercise test (after a 2 min warm-up at 20 W, followed by 10 W increases every min) until they felt exhaustion or reached their age predicted maximal HR. The peak oxygen consumption (VO_{2peak}) was defined at the highest value recorded during the test. Each individual VT was calculated by using regression analysis of the slopes of carbon dioxide production, oxygen uptake and the minute ventilation plot.

A blood sample was collected from the antecubital vein after overnight fasting. Serum total cholesterol, triglycerides, and plasma glucose were determined using standard enzymatic technique.

Aerobic Exercise Training Regimen

The subjects underwent aerobic exercise training 4-6 days per week (three supervised sessions and additional home-based sessions) for a total 12 weeks (Akazawa et al., 2012). Initially, the subjects performed cycling and walking sessions for 30 min/day at a relatively low intensity (60% of their individually determined maximal HR). As their exercise tolerance improved, the intensity and duration of aerobic exercise

was increased to 40–60 min/day at an intensity of 70-80% of the maximal HR.

Steady-state Aerobic Exercise Test

Before and after the exercise training program, the subjects performed a steady-state exercise test at their individual VT for 30 min using an electrically braked cycle ergometer (75XLIII; Combi Welness, Tokyo, Japan). An investigator monitored the subject's working load, oxygen uptake, heart rare, and rating of perceived exertion (RPE) during exercise and supervised each subject to perform the cycling exercise around the target intensities at 60 rpm.

Statistical Analyses

Data are expressed as mean ± SE. Student's *t*-test for paired data was used to evaluate the difference in the body mass, blood chemistry, VT, and VO_{2peak} at test and oxygen uptake, heart rate, and RPE during a single bout of exercise before and after exercise training. A two-way analysis of variance with repeated measures was performed to identify an interaction or main effect. A Dunnet post hoc test was used, when indicated for a significant main effect or interaction. Univariable correlation analyses were used to determine the relations between variables of interest. Statistical significance was set a priori at $P < 0.05$ for all comparisons.

Results

There were no significant differences in measurement of body mass, triglyceride and blood glucose between before and after the 12-week exercise training regimen (**Table 1**). However, after the regimen, total cholesterol was significantly decreased and individual VT and VO_{2peak} were significantly increased (**Table 1**).

The oxygen uptake and heart rate increased during exercise and increase in oxygen uptake and heart rate was higher after exercise training than that of before training, but RPE was not different from between before and after training regimen (**Table 2**).

There were no differences in baseline measures of brachial and aortic blood pressure, HR and cfPWV between before and after exercise training (**Table 3**). Participants completed a 30-min of aerobic cycling exercise test at the intensity of their specific VT before and after the training regimen. Measures of brachial and aortic blood pressure, HR, and cfPWV did not change following the single exercise bout before the training regimen. By use of relative change from the baseline (i.e., pre-exercise), aortic pulse pressures did not change significantly after the initial acute exercise bout, whereas following the training regimen aortic pulse pressures siginificantly decreased with the acute aerobic exercise bout ($P < 0.05$ vs. baseline; **Figure 1**). The relative changes in brachial pulse pressure with acute exercise bout did not alter significantly either before or after the training. Likewise, the single exercise bout augmentation pressure was not changed before the training regimen but was significantly reduced following the regimen (**Figure 2**). Significant reductions of these measurements did not last for 60 min.

Although cfPWV did not change significantly with acute exercise bout, the individual post-exercise cfPWV response (post

TABLE 1 | Subjects characteristics before and after exercise training.

	Before training	After training
Age (years)	61 ± 2	
Height (cm)	154 ± 1	
Weight (kg)	52 ± 2	51 ± 1
Total cholesterol (mg/dL)	231 ± 9	216 ± 7*
Triglyceride (mg/dL)	127 ± 42	138 ± 28
Blood glucose (mg/dL)	94 ± 2	92 ± 2
Oxygen uptake at VT (mL/kg/min)	12 ± 2	16 ± 2*
Peak oxygen uptake (mL/kg/min)	23 ± 1	26 ± 1*

*Data are means ± SE. VT, ventilatory threshold. *P < 0.05 vs. before training.*

TABLE 2 | Work load, oxygen uptake, heart rate, and rating of perceived exertion during 30 min steady-state exercise.

	Before training	After training
Work load (Watt)	31 ± 4	59 ± 3*
Oxygen uptake (mL/kg/min)	12 ± 1	17 ± 1*
Heart rate (bpm)	106 ± 5	125 ± 4*
Rating of perceived exertion	13 ± 1	13 ± 1

*Data are means ± SE. *P < 0.05 vs. before training.*

TABLE 3 | Hemodynamics responses to a single bout exercise before and after training.

		Baseline	P30	P60
BRACHIAL				
Systolic blood pressure (mmHg)	Before	116 ± 4	115 ± 3	115 ± 3
	After	115 ± 3	114 ± 4	116 ± 3
Diastolic blood pressure (mmHg)	Before	71 ± 3	71 ± 2	71 ± 2
	After	69 ± 3	70 ± 3	71 ± 3
Pulse pressure (mmHg)	Before	45 ± 3	43 ± 2	44 ± 2
	After	46 ± 2	44 ± 3	45 ± 3
AORTIC				
Systolic blood pressure (mmHg)	Before	108 ± 5	106 ± 3	106 ± 2
	After	106 ± 3	103 ± 4	105 ± 3
Diastolic blood pressure (mmHg)	Before	71 ± 3	71 ± 2	71 ± 2
	After	70 ± 2	70 ± 3	71 ± 3
Pulse pressure (mmHg)	Before	37 ± 4	34 ± 3	35 ± 3
	After	36 ± 3	33 ± 3	35 ± 3
Augmentation pressure (mmHg)	Before	16 ± 2	14 ± 2	15 ± 2
	After	17 ± 2	13 ± 2	15 ± 2
P1_height (mmHg)	Before	21 ± 2	21 ± 1	20 ± 2
	After	20 ± 1	20 ± 1	19 ± 1
Heart rate (beats/min)	Before	61 ± 2	63 ± 2	62 ± 2
	After	58 ± 2	63 ± 2	62 ± 2
Carotid-femoral PWV (cm/sec)	Before	949 ± 21	964 ± 29	970 ± 29
	After	950 ± 30	966 ± 28	987 ± 24

Data are means ± SE. P1, first systolic peak; PWV, Pulse wave velocity; P30, post 30 min; P60, post 60 min.

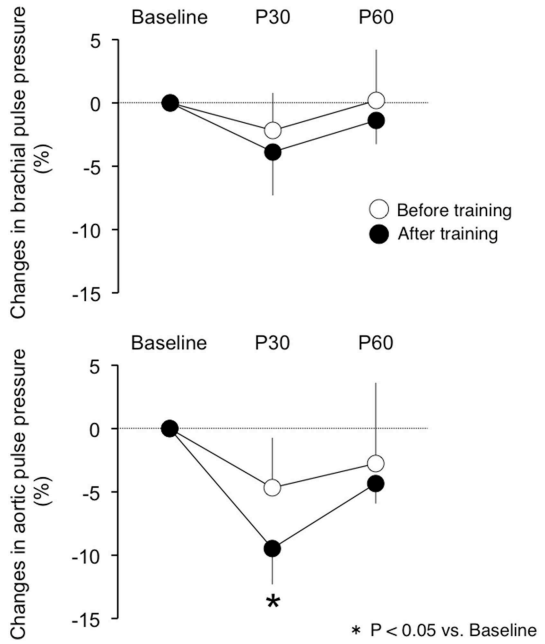

FIGURE 1 | The post-exercise response of (top) brachial pulse pressure and (bottom) aortic pulse pressure before and after exercise training. P30, post 30 min acute exercise; P60, post 60 min acute exercise.

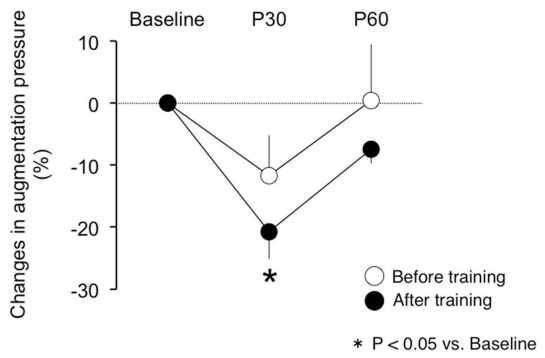

FIGURE 2 | The post-exercise response of aortic augmentation pressure before and after exercise training. P30, post 30 min acute exercise; P60: post 60 min acute exercise.

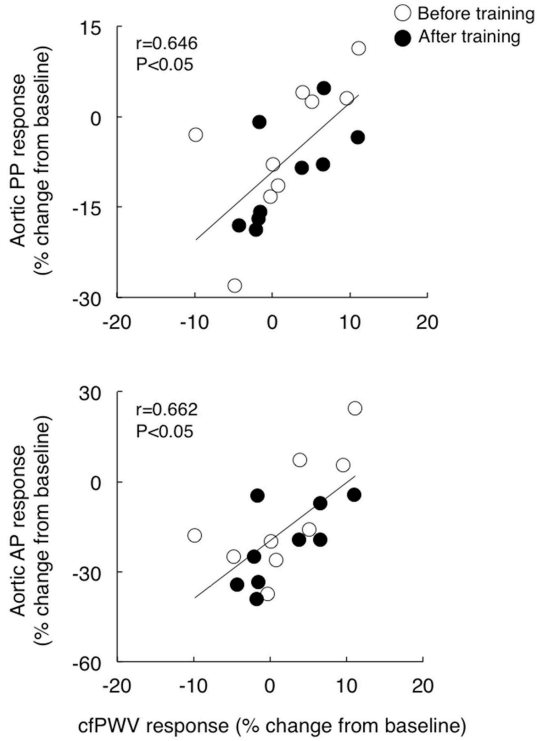

FIGURE 3 | The relations between the post-exercise response (relative change from the baseline to post 30 min) of carotid-femoral pulse wave velocity (cfPWV) and corresponding aortic pulse pressure (PP, top) and augmentation pressure (AP, bottom) response.

30 min) correlated with corresponding aortic pulse pressure and augmentation pressure responses ($r = 0.646$ and $r = 0.662$, respectively, **Figure 3**). Additionally, although baseline cfPWV did not change significantly following the exercise training regimen, the training-induced changes in post-exercise cfPWV responses correlated to the corresponding changes in the aortic pulse pressure and augmentation pressure response ($r = 0.788$ and $r = 0.716$, respectively, **Figure 4**).

Discussion

The present study aimed to determine the effect of aerobic exercise training on the acute central hemodynamic response to the single bout of aerobic exercise in postmenopausal women. The main findings are as follows: First, before the training intervention, aortic and brachial BP did not change significantly following 30 min of cycling at the individual VT measured before the training regimen. Second, after the intervention of 12-week exercise training regimen, aortic pulse pressure and augmentation pressure decreased following 30 min of cycling at the individual VT measured after the training regimen. These findings suggest that the central hemodynamic responses to acute aerobic exercise are enhanced by regular aerobic exercise training.

A transient reduction in brachial BP after acute aerobic exercise is reportedly observed in normotensive and hypertensive individuals (Halliwill, 2001; MacDonald, 2002). However, little is known about the central BP response to acute aerobic exercise. We recently reported a study that showed that the aortic pulse pressure decreased with moderate intensity exercise in a group of young healthy men (Sugawara et al., 2015). In the present study, we identified that postmenopausal women exhibited a reduction of aortic pulse pressure following a single bout of aerobic exercise without lowering brachial pulse pressure only after a 12-week training regimen. The present study develops this notion for the aging population. In the present study, the exercise work rate, oxygen consumption, and heart rate during a single bout exercise was increased after exercise training regimen. But, the increases in exercise

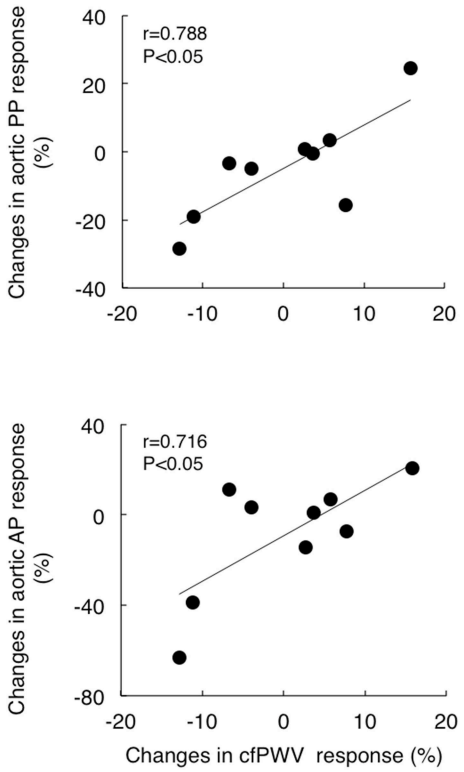

FIGURE 4 | The relations between training-induced change (from pre- to post-training) in post-exercise response (from the baseline to post 30 min) of carotid-femoral pulse wave velocity (cfPWV) and corresponding changes in aortic pulse pressure (PP, top) and augmentation pressure (AP, bottom) response.

intensity were not correlated with the central blood pressure response to acute exercise. Thus, it seems that the decrease in aortic blood pressure following acute exercise may be contributed to other than increase in exercise work volume and/or intensity.

In the present study, although there were no significant effects of acute exercise and regular exercise training on cfPWV, temporary cfPWV response to acute exercise is correlated with corresponding response of aortic pulse pressure and augmentation pressure to acute exercise before and after training (**Figure 3**). Furthermore, the training-induced change in cfPWV response to acute exercise bout was correlated with the corresponding changes in response of aortic pulse pressure and augmentation pressure to acute exercise (**Figure 4**). These results suggest the changes in arterial stiffness may play a role partially in post-exercise response of central aortic hemodynamics in postmenopausal women.

The underlying mechanism for reduction in aortic pulse pressure may be different between young and older populations. Theoretically, it is believed that the central arterial pressure waveform consists of a forward traveling wave and a later-arriving reflected wave. In young men, the post exercise attenuation of aortic pulse pressure was associated with the corresponding changes in aortic pulse wave velocity (Sugawara et al., 2015), suggesting the delay of the reflected wave mitigates its overlap on the incident wave and lowers aortic pulse pressure. Comparatively, in postmenopausal women, aortic pulse wave velocity did not change after the single aerobic exercise bout either before or after the 12-week exercise training regimen. Additionally, the amplitude of P1 (a surrogate measure of the incident wave amplitude) did not change, whereas augmentation pressure was diminished by the single exercise bout after the training regimen. Conclusively, the significant acute exercise-induced reduction of aortic pulse pressure may be attributed to the attenuated wave reflection rather than delayed return of the reflected wave.

We could speculate several possibilities regarding the mechanism responsible for decreased augmentation pressure in postmenopausal women. Regular endurance training improves endothelial function and capacity of dilation in trained vascular beds in the aging individual (Taddei et al., 2000). Based on the traditional concept that central arterial pressure is comprised of the incident wave (generated by left ventricle) and the reflected wave (emanated from points of impedance mismatch in the lower body) (Nichols and O'Rourke, 2005), the improved vasodilatory function in legs could buffer the incident wave effectively and attenuate the reflected wave. Alternatively, recent studies propose that aortic pressure is affected by, not only the aforementioned components, but also by the reservoir pressure (Wang et al., 2003; Davies et al., 2010). The arterial reservoir pressure is associated with the volume of blood stored in the aorta; it depends on the buffering capacity of the aorta, especially the proximal region (i.e., the ascending aorta and aortic arch). If this emerging idea is true, the ameliorated reservoir function of proximal aorta might be associated with the diminished aortic pressure augmentation. In this study, although cfPWV was not improved by the exercise training, cfPWV mainly reflects pulse wave velocity along the descending aorta and does not cover the ascending aorta and aortic arch.

In the present study, exercise training was found to slightly decrease brachial and central BP at baseline; however, an overall decrease in BP did not reach statistical significance. A meta-analysis had reported that exercise training decreases brachial systolic and diastolic BP by about 3-4 mmHg and 2-3 mmHg, respectively (Kelley and Sharpe Kelley, 2001). On the other hand, the effects of exercise training on central BP are not yet consistent. Laskey et al. (2013) reported that a 20-week exercise rehabilitation program decreased central aortic systolic pressure and pulse pressure at rest in patients with coronary heart disease. However, we and other investigators have shown that aerobic exercise training did not change central aortic hemodynamics in middle-aged and older populations (Tanaka et al., 2002; Sugawara et al., 2006, 2012). These inconsistent results may, in part, be due to the differences in exercise training protocols or subject characteristics among studies. Further studies are needed to clarify the effects of exercise training on central arterial BP at rest.

A number of studies have showed that aerobic exercise training decreases arterial stiffness (Montero et al., 2014). On the other hand, in the present study, we cannot observe the significant change in pulse wave velocity after 12-week training. It should be noted that several studies demonstrated that exercise

training did not affect cfPWV in healthy postmenopausal women (Seals et al., 2001; Sugawara et al., 2012). It is likely that elderly women show less response of training effects. Interestingly, Hayashi et al. (2008) suggested that habitual exercise activity decreases arterial stiffness in individual with TC or CC genotype of estrogen receptor-α, there is no significant effect of exercise on arterial stiffness in individual with TT genotype. These results suggest that training efficiency on arterial stiffness might be affected gene polymorphism. However, we could not clarify the precise mechanisms because this was not mechanistic study. The lack of the control group was also a limitation of the study. Randomized controlled trial study is warranted in future study.

In conclusion, we demonstrated that a single 30-min bout of moderate-intensity cycling did not induce a significant change in central aortic pressure in postmenopausal women; but, after a 12-week period of consistent aerobic training, a cycling exercise at relatively similar intensity evoked a significant reduction of aortic augmentation pressure and pulse pressure. These findings suggest that regular aerobic exercise training may improve the blunted central hemodynamic response to an acute aerobic exercise bout in postmenopausal women.

Author Contributions

NA drafted the manuscript. All authors contributed to the interpretation and discussion of the results. NA, SR, and JS contributed data acquisition and analysis. JS and SM provided the concept and design of the study and critically revised the manuscript.

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References

Akazawa, N., Choi, Y., Miyaki, A., Tanabe, Y., Sugawara, J., Ajisaka, R., et al. (2012). Curcumin ingestion and exercise training improve vascular endothelial function in postmenopausal women. *Nutr. Res.* 32, 795–799. doi: 10.1016/j.nutres.2012.09.002

Benetos, A., Rudnichi, A., Safar, M., and Guize, L. (1998). Pulse pressure and cardiovascular mortality in normotensive and hypertensive subjects. *Hypertension* 32, 560–564.

Blacher, J., Asmar, R., Djane, S., London, G. M., and Safar, M. E. (1999). Aortic pulse wave velocity as a marker of cardiovascular risk in hypertensive patients. *Hypertension* 33, 1111–1117.

Davies, J. E., Baksi, J., Francis, D. P., Hadjiloizou, N., Whinnett, Z. I., Manisty, C. H., et al. (2010). The arterial reservoir pressure increases with aging and is the major determinant of the aortic augmentation index. *Am. J. Physiol. Heart Circ. Physiol.* 298, H580–H586. doi: 10.1152/ajpheart.00875.2009

Halliwill, J. R. (2001). Mechanisms and clinical implications of post-exercise hypotension in humans. *Exerc. Sport Sci. Rev.* 29, 65–70. doi: 10.1097/00003677-200104000-00005

Hayashi, K., Maeda, S., Iemitsu, M., Otsuki, T., Sugawara, J., Tanabe, T., et al. (2008). Estrogen receptor-alpha genotype affects exercise-related reduction of arterial stiffness. *Med. Sci. Sports Exerc.* 40, 252–257. doi: 10.1249/mss.0b013e31815c04cf

Karamanoglu, M., O'Rourke, M. F., Avolio, A. P., and Kelly, R. P. (1993). An analysis of the relationship between central aortic and peripheral upper limb pressure waves in man. *Eur. Heart J.* 14, 160–167. doi: 10.1093/eurheartj/14.2.160

Kaufman, F. L., Hughson, R. L., and Schaman, J. P. (1987). Effect of exercise on recovery blood pressure in normotensive and hypertensive subjects. *Med. Sci. Sports Exerc.* 19, 17–20.

Kelley, G. A., and Sharpe Kelley, K. (2001). Aerobic exercise and resting blood pressure in older adults: a meta-analytic review of randomized controlled trials. *J. Gerontol. A Biol. Sci. Med. Sci.* 56, M298–M303. doi: 10.1093/gerona/56.5.M298

Kingwell, B. A., Berry, K. L., Cameron, J. D., Jennings, G. L., and Dart, A. M. (1997). Arterial compliance increases after moderate-intensity cycling. *Am. J. Physiol.* 273(5. Pt 2), H2186–H2191.

Laskey, W., Siddiqi, S., Wells, C., and Lueker, R. (2013). Improvement in arterial stiffness following cardiac rehabilitation. *Int. J. Cardiol.* 167, 2734–2738. doi: 10.1016/j.ijcard.2012.06.104

MacDonald, J. R. (2002). Potential causes, mechanisms, and implications of post exercise hypotension. *J. Hum. Hypertens.* 16, 225–236. doi: 10.1038/sj.jhh.1001377

Maeda, S., Tanabe, T., Otsuki, T., Sugawara, J., Ajisaka, R., and Matsuda, M. (2008). Acute exercise increases systemic arterial compliance after 6-month exercise training in older women. *Hypertens. Res.* 31, 377–381. doi: 10.1291/hypres.31.377

McEniery, C. M., Yasmin, Hall, I. R., Qasem, A., Wilkinson, I. B., Cockcroft, J. R., and ACCT Investigators. (2005). Normal vascular aging: differential effects on wave reflection and aortic pulse wave velocity: the Anglo-Cardiff Collaborative Trial (ACCT). *J. Am. Coll. Cardiol.* 46, 1753–1760. doi: 10.1016/j.jacc.2005.07.037

Montero, D., Roche, E., and Martinez-Rodriguez, A. (2014). The impact of aerobic exercise training on arterial stiffness in pre- and hypertensive subjects: a systematic review and meta-analysis. *Int. J. Cardiol.* 173, 361–368. doi: 10.1016/j.ijcard.2014.03.072

Nichols, W. W., and Edwards, D. G. (2001). Arterial elastance and wave reflection augmentation of systolic blood pressure: deleterious effects and implications for therapy. *J. Cardiovasc. Pharmacol. Ther.* 6, 5–21. doi: 10.1177/107424840100600102

Nichols, W. W., and O'Rourke, M. F. (2005). *Mc'Donald's Blood Flow in Arteries: Theoretical, Experimental, and Clinical principles. 5th Edn*. London: Arnold.

O'Rourke, M. F. (1999). Isolated systolic hypertension, pulse pressure, and arterial stiffness as risk factors for cardiovascular disease. *Curr. Hypertens. Rep.* 1, 204–211.

Roman, M. J., Devereux, R. B., Kizer, J. R., Lee, E. T., Galloway, J. M., Ali, T., et al. (2007). Central pressure more strongly relates to vascular disease and outcome than does brachial pressure: the Strong Heart Study. *Hypertension* 50, 197–203. doi: 10.1161/HYPERTENSIONAHA.107.089078

Safar, M. E., Blacher, J., Pannier, B., Guerin, A. P., Marchais, S. J., Guyonvarc'h, P. M., et al. (2002). Central pulse pressure and mortality in end-stage renal disease. *Hypertension* 39, 735–738. doi: 10.1161/hy0202.098325

Seals, D. R., Tanaka, H., Clevenger, C. M., Monahan, K. D., Reiling, M. J., Hiatt, W. R., et al. (2001). Blood pressure reductions with exercise and sodium restriction in postmenopausal women with elevated systolic pressure: role of arterial stiffness. *J. Am. Coll. Cardiol.* 38, 506–513. doi: 10.1016/S0735-1097(01)01348-1

Sugawara, J., Akazawa, N., Miyaki, A., Choi, Y., Tanabe, Y., Imai, T., et al. (2012). Effect of endurance exercise training and curcumin intake on central arterial hemodynamics in postmenopausal women: pilot study. *Am. J. Hypertens.* 25, 651–656. doi: 10.1038/ajh.2012.24

Sugawara, J., Hayashi, K., and Tanaka, H. (2010). Distal shift of arterial pressure wave reflection sites with aging. *Hypertension* 56, 920–925. doi: 10.1161/HYPERTENSIONAHA.110.160549

Sugawara, J., Komine, H., Miyazawa, T., Imai, T., and Ogoh, S. (2015). Influence of single bout of aerobic exercise on aortic pulse pressure. *Eur. J. Appl. Physiol.* 115, 739–746. doi: 10.1007/s00421-014-3061-0

Sugawara, J., Otsuki, T., Tanabe, T., Hayashi, K., Maeda, S., and Matsuda, M. (2006). Physical activity duration, intensity, and arterial stiffening in postmenopausal women. *Am. J. Hypertens.* 19, 1032–1036. doi: 10.1016/j.amjhyper.2006.03.008

Taddei, S., Galetta, F., Virdis, A., Ghiadoni, L., Salvetti, G., Franzoni, F., et al. (2000). Physical activity prevents age-related impairment in nitric oxide availability in elderly athletes. *Circulation* 101, 2896–2901. doi: 10.1161/01.CIR.101.25.2896

Tanaka, H., Seals, D. R., Monahan, K. D., Clevenger, C. M., DeSouza, C. A., and Dinenno, F. A. (2002). Regular aerobic exercise and the age-related increase in carotid artery intima-media thickness in healthy men. *J. Appl. Physiol. (1985)* 92, 1458–1464. doi: 10.1152/japplphysiol.00824.2001

Tomiyama, H., Yamashina, A., Arai, T., Hirose, K., Koji, Y., Chikamori, T., et al. (2003). Influences of age and gender on results of noninvasive brachial-ankle pulse wave velocity measurement–a survey of 12517 subjects. *Atherosclerosis* 166, 303–309. doi: 10.1016/S0021-9150(02)00332-5

Wang, J. J., O'Brien, A. B., Shrive, N. G., Parker, K. H., and Tyberg, J. V. (2003). Time-domain representation of ventricular-arterial coupling as a windkessel and wave system. *Am. J. Physiol. Heart Circ. Physiol.* 284, H1358–H1368. doi: 10.1152/ajpheart.00175.2002

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Neuro-mechanical determinants of repeated treadmill sprints - Usefulness of an "hypoxic to normoxic recovery" approach

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To improve our understanding of the limiting factors during repeated sprinting, we manipulated hypoxia severity during an initial set and examined the effects on performance and associated neuro-mechanical alterations during a subsequent set performed in normoxia. On separate days, 13 active males performed eight 5-s sprints (recovery = 25 s) on an instrumented treadmill in either normoxia near sea-level (SL; FiO_2 = 20.9%), moderate (MH; FiO_2 = 16.8%) or severe normobaric hypoxia (SH; FiO_2 = 13.3%) followed, 6 min later, by four 5-s sprints (recovery = 25 s) in normoxia. Throughout the first set, along with distance covered [larger sprint decrement score in SH (−8.2%) compared to SL (−5.3%) and MH (−7.2%); $P < 0.05$], changes in contact time, step frequency and root mean square activity (surface electromyography) of the quadriceps (*Rectus femoris* muscle) in SH exceeded those in SL and MH ($P < 0.05$). During first sprint of the subsequent normoxic set, the distance covered (99.6, 96.4, and 98.3% of sprint 1 in SL, MH, and SH, respectively), the main kinetic (mean vertical, horizontal, and resultant forces) and kinematic (contact time and step frequency) variables as well as surface electromyogram of quadriceps and plantar flexor muscles were fully recovered, with no significant difference between conditions. Despite differing hypoxic severity levels during sprints 1–8, performance and neuro-mechanical patterns did not differ during the four sprints of the second set performed in normoxia. In summary, under the circumstances of this study (participant background, exercise-to-rest ratio, hypoxia exposure), sprint mechanical performance and neural alterations were largely influenced by the hypoxia severity in an initial set of repeated sprints. However, hypoxia had no residual effect during a subsequent set performed in normoxia. Hence, the recovery of performance and associated neuro-mechanical alterations was complete after resting for 6 min near sea level, with a similar fatigue pattern across conditions during subsequent repeated sprints in normoxia.

Keywords: repeated-sprint ability, running mechanics, hypoxia, electromyography, recovery

Introduction

Intense physical efforts performed at or near maximal speeds are often crucial for successful participation in intermittent sports (e.g., team or racket sports). For instance, top-level soccer players complete more high-intensity running or sprinting than their lower-level counterparts (Mohr et al., 2003, 2008). However, irrespectively of competitive standard, the volume of all high-intensity actions decline over the course of a game, reflecting muscle fatigue development (Mohr et al., 2008). Although, still debated (Carling, 2013), the repeated-sprint ability (RSA) is commonly viewed as an important marker of successful physical performance in these disciplines.

While RSA has been increasingly investigated over the last decade, to date, most of the available studies focused only on the physiological features of this fitness component. Evaluation of the biomechanical aspects of running RSA have insofar been limited to either indirect measures of stride characteristics (i.e., pressure insoles) (Girard et al., 2011a; Brocherie et al., 2015) or direct sprint kinetics/kinematics assessments (i.e., force platforms), but only for a discrete number of steps at various intervals during the sprint distance (Girard et al., 2011b). Using instrumented, sprint treadmills makes now possible to deepen our knowledge about the biomechanical manifestation of fatigue during repeated sprinting (Morin et al., 2011). For instance, through direct measurement of ground reaction forces, Girard et al. (2015a) reported significant decrease in propulsive power and step frequency with fatigue while contact time and step length increased, when five maximal 5-s sprints with incomplete recoveries (25 s) were repeated.

Peripheral mechanisms, that include limitation in energy supply and the intramuscular accumulation of metabolic by-products, have been traditionally associated to fatigue development during repeated sprinting (Girard et al., 2011c). Consideration of neural factors (i.e., neural drive and muscle recruitment strategies) as significant contributors to fatigue etiology during RSA protocols stem from parallel reductions in amplitude of quadriceps surface electromyography (EMG) signals (i.e., a reasonable proxy for net motor unit activity) and in sprint performance (Mendez-Villanueva et al., 2008; Billaut et al., 2013; Bowtell et al., 2014; Brocherie et al., 2015). For instance, Brocherie et al. (2015) demonstrated a disproportionate decrease in motor unit recruitment inferred via EMG signaling [Root Mean Square (RMS) activity] of *Rectus femoris* and *Biceps femoris* muscles over sprint times when professional football players completed the repeated anaerobic sprint test on artificial turf. Although, muscle activation capacity of plantar flexors decreases from pre- to post-RSA running (Perrey et al., 2010), the question of whether this muscle group is subjected to similar neural adjustments than those seen for the quadriceps during actual sprint repetitions remains undetermined.

When attempting to evaluate RSA and its fatigue-causing factors, a single set of a fixed number of 5–15 sprints (i.e., usually of 5–10 s) with (incomplete) recovery of less than 30 s (i.e., usually passive) has most commonly been used (Girard et al., 2011c). Admittedly, while valuable knowledge on how fatigue manifests and the potential contribution of neural factors can be gained from such RSA tests' format, derived information remains mainly descriptive. Innovative analysis methods that are based on the comparison of fatigue responses and recovery of performance during and between sets of repeated sprints, respectively, have emerged (Girard et al., 2015b). By linking the aforementioned changes to muscle metabolism and neuromuscular function, such approaches support the idea that previous repeated-sprint exercise has a negative "carry-over" impact on physiological strain, perception of effort and performance during the next bout of activity (Mendez-Villanueva et al., 2007, 2012; Billaut et al., 2013). With this in mind, it is surprising that little attention has been directed toward the usefulness of the "recovery of performance approach" to shed more light on how running mechanics and muscle activation patterns are altered during RSA run-based tests.

Extreme environments such as hypoxia [i.e., a reduction in environmental oxygen (O_2) availability] are known to lead to premature fatigue and exacerbated cardiorespiratory and perceptual responses during repeated-sprint exercise (Billaut et al., 2013; Bowtell et al., 2014; Goods et al., 2014). By majoring RSA-induced demands (and thereby recovery requirements) on the neuromuscular system during an initial set (i.e., larger changes within the central nervous system with severer hypoxic levels), it seems reasonable to speculate that performance decrement during a subsequent repeated-sprint exercise would be exacerbated. Accordingly, modifying the ensuing recovery rate of repeated-sprint performance from previous strenuous exercise highlights a context whereby neuro-mechanical determinants of RSA running performance could be explored from a new perspective (Minett and Duffield, 2014).

Our intention was therefore to manipulate hypoxia severity during an initial repeated-sprint set and examine the effect on sprinting performance, running mechanics (kinetics and kinematics) and lower-limbs neuromuscular activity (surface EMG activity) during a subsequent set performed in normoxia. We hypothesized that, with severer hypoxia levels during a first repeated-sprint set expected to major RSA-induced demands placed on the neuromuscular system, larger recovery requirements and fatigue-related residual or "carry-over" effects from the previous set would, in turn, negatively influence fatigability during the completion of a second set performed in normoxia.

Methods

Subjects

Thirteen male recreational team- (i.e., football, rugby, basketball) and racket- (i.e., tennis, squash) sport players (Mean ± SD: 31.2 ± 4.8 years; 178.4 ± 6.6 cm; 74.3 ± 8.2 kg) participated in the study. In the 6 months preceding the study, subjects trained on average 4.5 ± 2.5 $h.wk^{-1}$, which included activity-specific training (i.e., technical and tactical skills), aerobic and anaerobic training (i.e., on- and off-court/field exercises) and basic strength training. Although, training content of the tested athletes largely focused on accelerated runs, their sprinting skills are deemed to be "moderate" compared to "elite" (i.e., national

to international level) sprinters (Rabita et al., 2015) and/or team-sport athletes (Brocherie et al., 2015). All subjects were born and raised at <1000 m and had not traveled to elevations >1000 m in the 3 months prior to investigation. They gave their informed, written consent preceding the commencement of the experiment. Experimental protocol was conducted according to the Declaration of Helsinki for use of Human Subjects and approved by the Ethics Committee of *Shafallah Medical Genetics Center*.

Experimental Procedure

About 1 week prior to testing, subjects undertook a complete preliminary session where they performed short (<5 s) "familiarization" treadmill sprints at increasing intensities while wearing a facemask for habituation (i.e., the hypoxic system was turned off at this occasion), with full recovery and until being comfortable with treadmill maximal sprint technique (which generally required 7–10 trials). Subjects then performed three maximal 5-s single sprints separated by 2 min of passive rest. All participants satisfied the criteria of having a coefficient of variation <2.2% for distance covered across three successive trials (Girard et al., 2015c). After 10 min of rest, the complete RSA test was completed. Strong verbal encouragement was given during all maximal efforts.

Subjects then came to the laboratory (well-ventilated at a constant temperature of ∼25°C and 40% relative humidity) for three experimental sessions (∼1 h; counterbalanced randomized crossover design in double-blind fashion), with at least 3–4 days apart, including a repeated-sprint running protocol on a treadmill sprint ergometer. They performed their trials at the same time of the day (±1 h) and wore similar sports gear (running shoes, short, and T-shirt). They were instructed to maintain their normal diet (i.e., avoiding any nutritional supplements or alcohol consumption), sleeping (i.e., ≥7 h/night) and training (i.e., avoiding vigorous exercise 24 h before every trial) habits during the 1–2 weeks period of testing to prevent any possible interference on their sprinting abilities. Subjects were instructed to drink 4–6 mL of water per kilogram of body mass every 2.5 h on the day before each experimental session to ensure euhydration at the start of exercise. They were permitted to drink *ad libitum* during the warm-up procedure.

Repeated-sprint Exercise Protocol

The exercise protocol consisted of performing first eight, 5-s "all-out" sprints interspersed with 25 s of passive rest and randomly conducted near sea level (SL; FiO_2 ∼20.9%), at moderate and severe simulated altitudes (normobaric hypoxia) of 1800 m (MH; FiO_2 ∼16.6%) and 3600 m (SH; FiO_2 ∼13.0%), respectively. This was followed, after 6 min of passive rest (i.e., subjects breathed ambient air), by four, 5-s "all-out" sprints also interspersed by 25 s of passive rest but always performed at SL. During recovery periods, subjects stood on the treadmill. Before all tests, subjects completed a standardized warm-up (i.e., on the instrumented treadmill with subjects breathing ambient air) consisting of 10 min of running at 10 km.h^{-1}, followed by 15 min of sprint-specific muscular warm-up exercises [i.e., 3 × (high knee, high heels, butt-kick, skipping for ∼10 s with 30-s walking in between), followed by 3 × (3 steps accelerations at a subjective "sense of effort" of 7, 8, and 9), then by 2 × (3-s sprints at a subjective "sense of effort" of 8 and 9] (Christian et al., 2014). Afterwards, three maximal 5-s single sprints (i.e., the best of these three trials was used as the criterion score), separated by 2 min of passive rest, were completed. Finally, after a facemask connected to a portable hypoxic generator has been attached on subjects, they were allowed 5-min of free cool down prior to the repeated-sprint protocol. Testing protocols were run in a double-blind fashion in that subjects and one investigator were blinded toward the environmental condition of the initial set. The efficacy of the subjects' blinding procedure was evaluated after each experimental session by questionnaires in which subjects were asked whether they believed to be exercising at SL, MH, or SH. We are confident that the blinding procedure was efficient, as only four athletes were able to correctly identify the order of treatment.

Altitude Simulation

Normobaric hypoxia was obtained by mixing nitrogen into ambient air under control of FiO_2 (Altitrainer, SMTec SA, Nyon, Switzerland). This gas-mixing system enriches the inspired air by adding a fixed quantity of nitrogen via a 30 L mixing chamber, with the dilution being constantly controlled by a PO_2 probe (with a precision of ± 0.82 Torr and safety set at FiO_2 = 9.7%). This device allows the production of large quantities of a hypoxic gas mixture (up to 200 L.min^{-1}), with an easily adjustable O_2 fraction over a large range, and a short response time (between 15 and 45 s), expressed either by the equivalent altitude or by the O_2 partial pressure, taking into account the barometric pressure. For blinding purposes, subjects who always breathed through the same set-up (also in normoxia), inhaled the mixture contained in the buffer tank through a Hans Rudolph two-way respiratory valve. Subjects were instrumented with the facemask 5 min before the repeated-sprint exercise (i.e., after the three "reference" sprints at warm-up termination) until the end of the first set of eight sprints.

Instrumented Sprint Treadmill

The sprints were performed on an instrumented motorized treadmill (ADAL3D-WR, Medical Development—HEF Tecmachine, Andrézieux-Bouthéon, France). Briefly, it is mounted on a highly rigid metal frame fixed to the ground through four piezoelectric force transducers (KI 9077b; Kistler, Winterthur, Switzerland) and installed on a specially engineered concrete slab to ensure maximal rigidity of the supporting ground. This motorized treadmill allows subjects to sprint and produce realistic acceleration and high running velocities (Morin et al., 2010). A single-pass waist and a stiff rope (1 cm in diameter, ∼2 m length) were used to tether subjects to the 0.4-m vertical rail anchored to the wall behind them. When correctly attached, subjects were required to lean forward in a typical and standardized crouched sprint-start position with their left foot forward. This starting position was used and standardized all along the sprint series. After a 5-s countdown ("5 s, 3-2-1-Go" given by both visual and audio instructions by

the same investigator), the treadmill was released, and the belt began to accelerate as subjects applied a positive horizontal force.

Mechanical Variables

Data were continuously sampled at 1000 Hz over the sprints, and after appropriate filtering (Butterworth-type 30 Hz low-pass filter), instantaneous data of vertical, net horizontal and total (i.e., resultant) ground reaction forces were averaged for each support phase (vertical force above 30 N) over the 5-s sprints, and expressed in body weight (BW). These data were completed by measurements of the main step kinematic variables: contact time, aerial time, step frequency, and step length.

Electromyography

EMG signals from superficial *Rectus femoris*, *Vastus lateralis, Biceps femoris*, *Gastrocnemius medialis*, *Gastrocnemius lateralis*, and *Tibialis anterior* of the right lower limb were recorded using pre-amplified bi-polar surface EMG (Delsys, Trigno Wireless, Boston, Massachusetts, USA) with an inter-electrode (center-to-center) distance of 20 mm and placed according to the surface electromyography for the non-invasive assessment of muscles (SENIAM) project's recommendations. Before electrode placement, the skin was lightly abraded and washed to remove surface layers of dead skin, hair, and oil. All electrodes were secured with elastic cohesive bandage to reduce any movement of electrodes during sprinting or artifact in the signal. The position of the EMG electrodes was marked with indelible ink to ensure that they were placed in the same location during subsequent visits. The myoelectric signal was amplified (gain = 1000×) and filtered (bandwidth frequency = 20–450 Hz) to minimize extraneous noise and possible movement artifacts in the low-frequency region and to eliminate aliasing and other artifacts in the high-frequency region. Surface EMG signals were recorded continuously during each 5-s sprint with a sampling frequency of 2000 Hz using a dedicated acquisition system (CED 1401, Cambridge Electronic Design, Cambridge, UK) and analyzed offline (Spike2 v3.21; Cambridge Electronic Design, Cambridge, UK). The activity of each muscle was determined by measuring the mean value of the RMS signal between the onset and the end of the burst for each 5-s sprint. For each individual, a burst of muscle activity was identified as the amplitude of muscle activity exceeding 15% of peak activation for more than 10% of the stride (Brocherie et al., 2015). To investigate the difference in EMG frequency between the three conditions, the filtered EMG data from each sprint were further transformed into the frequency domain using a fast Fourier transformation and the median power frequency (MPF) of the resulting power spectrum density was calculated (Matsuura et al., 2006). The RMS and MPF were normalized to the first sprint value of each condition, which was assigned the value of 100% (Mendez-Villanueva et al., 2012; Brocherie et al., 2015).

Responses to Exercise

Heart rate (HR) and pulse O_2 saturation (SpO_2) were monitored and estimated, respectively, via a Polar transmitter-receiver (Wearlink T-31, Polar Electro Oy, Kempele, Finland) and non-invasive pulse oximetry using a finger probe (Palmsat 2500, NONIN Medical Inc., Plymouth, MI, USA). The subjects were unable to view any of the HR or SpO_2 values since receivers were attached on the handrails of the treadmill facing one experimenter. Together with HR and SpO_2, ratings of perceived exertion (RPE) were recorded using the Borg 6–20 scale (i.e., 6 = no exertion at all, 20 = maximal exertion) exactly 10 s following each sprint (i.e., peak values likely to be obtained), where subjects were instructed to reflect on their perception of overall peripheral discomfort during the preceding exercise bout (Christian et al., 2014). In addition, SpO_2 was recorded between before the warm-up and 4 min into recovery between the two repeated-sprint sets. A capillary blood sample was taken from the fingertip and analyzed for blood lactate concentration with a portable analyzer (Lactate Pro LT-1710, Arkray, Japan) before the warm-up, 2 min after the first set of 8 sprints and 2 min after the second set of 4 sprints.

Data Analysis and Statistics

Subjects completed between 15 and 18 steps during each 5-s sprint. After excluding the last two ground contacts, the remaining three consecutive steps were used for final analysis of sprint kinetics/kinematics (Brocherie et al., 2015). While subjects performed a total of 12 sprints, only responses to exercise, running mechanical and surface EMG data collected for sprint number 1, 4, 8, 9, and 12 were considered for the main analysis. For the main running mechanical variables, the average of sprints number 1–4, 5–8, and 9–12 have also been compared.

Values are expressed as mean ± SD. Two-Way repeated-measures analysis of variance (ANOVAs) [Time (Sprints 1, 4, 8, 9, and 12 or Sprints number 1–4, 5–8, and 9–12) × Condition (SL, MH, and SH)] were used to compare investigated variables. To assess assumptions of variance, Mauchly's test of sphericity was performed using all ANOVA results. A Greenhouse-Geisser correction was performed to adjust the degree of freedom if an assumption was violated, while a Bonferroni *post-hoc* multiple comparison was performed if a significant main effect was observed. For each ANOVA, partial eta-squared was calculated as measures of effect size. Values of 0.01, 0.06, and above 0.14 were considered as small, medium, and large, respectively. All statistical calculations were performed using SPSS statistical software V.21.0 (IBM Corp., Armonk, NY, USA). The significance level was set at $P < 0.05$.

Results

Responses to Exercise

Responses to exercise across the three conditions are depicted in **Figure 1**. During the initial set of sprints, SpO_2 was significantly reduced for each simulated altitude ascent ($P < 0.05$). Lower SpO_2 values were recorded for both sprints 4 and 8 (no difference) vs. sprint 1 in MH and SH, while no change occurred at SL. In response to sprint 1, HR was significantly higher in MH and SH compared to SL ($P < 0.05$), while RPE values were similar. Both HR and RPE increased significantly from sprint 1–4 ($P < 0.05$), while only RPE further increased at sprint 8 in reference to sprint 4 ($P < 0.05$), yet with similar values across conditions. Compared to prior to the warm-up (96.9 ±

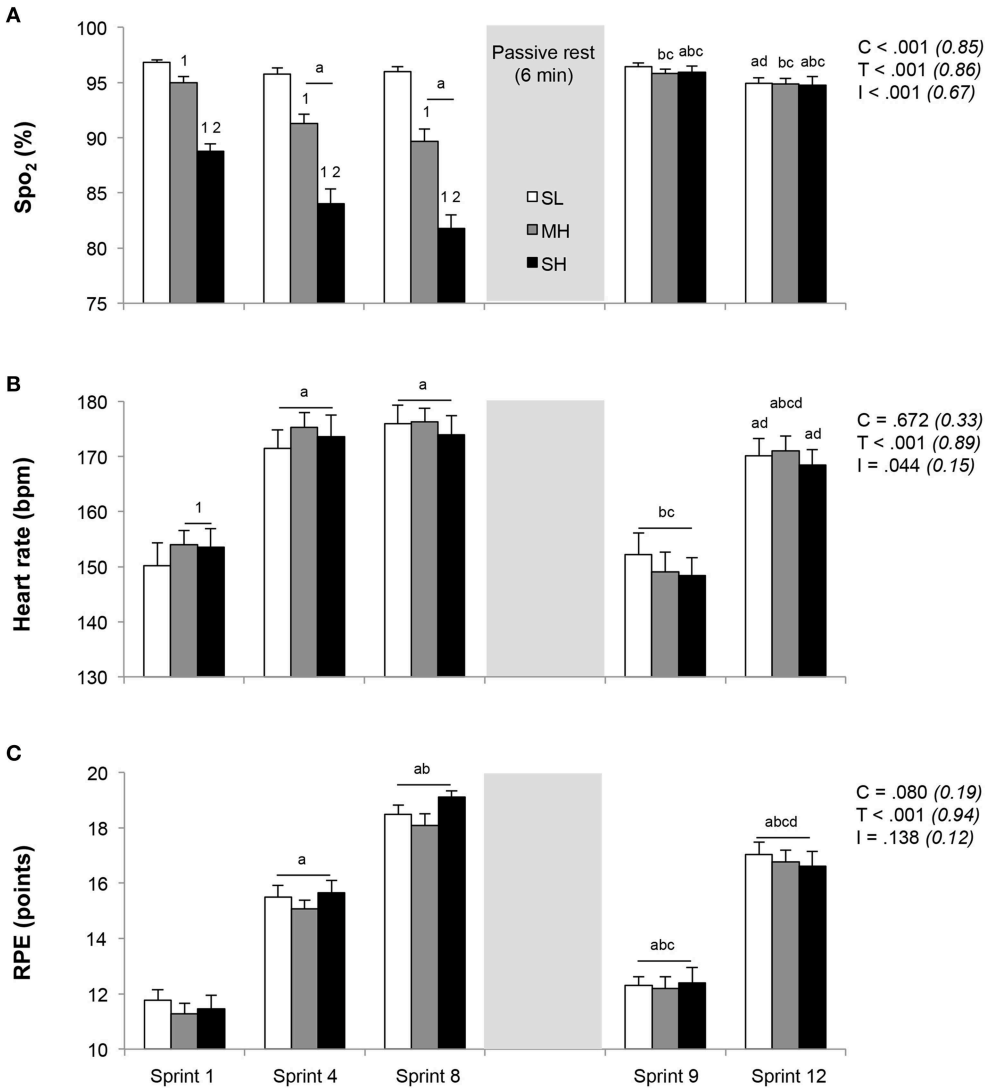

FIGURE 1 | Changes in exercise responses (A, SpO_2; B, heart rate; C, RPE). Mean ± SD ($n = 13$). The repeated-sprint exercise protocol included a first set of eight sprints performed at sea level (SL), moderate (MH), or severe hypoxia (SH), while the second set of four sprints was always performed at SL. SpO_2, arterial oxygen saturation (estimated by pulse oxymetry); RPE, rating of perceived exertion. C, T, and I, respectively refer to ANOVA main effects of condition, time, and interaction between these two factors with *P*-value and partial eta-squared into brackets. [a], [b], [c], and [d] significantly different from sprint 1, 4, 8, and 9, respectively ($P < 0.05$). [1] and [2] significantly different from SL and MH, respectively ($P < 0.05$).

0.4%), SpO_2 were not different among conditions 4 min into recovery between the two repeated-sprint sets (96.2 ± 0.5%; all conditions compounded, $P > 0.05$). During sprint 9, after 6 min of rest, SpO_2, HR, and RPE values recovered significantly in relation to those achieved in sprint 4 and 8 ($P < 0.05$), with no difference between conditions. Whereas RPE values remained elevated compared to those measured in response to sprint 1, HR values recorded after sprint 1 and 9 were not different. At sprint 12, HR, and RPE values did not differ between conditions, while RPE was larger than in sprint 4 ($P < 0.05$).

From pre- to +2 min post-set 1, the execution of the initial set of 8 sprints resulted in similar increases in blood lactate concentration (SL: 1.4 ± 0.4 vs. 9.9 ± 1.7 mmol.L^{-1}, MH: 1.4 ± 0.4 vs. 10.4 ± 1.8 mmol.L^{-1}, and SH: 1.4 ± 0.4 vs. 10.7 ± 2.1 mmol.L^{-1}; $P < 0.001$), irrespectively of the environmental condition. There was a further global increase of blood lactate concentration values (10.8 ± 1.9, 11.2 ± 1.7 and 10.6 ± 2.2 mmol.L^{-1} in SL, MH, and SH, respectively; $P<0.05$) recorded +2 min post-set 2 (i.e., after the completion of 4 additional normoxic sprints) in reference to post-set 1.

Sprint Performance and Running Kinetics

Distance ran and associated running kinetics during the repeated-sprint exercise are displayed in **Figure 2**. No difference was found in distance ran during the first sprint between SL, MH and SH (24.2 ± 1.4, 24.1 ± 1.5, and 24.2 ± 2.0 m, respectively).

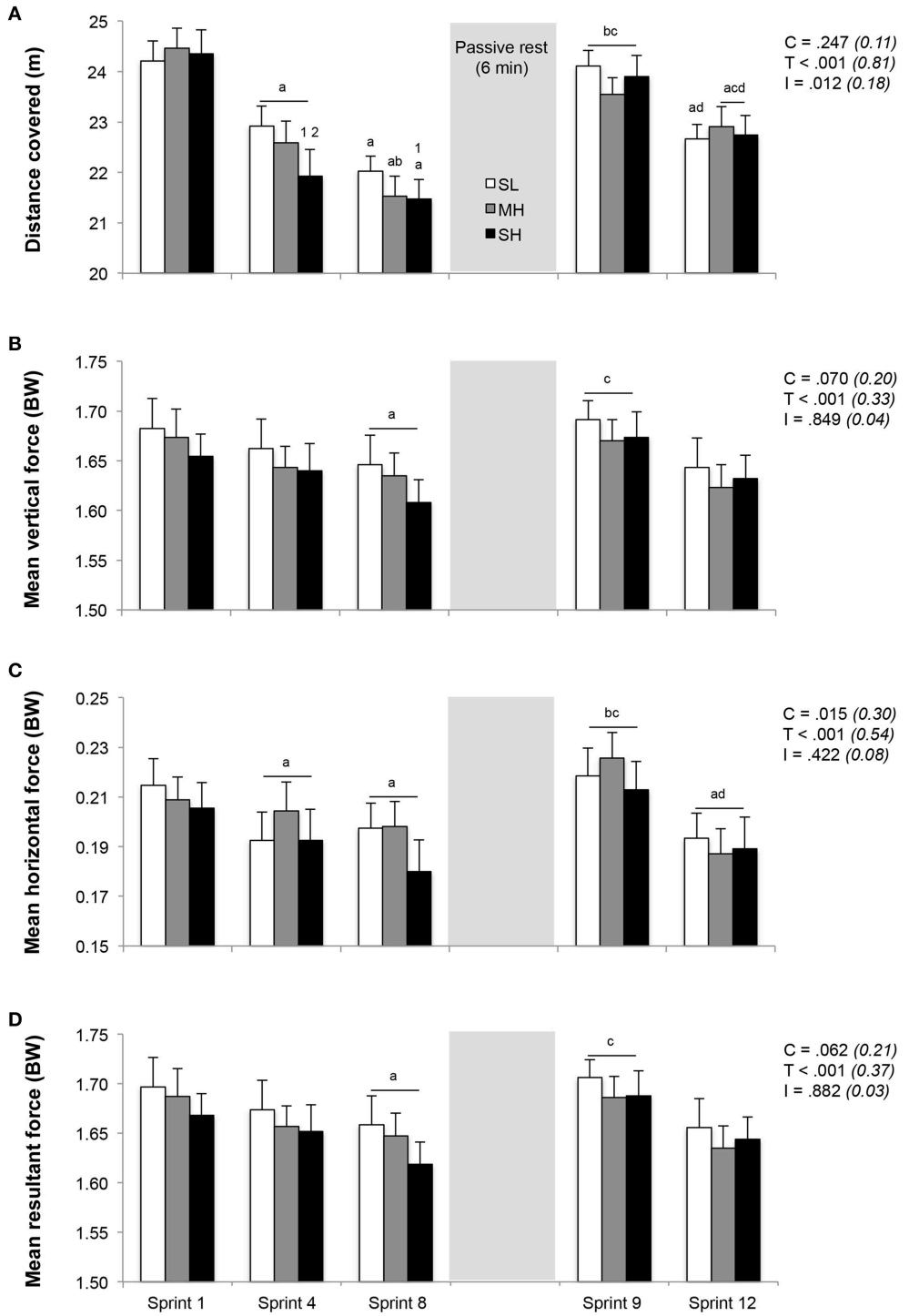

FIGURE 2 | Changes in distance covered (A) and stride kinetics (B, mean vertical force; C, mean horizontal force; D, mean resultant force). Mean ± SD ($n = 13$). The repeated-sprint exercise protocol included a first set of eight sprints performed at sea level (SL), moderate (MH), or severe hypoxia (SH), while the second set of four sprints was always performed at SL. C, T, and I, respectively refer to ANOVA main effects of condition, time and interaction between these two factors with *P*-value and partial eta-squared into brackets. [a], [b], [c], and [d] significantly different from sprint 1, 4, 8, and 9, respectively ($P < 0.05$). [1] and [2] significant different from SL and MH, respectively ($P < 0.05$).

However, sprint performance decreased to a larger extent in SH compared to SL, as evidenced by larger reductions in distance ran during sprint 4 ($-9.9 \pm 5.2\%$ vs. $-5.3 \pm 2.8\%$; $P < 0.05$) and 8 ($-11.7 \pm 5.2\%$ vs. $-8.9 \pm 4.1\%$; $P < 0.05$) in reference to sprint 1. Horizontal, but not vertical and total forces, significantly decreased from sprint 1 to 4 ($P < 0.05$). During sprint 8, values

for vertical, horizontal and total forces were significantly lower (all conditions pooled; −2.3±1.9%, −8.6±6.5%, and −2.4±1.9%, respectively; $P < 0.05$) in reference to sprint 1.

During sprint 9, following 6 min of rest, sprint performance and running kinetics recovered significantly, as evidenced by larger values compared to those reached during sprint 8 (distance ran and horizontal forces; $P < 0.05$). Sprint 9 values did not differ from those achieved during sprint 1 and were similar between conditions. Over the last 4 sprints (sprint 9–12), distance ran and horizontal forces decreased similarly by an average of −4.5 ± 2.5% and −13.1±9.6% (all conditions pooled; $P < 0.05$), while the decrease in vertical forces (−2.6±4.9%) and total forces (−2.8 ± 4.9%) were not significant.

Running Kinematics

Running kinematics across the repeated sprints are displayed in **Figure 3**. Whereas step length remained unchanged, both contact and aerial times lengthened and step frequency decreased from sprint 1 to 4. During sprint 4, the increase in contact time and the decrease in step frequency were significantly larger in SH compared to MH ($P < 0.05$). From sprint 1 to sprint 8, the increase in contact time (+14.5 ± 6.1% vs. +11.2 ± 6.8% and +12.4 ± 5.1%; $P < 0.05$) and decrease in step frequency (−9.7 ± 4.2% vs. −7.2 ± 3.7% and −8.1 ± 2.7%; $P < 0.05$) were larger in SH compared to SL and MH. Independently of the condition, aerial time lengthened (+4.2 ± 2.9%; $P < 0.05$) and step length decreased (−2.5 ± 3.0%; $P < 0.05$) from sprint 1 to 8. After 6 min of rest between sprints 8 and 9, sprint kinematic values during sprint 9 were not statistically different from those recorded during sprint 1, with also no significant difference between conditions. During subsequent sprints (9–12), irrespectively of the condition, contact time (+10.2 ± 5.2%) and aerial time (+3.8 ± 3.3%) lengthened ($P < 0.05$), step frequency (−6.2 ± 2.6%) decreased ($P < 0.05$) and step length (+1.2 ± 3.0%) remained unchanged.

Compared to SL and MH, contact time and step frequency values corresponding to the average of sprints 1 to 4 and sprints 5 to 8 differed under SH (**Table 1**; $P < 0.05$). The averaged values of distance covered, kinetics and kinematics for sprints 9–12 were similar across conditions and were not statistically different than the average of sprints 1–4.

Surface EMG Activity

Temporal profiles of the EMG amplitude (RMS) and frequency spectrum (MPF) for the six investigated muscles are shown in **Tables 2**, **3**. With the exception of *Rectus femoris* RMS activity displaying lower values in SH compared to SL for sprint 8 ($P < 0.05$), all other investigated muscles RMS and MPF values fell significantly over time ($P < 0.05$), independently of the condition. The decrease in RMS activity from sprint 1 to 4, expressed as a percentage of sprint 1 value, was significant for *Vastus lateralis*, *Rectus femoris*, *Gastrocnemius lateralis,* and *Tibialis anterior* muscles ($P < 0.05$), while all muscles displayed lower values in all conditions during sprint 8 (**Table 2**). After 6 min of rest, a recovery in the RMS activity of all muscles (except for *Biceps femoris*) occurred during sprint 9, which was not statistically different than that in sprint 1. During sprint 12, RMS activities for all muscles were lower than those of sprints 4 (except for *Biceps femoris*) and 9 ($P < 0.05$). When compared to sprint 1 (100%), MPF values were reduced during sprint 8 for *Vastus lateralis* and *Rectus femoris*, during sprint 9 for *Gastrocnemius lateralis* and *Tibialis anterior* and during sprint 12 for *Biceps femoris* and *Gastrocnemius medialis* (**Table 3**; $P < 0.05$).

Discussion

Different Levels of Acute Hypoxia Alter RSA and Neuro-mechanical Adjustments

SpO_2 values were increasingly lower as O_2 availability decreased, yet cardio-vascular (HR) and perceptual (RPE) loads associated with performing repeated treadmill sprints were not incrementally higher, which may be due in part to the lower work performed at SH and the "all out" nature of the present exercise (Balsom et al., 1994). Hence, fatigue-induced decrement in sprint distance was significantly exacerbated in SH relative to SL, while sprint performance was relatively resilient to MH exposure. Single (i.e., sprint 1 in the present study) sprint performance is known to be unaffected by differing hypoxia levels (Billaut et al., 2013). For instance, treadmill sprint performance for efforts lasting 60 s or less is not adversely affected at altitude ($FiO_2 = 13\%$) (Weyand et al., 1999). This may relate to an enhanced anaerobic energy release to compensate for the reduced aerobic ATP production (Calbet et al., 2003; Ogawa et al., 2007). However, earlier and larger performance decrements usually occur when consecutive sprints are performed in O_2-deprived environments with hypoxia-related effects becoming more evident above 3000 m (Bowtell et al., 2014; Goods et al., 2014).

During set 1 of all trials, the temporal aspects of the stride cycles shifted toward an increase in contact and aerial times, along with reductions in step frequency. Collectively, it demonstrates a deteriorated ability to tolerate ground impact/stretch loads as fatigue develops with sprint repetitions. In line with these findings, similar impairments in sprint kinematics have been connected with progressively slower sprint performance during over-ground [i.e., 6 × 20 m – 20 s of passive recovery in U19 footballers (Girard et al., 2011a); 6 × 35 m – 10 s of passive recovery in elite footballers (Brocherie et al., 2015); 12 × 40 m –30 s of passive recovery in team- and racquet-sports athletes (Girard et al., 2011b)] or treadmill [i.e., 5 × 5-s sprints–25 s of passive recovery (Girard et al., 2015a); 3 sets of 5 × 6-s sprints–24 s of passive recovery between sprints and 3 min between sets (Morin et al., 2011) in athletes with a team-sport background] repeated sprints. Furthermore, the larger magnitude of repeated-sprint performance alterations seen at SH compared to SL and MH was due to exacerbated increases in contact time and decreases in step frequency in the severer hypoxic condition. Slower sprints and less efficient stride characteristics in SH compared to SL or MH appear to be the result of individuals applying less forward-oriented forces. In line with previous literature (Morin et al., 2011), our primary biomechanical rationale for this conclusion is based on the fact that the magnitude of reductions for horizontal forces was three times larger than for resultant (total) forces.

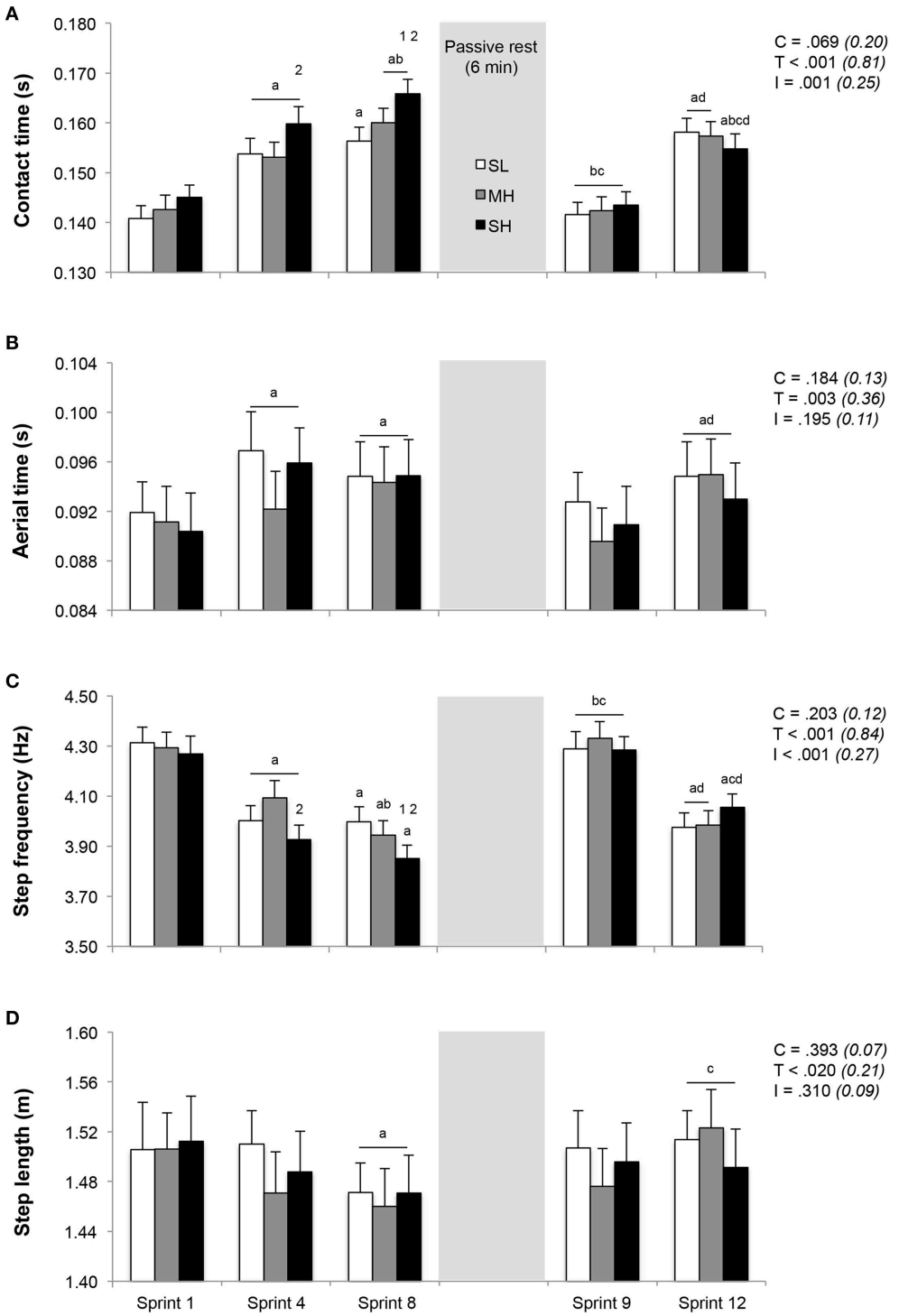

FIGURE 3 | Changes in stride kinematics (A, contact time; B, aerial time; C, step frequency; D, step length). Mean ± SD ($n = 13$). The repeated-sprint exercise protocol included a first set of eight sprints performed at sea level (SL), moderate (MH) or severe hypoxia (SH), while the second set of four sprints was always performed at SL. C, T, and I, respectively refer to ANOVA main effects of condition, time and interaction between these two factors with *P*-value and partial eta-squared into brackets. [a], [b], [c], and [d] significantly different from sprint 1, 4, 8, and 9, respectively ($P < 0.05$). [1] and [2] significant different from SL and MH, respectively ($P < 0.05$).

Remarkably, most of the alteration in performance and accompanying running mechanics was observed within the first half of the first set (sprints 1–4) with smaller changes during the second part (sprints 5–8). During the completion of ten, 10-s sprints with 180 s of recovery the rate of decline in total work was also greater during the first 5 sprints compared to the last

TABLE 1 | Sprint performance, kinetics, and kinematics averaged for sprints 1–4, 5–8, and 9–12.

Variables	Average of sprints			ANOVA *p*-value *(partial eta-squared)*		
	1–4	5–8	9–12	Condition	Time	Interaction
DISTANCE (M)						
SL	23.48 ± 1.33	22.32 ± 1.06	23.32 ± 1.53	< 0.001	0.091	0.119
MH	23.31 ± 1.36	22.04 ± 1.32	23.24 ± 1.36	*(0.81)*	*(0.18)*	*(0.14)*
SH	22.92 ± 1.51	21.71 ± 1.27	23.30 ± 1.29			
VERTICAL FORCE (BW)						
SL	1.67 ± 0.09	1.65 ± 0.09	1.67 ± 0.10	0.003	0.208	0.292
MH	1.67 ± 0.09	1.64 ± 0.08	1.65 ± 0.09	*(0.39)*	*(0.12)*	*(0.10)*
SH	1.66 ± 0.08	1.63 ± 0.09	1.66 ± 0.09			
HORIZONTAL FORCE (BW)						
SL	0.20 ± 0.04	0.20 ± 0.04	0.20 ± 0.04	0.004	0.013	0.418
MH	0.21 ± 0.04	0.20 ± 0.03	0.20 ± 0.04	*(0.37)*	*(0.31)*	*(0.07)*
SH	0.20 ± 0.04	0.18 ± 0.04	0.20 ± 0.04			
RESULTANT FORCES (BW)						
SL	1.69 ± 0.09	1.66 ± 0.09	1.68 ± 0.10	0.003	0.178	0.385
MH	1.68 ± 0.09	1.65 ± 0.08	1.67 ± 0.09	*(0.38)*	*(0.13)*	*(0.08)*
SH	1.67 ± 0.08	1.64 ± 0.09	1.68 ± 0.09			
CONTACT TIME (S)						
SL	0.148 ± 0.010	0.155 ± 0.008[a]	0.151 ± 0.011	<0.001	0.015	<0.001
MH	0.148 ± 0.010	0.158 ± 0.009[a]	0.150 ± 0.010[ab]	*(0.82)*	*(0.30)*	*(0.36)*
SH	0.152 ± 0.010[12]	0.163 ± 0.010[a12]	0.150 ± 0.009[ab]			
AERIAL TIME (S)						
SL	0.095 ± 0.010	0.096 ± 0.011	0.094 ± 0.010	0.026	0.583	0.886
MH	0.093 ± 0.010	0.095 ± 0.009	0.093 ± 0.010	*(0.26)*	*(0.04)*	*(0.02)*
SH	0.094 ± 0.010	0.096 ± 0.011	0.093 ± 0.010			
STEP FREQUENCY (HZ)						
SL	4.14 ± 0.22	4.01 ± 0.21[a]	4.11 ± 0.24[b]	<0.001	0.039	0.002
MH	4.17 ± 0.24	3.98 ± 0.22[a]	4.14 ± 0.25[b]	*(0.80)*	*(0.24)*	*(0.30)*
SH	4.07 ± 0.22[12]	3.88 ± 0.17[a1]	4.14 ± 0.21[b]			
STEP LENGTH (M)						
SL	1.51 ± 0.11	1.48 ± 0.09	1.51 ± 0.12	0.008	0.410	0.987
MH	1.49 ± 0.11	1.47 ± 0.10	1.50 ± 0.11	*(0.33)*	*(0.07)*	*(0.01)*
SH	1.49 ± 0.11	1.47 ± 0.10	1.50 ± 0.11			

Mean ± SD (n = 13). The repeated-sprint exercise protocol included a first set of eight sprints performed at sea level (SL), moderate (MH), or severe hypoxia (SH), while the second set of four sprints was always performed at SL.

[a,b] *Significant different from average of sprint 1–4 and 5–8, respectively (P < 0.05).*

[1,2] *Significant different from SL and MH, respectively (P < 0.05).*

5 sprints (−5.2% vs. −3.3%) (Pearcey et al., 2015). In this later study, neuromuscular fatigue in the first 5 sprints was mainly peripheral, whereas in the last 5 sprints it was both peripheral and central. By assessing the development of fatigability during repeated-sprint running exercise (12 × 30 m–30 s rest), it has also been reported that significant peripheral and central knee extensor fatigue becomes evident after just two maximal sprints (Goodall et al., 2015). In our study, the etiology of neuromuscular fatigability (i.e., using peripheral and/or magnetic stimulations) during or after repeated sprinting has not been specifically investigated. Using such stimulation procedure and exposure to acute moderate hypoxia (i.e., FiO_2 = 13.8%; Billaut et al., 2013) or the induction of pre-existing locomotor muscle fatigue (i.e., following a 10-min neuromuscular electrical stimulation protocol of the quadriceps; Hureau et al., 2014) it was, however, evidenced that feedbacks from fatiguing muscles play an important role in the determination of central motor drive and force output during RSA protocols; i.e., the development of peripheral muscle fatigue would be confined to a certain level so as not to surpass a sensory tolerance limit.

During the first repeated-sprint set, RMS activity values of all investigated muscles decreased significantly over time, confirming that neural factors may have played a role in fatigue-related decrement in sprint performance (Bowtell et al., 2014;

TABLE 2 | Surface EMG root mean square (RMS) activity.

Variables (% sprint 1)	Sprints				ANOVA *p*-value *(partial eta-squared)*		
	4	8	9	12	Condition	Time	Interaction
RMS *vastus lateralis*							
SL	90.9 ± 9.5[a]	85.0 ± 17.0[a]	92.4 ± 16.8[c]	86.1 ± 17.2[ad]	0.987	<0.001	0.923
MH	89.2 ± 11.7[a]	86.3 ± 11.7[a]	94.3 ± 9.4[c]	84.9 ± 14.4[ad]	*(0.01)*	*(0.52)*	*(0.03)*
SH	88.8 ± 11.8[a]	83.0 ± 13.1[a]	96.1 ± 5.8[c]	84.8 ± 6.0[ad]			
RMS *rectus femoris*							
SL	88.8 ± 9.2[a]	84.8 ± 9.1[ab]	98.1 ± 11.7[c]	88.1 ± 9.9[ad]	0.166	<0.001	0.036
MH	96.8 ± 10.2[a]	85.5 ± 13.3[ab]	94.6 ± 14.2[c]	84.3 ± 17.0[ad]	*(0.14)*	*(0.67)*	*(0.15)*
SH	84.1 ± 10.6[a]	75.5 ± 9.9[ab1]	91.1 ± 13.7[c]	81.8 ± 12.4[ad]			
RMS *biceps femoris*							
SL	92.9 ± 10.4	87.1 ± 13.3[a]	94.7 ± 9.0[a]	85.1 ± 18.6[abd]	0.906	<0.001	0.535
MH	98.9 ± 9.2	91.6 ± 9.2[a]	90.2 ± 12.9[a]	85.4 ± 15.3[abd]	*(0.01)*	*(0.53)*	*(0.06)*
SH	93.3 ± 9.4	89.2 ± 9.5[a]	94.2 ± 11.7[a]	85.6 ± 15.5[abd]			
RMS *gastrocnemius medialis*							
SL	93.8 ± 5.9[a]	86.2 ± 10.2[ab]	99.6 ± 9.9[c]	91.9 ± 18.2[acd]	0.336	<0.001	0.553
MH	93.2 ± 11.2[a]	82.6 ± 13.9[ab]	92.4 ± 12.9[c]	84.5 ± 18.9[acd]	*(0.09)*	*(0.61)*	*(0.06)*
SH	90.3 ± 9.7[a]	81.9 ± 8.4[ab]	95.7 ± 9.5[c]	91.5 ± 11.6[acd]			
RMS *gastrocnemius lateralis*							
SL	94.5 ± 12.0	86.8 ± 10.4[ab]	98.0 ± 8.8[c]	88.3 ± 13.1[ad]	0.725	<0.001	0.657
MH	95.4 ± 10.3	82.5 ± 17.1[ab]	99.1 ± 14.9[c]	87.4 ± 13.6[ad]	*(0.02)*	*(0.63)*	*(0.05)*
SH	89.7 ± 12.1	83.0 ± 13.8[ab]	97.6 ± 8.1[c]	88.2 ± 13.4[ad]			
RMS *tibialis anterior*							
SL	85.6 ± 13.2[a]	72.5 ± 13.9[ab]	98.4 ± 10.8[c]	83.4 ± 16.1[ad]	0.302	<0.001	0.039
MH	87.3 ± 10.4[a]	84.4 ± 15.5[ab]	96.6 ± 10.8[c]	85.1 ± 10.8[ad]	*(0.10)*	*(0.71)*	*(0.15)*
SH	83.9 ± 13.3[a]	76.3 ± 13.2[ab]	90.0 ± 10.0[c]	80.5 ± 13.7[ad]			

Mean ± SD (n = 13). The repeated-sprint exercise protocol included a first set of 8 sprints performed at sea level (SL), moderate (MH), or severe hypoxia (SH), while the second set of 4 sprints was always performed at SL.

[a], [b], [c], and [d] significant different from sprint 1, 4, 8, and 9, respectively ($P < 0.05$).

[1] significant different from SL ($P < 0.05$).

Brocherie et al., 2015). This emphasis a decreased number of motor units activated and/or firing rates of the recruited motor units in exercising quadriceps and plantar flexor muscles, yet with no possible distinction between these two phenomena. Our results also feature an earlier and larger central down-regulation of skeletal muscle recruitment in SH compared to SL or MH, even though this observation is restricted to the *Rectus femoris* muscle only. Exacerbated performance decrements under severe hypoxia are likely to be explained by a reduced neural drive to the active musculature, arising secondary to a stronger reflex inhibition due to brain hypoxia (i.e., decreased brain oxygenation independently of afferent feedback and peripheral fatigue; Millet et al., 2012a) or a hypoxia-induced increased level of intramuscular metabolites known to stimulate group III-IV muscle afferents (Hogan et al., 1999). Although, hypoxia exposure would exacerbate exercise-induced demand placed upon the central nervous system to explain premature fatigue, it is important to emphasize that local metabolic factors (not measured here) may also be responsible for the greater fatigue incurred in SH vs. other conditions.

Reductions in MPF during exercise are indicative of a slowdown of muscle fiber action potential conduction velocity (Lindstrom et al., 1970). In the present study, the values of MPF from the *Vastus lateralis* and *Rectus femoris* muscles during sprint 8 were significantly lower than in sprint 1, while there was no condition effect. This result differs from that of Matsuura et al. (2006) who suggested, based on lower MPF values during repeated cycling sprints with 35-s vs. 350-s recovery periods, that a severer metabolic state (i.e., increased hypoxia severity) induces preferred recruitment of slow twitch motor units.

Restoration of Sprint Mechanical Performance between Repeated-sprint Sets

Conceivably, perceptual recovery, which is known to interact with both feed-forward/feed-back mechanisms, may well affect athlete's willingness to maintain maximal efforts during successive sprint actions. Despite distance covered and resulting HR not being different, RPE values were elevated during the second compared to the first repeated-sprint set at similar time points (sprint 9 vs. 1 and 12 vs. 4). This indicates that perception of peripheral discomfort may not be the major performance regulator during RSA running protocols. Also in line with this assumption are the well-preserved quadriceps muscle activation and associated power output that occurred during two 4-s maximal cycling bouts under hypoxic (FiO_2 =

TABLE 3 | Surface EMG median power frequency (MPF).

Variables (% sprint 1)	Sprints				ANOVA *p*-value *(partial eta-squared)*		
	4	8	9	12	Condition	Time	Interaction
RMS *vastus lateralis*							
SL	96.1 ± 10.0[a]	94.2 ± 13.0	97.7 ± 10.9	94.3 ± 11.9	0.977	0.024	0.923
MH	95.4 ± 13.0[a]	95.0 ± 12.6	98.0 ± 12.3	93.9 ± 16.0	*(0.02)*	*(0.21)*	*(0.03)*
SH	99.2 ± 8.4[a]	93.2 ± 10.0	94.4 ± 13.8	92.9 ± 14.4			
RMS *rectus femoris*							
SL	99.9 ± 5.2[a]	96.4 ± 7.6[ab]	98.8 ± 8.6[c]	99.0 ± 10.6	0.186	<0.001	0.167
MH	89.6 ± 10.9[a]	88.8 ± 8.8[ab]	97.5 ± 11.6[c]	95.1 ± 14.1	*(0.14)*	*(0.42)*	*(0.13)*
SH	94.1 ± 10.3[a]	87.6 ± 13.1[ab]	94.2 ± 13.2[c]	89.3 ± 16.4			
RMS *biceps femoris*							
SL	96.2 ± 7.2	95.9 ± 10.9	90.0 ± 16.4	90.1 ± 18.7	0.208	0.046	0.509
MH	99.3 ± 12.2	94.8 ± 13.0	95.1 ± 15.6	94.4 ± 11.8	*(0.12)*	*(0.23)*	*(0.07)*
SH	105.1 ± 6.1	101.7 ± 10.8	94.2 ± 11.2	97.1 ± 14.9			
RMS *gastrocnemius medialis*							
SL	98.7 ± 9.3	99.4 ± 9.2	92.9 ± 12.3	93.3 ± 8.8[a]	0.886	0.027	0.341
MH	94.8 ± 7.4	92.7 ± 10.6	96.0 ± 11.1	95.2 ± 11.5[a]	*(0.01)*	*(0.20)*	*(0.09)*
SH	97.8 ± 10.0	97.4 ± 10.9	95.0 ± 9.8	94.3 ± 10.0[a]			
RMS *gastrocnemius lateralis*							
SL	96.8 ± 5.1	99.9 ± 9.3	95.1 ± 7.7[a]	98.9 ± 12.2	0.264	0.011	0.607
MH	97.4 ± 6.3	94.4 ± 11.5	94.9 ± 12.6[a]	94.1 ± 8.3	*(0.11)*	*(0.23)*	*(0.06)*
SH	95.8 ± 9.4	94.6 ± 10.7	91.7 ± 12.3[a]	91.2 ± 11.2			
RMS *tibialis anterior*							
SL	97.2 ± 9.3	99.7 ± 10.8	96.3 ± 6.9	98.2 ± 11.5	0.663	0.011	0.605
MH	97.5 ± 5.9	94.5 ± 10.1	96.3 ± 15.8	93.3 ± 13.9	*(0.03)*	*(0.23)*	*(0.06)*
SH	97.9 ± 5.6	95.7 ± 11.0	91.8 ± 8.4	96.7 ± 6.5			

Mean ± SD (n = 13). The repeated-sprint exercise protocol included a first set of 8 sprints performed at sea level (SL), moderate (MH) or severe hypoxia (SH), while the second set of 4 sprints was always performed at SL.

[a], [b], and [c] significant different from sprint 1, 4, and 8, respectively ($P < 0.05$).

13%) and normoxic conditions, despite higher overall perceived peripheral discomfort and perceived difficulty breathing (Christian et al., 2014). Nevertheless, the role of effort perception during recovery should not be disregarded as, for instance, the magnitude of the core temperature decrease and the subjective perception of recovery following cold water immersion after an intense conditioning session have been related to performance enhancement in a repeated 40 m sprint protocol undertaken 24 h later (Cook and Beaven, 2013). Nonetheless, future studies should isolate perceptual responses to recovery as mitigating of improved performance.

After the 6 min of passive rest between sprint 8 and sprint 9, the temporal aspects of the stride cycle (contact and aerial times, step frequency and step length values) and force production characteristics (mean horizontal and resultant forces) during sprint 9 recovered from those recorded during sprint 8 and were not different from sprint 1. When physical education students performed four sets of five, 6-s sprints (24 s of passive recovery between sprints, 3 min of rest between sets), Morin et al. (2011) observed that the level of performance was almost systematically higher at the beginning of sets 2, 3, and 4 than at the end of sets 1, 2, and 3. Thus, to preserve RSA performance it is practically important to apply large forward-oriented total force against the ground and minimize the decrease in step frequency (i.e., increase in contact time). Furthermore, despite differing hypoxic severity levels during sprints 1–8, distance covered as well as the main kinetic and kinematic variables measured at sprint 9 were restored near sprint 1 in all conditions. Interestingly, SpO_2 values recorded for sprint 9 were similar to those of sprint 1 in the SL condition, with also no difference between conditions for the average of the four sprint repetitions of the second normoxic set. In fact, restoration of SpO_2 levels near baseline SL values is virtually complete after 6 min of normoxic exposure. Collectively, it shows that hypoxia level of an initial sprint bout may not blunt the post-exercise recovery of single and repeated-sprint performance and its mechanical basis.

Restoration of EMG indices (RMS and MPF values) appear to align with sprint mechanical performance recovery between sprints 8 and 9, with no difference when comparing initial efforts of the two repeated-sprint sets (sprints 1 vs. 9). This reinforces that the ability to fully activate the contracting musculature and/or optimal inter-muscle recruitment strategies are important regulators of RSA. These results, however, are in disagreement with those reporting that EMG amplitude remained depressed (∼12%), after 6 min of rest, during the initial repetition of

the second exercise set, despite mechanical performance being matched for first sprint of the two repeated-sprint series (Mendez-Villanueva et al., 2007). Compared to normoxia, cycling performance and quadriceps muscle activation during a multiple sets RSA protocol (three sets of five 5-s cycling sprints with 25 s of passive recovery between sprints and 120 s of rest between sets) was lower in moderate hypoxia (FiO_2 ~0.14), with also incomplete apparent recoveries of performance between the last repetition of sets 1 and 2 (i.e., sprints 5 and 10) and the initial repetition of sets 2 and 3 (i.e., sprints 6 and 11) (Billaut et al., 2013). Accordingly, it is difficult to directly compare our results with other experimental/environmental conditions. In the present study, restoration of sprint mechanical performance following prior repeated sprints at differing hypoxia severity resulted from the recovery of muscle recruitment patterns, which implicates a role for central mechanisms in the regulation of post-exercise recovery. However, the role of peripheral recovery should not be overlooked since it was demonstrated, using a similar exercise protocol, that phosphocreatine re-synthesis was associated with total work done during the first sprint of the second set ($r = 0.79$, $P < 0.05$) and total work done during the five sprints of the second set ($r = 0.67$, $P < 0.05$) (Mendez-Villanueva et al., 2012).

Hypoxia has no Residual Effect during a Subsequent Normoxic Repeated-sprint Performance

An important determinant of fatigue during repeated sprinting is the initial (i.e., first sprint) mechanical output, which has consistently been positively correlated with performance decrement over subsequent sprints (Girard et al., 2011c). In this study, similar performance was observed during initial sprints of both sets with no difference between conditions. Furthermore, the averaged values of distance covered, kinetics and kinematics for the four sprints of the second set (i.e., sprints 9–12; fatigued muscles) were similar across conditions and were not statistically different in reference to the average of the first four sprints of the initial set (i.e., sprints 1–4; non-fatigued muscles). With this in mind, our results indicate that recent muscle activation (completion of the first set) does not alter the muscle recruitment pattern and fatigability during a second set of repeated sprints completed near sea level after a 6 min (normoxic) resting period. These results contrast with those of Mendez-Villanueva et al. (2007) who indicated that after 6 min of rest following 10, 6-s cycling sprints, participants were able to reproduce during sprint 11 the mechanical performance achieved during sprint 4, but not RSA. In the above study, greater fatigability was evident in the five repetitions of the second (i.e., sprints 11–15) vs. the first set (i.e., sprints 4–8), suggesting different recovery time courses after single sprint and RSA performances. Despite severer hypoxia levels during a first repeated-sprint exercise bout, majoring exercise-induced demands placed on the neuromuscular system (i.e., contact times and step frequencies for sprints 5–8 differed from sprints 1 to 4 and 9 to 12), there was no apparent fatigue-related residual or "carry-over" effects from this previous set. Hence, RSA was similar across conditions during the completion of a second set of normoxic repeated sprints.

With different exercise-to-rest ratios influencing, to a large extent, the oxidative vs. glycolytic component (Tabata et al., 1997), RSA may not be similarly affected by hypoxia exposure, which complicates comparison of our results with those of previous studies. Although, evidence is currently lacking, it is anticipated that narrower exercise-to-rest ratios (1:2–1:4 vs. 1:5 as used here) and severer hypoxic conditions, inducing a decreased O_2 availability and an increased reliance on O_2-independent glycolysis for ATP resynthesis together with a larger recruitment of fast-twitch fibers, may exacerbate sprint performance decrements. While similar blood lactate concentration levels observed here may suggest otherwise, whether glycolytic vs. aerobic contributions actually differed between our three conditions would need to be confirmed from muscle oxygenation, phosphocreatine metabolism and/or pH recordings. RSA protocols using different exercise-to-rest ratios and hypoxia levels in the same group of participants would also be helpful in this instance. The resting period duration between the two sets is obviously a key parameter for any type of multiple repeated-sprint sets. In the "hypoxia-to-normoxia recovery" protocols, this duration is paramount as it determines SpO_2 levels at the start of the second set. In this study, SpO_2 values measured 4 min into recovery between the two repeated-sprint sets returned near baseline and did not differ between conditions. It is, however, likely that SpO_2recovery to initial values was even shorter. Hence, Krivoshchekov et al. (2014) reported that the SpO_2 recovery response after an acute exposure to normobaric hypoxia ($FiO_2 = 0.10$) decreasing SpO_2 to 85% was ~120 s.

Limitations

Before concluding, we must acknowledge several limitations that may affect generalization of our findings. Firstly, although the data were collected continuously (step-by-step), our analysis was concentrated on 3 steps at top speed (i.e., usually corresponding to the 20 m mark of the sprints). It is noteworthy, however, that considering all steps or only a few steps during early, middle or late phases of 5-s sprints provides similar mechanical outcomes during repeated treadmill sprinting, although acceleration induces noticeable differences between the sections studied (Girard et al., 2015a). It must also be appreciated that running speed reached on our treadmill is 15–20% slower than over-ground, even though changes in running mechanics are relatively similar (Rabita et al., 2015) and overall sprint performance is highly correlated between these two sprinting modes (Morin and Sève, 2011). Although, an effect on performance induced by EMG electrodes or mask breathing cannot be completely ruled out, the use of wireless technology and the fact that resistance and increase in dead space are negligible (Sheel, 2002) suggests that their influence did not modify the main findings of the present study.

Secondly, several concerns may affect EMG analysis and include: (1) surface EMG amplitude cancellation; (2) the stability of neuromuscular propagation and sarcolemmal excitability (i.e., absence of supra-maximal stimulation to evoke a M-wave for normalization of the EMG signal); (3)

fatigue-related reflex inhibition (i.e., reflex effects in the spinal cord). With differences <5%, the surface EMG signal may not be sufficiently sensitive to measure meaningful (i.e., clinically relevant) difference in muscle activation between conditions. Therefore, declines in the magnitude of efferent descending motor outflow, as a key factor in neuromuscular recovery following repeated sprints, would need to be confirmed through the use of multiple neurophysiological measures (TMS, EEG) during resting intervals. The kinetics of muscle oxygenation (NIRS) would also be valuable for better comparing the metabolic differences between hypoxic conditions.

Thirdly, we have used three known values of FiO_2 as hypoxic stimulus. For exposure to the same simulated altitude (FiO_2 = 10%), however, it is conceded that there is a larger inter-individual variability in the degree of arterial hypoxemia compared to clamped values of SpO_2 (at 75%) (Hamlin et al., 2010). While clamping of SpO_2 would likely cause a more consistent hypoxic stimulus across individuals, it remains to be demonstrated that it will also induce a better heterogeneity in neuro-mechanical responses to repeated sprinting. Furthermore, hypobaric hypoxia has been shown to induce severer physiological responses (SpO_2 and HR) than normobaric hypoxia (Millet et al., 2012b). One may therefore speculate that the performance and mechanical alterations would be larger at natural altitude than in the present laboratory study. Direct comparisons of repeated sprint exercises between normobaric and hypobaric hypoxia are required.

Finally, in our study, we implemented 6 min of rest between the two sets of repeated treadmill sprints, so as to compare our results with previous findings (Mendez-Villanueva et al., 2007, 2012). However, not only are the acute neuro-mechanical adjustments and the ensuing recovery of SpO_2 and performance influenced by the duration/nature of the between-sets normoxic rest period but also the details of the RSA protocols (e.g., exercise-to-rest ratio, exercise mode, environment encountered; Girard et al., 2011c) and participants' background (e.g., training status, "aerobic" vs. "anaerobic" profile, gender; Calbet et al., 2003). Given the task-dependency of the effects of fatigue, our conclusions must remain specific to the circumstances of this study and would need to be confirmed using other RSA protocols and participants.

Conclusion

To improve our understanding of neuro-mechanical determinants of RSA, we manipulated the hypoxia severity during an initial set of repeated sprints and examined the effect on alterations in performance, running mechanics and lower-limbs neuromuscular activity during a subsequent set completed in normoxia. Under the circumstances of this study (participants' background, exercise-to-rest ratio, hypoxia exposure), the magnitude of performance and neuro-mechanical alterations (kinetics, kinematics, EMG indices) and the severity of physiological and perceptual responses were larger in SH compared to SL and MH. The novel findings from our "recovery of performance" approach are that recoveries of performance and neuro-mechanical alterations are almost complete after resting for 6 min near sea level, with also a similar fatigue pattern across conditions during subsequent repeated sprints in normoxia. To preserve RSA performance, it is therefore important to apply large forward-oriented ground reaction force and minimize the decrease in step frequency (i.e., increase in contact time), which at least in part result from more optimal neural drive strategies. However, no singular factor may represent a direct causative mechanism determining RSA so that studying the potential for other drivers of recovery (e.g., muscle damage or metabolic factors) may also be relevant.

Author Contributions

Conceived and designed the experiments: OG and FB. Performed experiments: OG and FB. Analyzed data: OG, FB, and JM. Interpreted results of research: OG, FB, JM, and GM. Drafted manuscript and prepared tables/figures: OG. Edited critically revised paper and approved final version of manuscript: OG, FB, JM, and GM.

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References

Balsom, P. D., Gaitanos, G. C., Ekblom, B., and Sjödin, B. (1994). Reduced oxygen availability during high intensity intermittent exercise impairs performance. *Acta Physiol. Scand*. 152, 279–285. doi: 10.1111/j.1748-1716.1994.tb09807.x

Billaut, F., Kerris, J. P., Rodriguez, R. F., Martin, D. T., Gore, C. J., and Bishop, D. J. (2013). Interactions of central and peripheral factors using repeated sprints at different levels of arterial O_2 saturation. *PLoS ONE* 8:e77297. doi: 10.1371/journal.pone.0077297

Bowtell, J. L., Cooke, K., Turner, R., Mileva, K. N., and Sumners, D. P. (2014). Acute physiological and performance responses to repeated sprints in varying degrees of hypoxia. *J. Sci. Med. Sport*. 17, 399–403. doi: 10.1016/j.jsams.2013.05.016

Brocherie, F., Millet, G. P., and Girard, O. (2015). Neuro-mechanical and metabolic adjustments to the repeated anaerobic sprint test in professional football players. *Eur. J. Appl. Physiol*. 115, 891–903. doi: 10.1007/s00421-014-3070-z

Calbet, J. A., De Paz, J. A., Garatechea, N., Cabeza de Veca, S., and Chavarren, J. (2003). Anaerobic energy provision does not limit Wingate exercise performance in endurance-trained cyclists. *J. Appl. Physiol*. 94, 668–676. doi: 10.1152/japplphysiol.00128.2002

Carling, C. (2013). Interpreting physical performance in professional soccer match-play: should we be more pragmatic in our approach? *Sports Med.* 43, 655–663. doi: 10.1007/s40279-013-0055-8

Christian, R. J., Bishop, D. J., Billaut, F., and Girard, O. (2014). The role of sense of effort on self-selected cycling power output. *Front Physiol.* 5:115. doi: 10.3389/fphys.2014.00115

Cook, C. J., and Beaven, C. M. (2013). Individual perception of recovery is related to subsequent sprint performance. *Br. J. Sports Med.* 47, 705–709. doi: 10.1136/bjsports-2012-091647

Girard, O., Brocherie, F., and Millet, G. P. (2015b). Can analysis of performance and neuromuscular recoveries from repeated sprints shed more light on its fatigue-causing mechanisms? *Front. Physiol.* 6:5. doi: 10.3389/fphys.2015.00005

Girard, O., Brocherie, F., Morin, J.-B., Degache, F., and Millet, G. P. (2015a). Comparison of four sections for analysing running mechanics alterations during repeated treadmill sprints. *J. Appl. Biomech.* doi: 10.1123/jab.2015-0049. [Epub ahead of print].

Girard, O., Brocherie, F., Morin, J.-B., and Millet, G. P. (2015c). Intra- and inter-session reliability of running mechanics during treadmill sprints. *Int. J. Sports Physiol. Perf.* doi: 10.1123/ijspp.2015-0145. [Epub ahead of print].

Girard, O., Mendez-Villanueva, A., and Bishop, D. J. (2011c). Repeated-sprint ability—Part I. *Sports Med.* 41, 673–694. doi: 10.2165/11590550-000000000-00000

Girard, O., Micallef, J. P., and Millet, G. P. (2011b). Changes in spring-mass model characteristics during repeated running sprints. *Eur. J. Appl. Physiol.* 111, 125–134. doi: 10.1007/s00421-010-1638-9

Girard, O., Racinais, S., Kelly, L., Millet, G. P., and Brocherie, F. (2011a). Repeated sprinting on natural grass impairs vertical stiffness but does not alter plantar loading in soccer players. *Eur. J. Appl. Physiol.* 111, 2547–2555. doi: 10.1007/s00421-011-1884-5

Goodall, S., Charlton, K., Howatson, G., and Thomas, K. (2015). Neuromuscular fatigability during repeated-sprint exercise in male athletes. *Med. Sci. Sports Exerc.* 47, 528–536. doi: 10.1249/MSS.0000000000000443

Goods, P. S. R., Dawson, B. T., Landers, G. J., Gore, C. J., and Peeling, P. (2014). Effect of different simulated altitudes on repeat-sprint performance in team-sport athletes. *Int. J. Sports Physiol. Perform.* 9, 857–862. doi: 10.1123/ijspp.2013-0423

Hamlin, M. J., Marshall, H. C., Hellemans, J., and Ainslie, P. N. (2010). Effect of intermittent hypoxia on muscle and cerebral oxygenation during a 20-km time trial in elite athletes: a preliminary report. *Appl. Physiol. Nutr. Metab.* 35, 548–559. doi: 10.1139/H10-044

Hogan, M. C., Richardson, R. S., and Haseler, L. J. (1999). Human muscle performance and PCr hydrolysis with varied inspired oxygen fractions: a 31P-MRS study. *J. Appl. Physiol.* 86, 1367–1373.

Hureau, T. J., Olivier, N., Millet, G. Y., Meste, O., and Blain, G. M. (2014). Exercise performance is regulated during repeated sprints to limit the development of peripheral fatigue beyond a critical threshold. *Exp. Physiol.* 99, 951–963. doi: 10.1113/expphysiol.2014.077974

Krivoshchekov, S. G., Balioz, N. V., Nekipelova, N. V., and Kapilevich, L. V. (2014). Age, gender, and individually-typological features of reaction to sharp hypoxic influence. *Hum. Physiol.* 40, 34–45. doi: 10.1134/S0362119714060061

Lindstrom, L., Magnusson, R., and Petersén, I. (1970). Muscular fatigue and action potential conduction velocity changes studied with frequency of EMG signals. *Electromyography* 10, 341–356.

Matsuura, R., Ogata, H., Yunoki, T., Arimitsu, T., and Yano, T. (2006). Effect of blood lactate concentration and the level of oxygen uptake immediately before a cycling sprint on neuromuscular activation during repeated cycling sprints. *J. Physiol. Anthropol.* 25, 267–273. doi: 10.2114/jpa2.25.267

Mendez-Villanueva, A., Edge, J., Suriano, R., Hamer, P., and Bishop, D. J. (2012). The recovery of repeated-sprint exercise is associated with PCr resynthesis, while muscle pH and EMG amplitude remain depressed. *PLoS ONE* 7:e51977. doi: 10.1371/journal.pone.0051977

Mendez-Villanueva, A., Hamer, P., and Bishop, D. J. (2007). Fatigue responses during repeated sprints matched for initial mechanical output. *Med. Sci. Sports Exerc.* 39, 2219–2225. doi: 10.1249/mss.0b013e31815669dc

Mendez-Villanueva, A., Hamer, P., and Bishop, D. J. (2008). Fatigue in repeated-sprint exer- cise is related to muscle power factors and reduced neuromuscular activity. *Eur. J. Appl. Physiol.* 103, 411–419. doi: 10.1007/s00421-008-0723-9

Millet, G. P., Faiss, R., and Pialoux, V. (2012b). Point: hypobaric hypoxia induces different physiological responses from normobaric hypoxia. *J. Appl. Physiol*, 112, 1783–1784. doi: 10.1152/japplphysiol.00067.2012

Millet, G. Y., Muthalib, M., Jubeau, M., Laursen, P. B., and Nosaka, K. (2012a). Severe hypoxia affects exercise performance independently of afferent feedback and peripheral fatigue. *J. Appl. Physiol.* 112, 1335–1344. doi: 10.1152/japplphysiol.00804.2011

Minett, G. M., and Duffield, R. (2014). Is recovery driven by central or peripheral factors? A role for the brain in recovery following intermittent-sprint exercise. *Front. Physiol.* 5:24. doi: 10.3389/fphys.2014.00024

Mohr, M., Krustrup, P., Andersson, H., Kirkendal, D., and Bangsbo, J. (2008). Match activities of elite women soccer players at different performance levels. *J. Strength Cond. Res.* 22, 341–349. doi: 10.1519/JSC.0b013e318165fef6

Mohr, M., Krustrup, P., and Bangsbo, J. (2003). Match performance of high-standard soccer players with special reference to development of fatigue. *J. Sports Sci*, 21, 519–528. doi: 10.1080/0264041031000071182

Morin, J. B., Samozino, P., Bonnefoy, R., Edouard, P., and Belli, A. (2010). Direct measurement of power during one single sprint on treadmill. *J. Biomech.* 43, 1970–1975. doi: 10.1016/j.jbiomech.2010.03.012

Morin, J.-B., Samozino, P., Edouard, P., and Tomazin, K. (2011). Effect of fatigue on force production and force application technique during repeated sprints. *J. Biomech.* 44, 2719–2723. doi: 10.1016/j.jbiomech.2011.07.020

Morin, J.-B., and Sève, P. (2011). Sprint running performance: comparison between treadmill and field conditions. *Eur. J. Appl. Physiol.* 111, 1695–1703. doi: 10.1007/s00421-010-1804-0

Ogawa, T., Hayashi, K., Ichinose, M., Wada, H., and Nishiyasu, T. (2007). Metabolic response during intermittent graded sprint running in moderate hypobaric hypoxia in competitive middle-distance runners. *Eur. J. Appl. Physiol.* 99, 39–46. doi: 10.1007/s00421-006-0315-5

Pearcey, G. E., Murphy, J. R., Behm, D. G., Hay, D. C., Power, K. E., and Button, D. C. (2015). Neuromuscular fatigue of the knee extensors during repeated maximal intensity intermittent-sprints on a cycle ergometer. *Muscle Nerve* 51, 569–579. doi: 10.1002/mus.24342

Perrey, S., Racinais, S., Saimouaa, K., and Girard, O. (2010). Neural and muscular adjustments following repeated running sprints. *Eur. J. Appl. Physiol.* 109, 1027–1036. doi: 10.1007/s00421-010-1445-3

Rabita, G., Dorel, S., Slawinski, J., Sàez-de-Villarreal, E., Couturier, A., Samozino, P., et al. (2015). Sprint mechanics in world-class athletes: a new insight into the limits of human locomotion. *Scand. J. Med. Sci. Sports* 25, 583–594. doi: 10.1111/sms.12389

Sheel, A. W. (2002). Respiratory muscle training in healthy individuals: physiological rationale and implications for exercise performance. *Sports Med.* 32, 567–581. doi: 10.2165/00007256-200232090-00003

Tabata, I., Irisawa, K., Kouzaki, M., Ishimura, K., Ogita, F., and Miyachi, M. (1997). Metabolic profile of high intensity intermittent exercises. *Med. Sci. Sports Exerc.* 29, 390–395. doi: 10.1097/00005768-199703000-00015

Weyand, P. G., Lee, C. S., Martinez-Ruiz, R., Bundle, M. W., Bellizzi, M. J., and Wright, S. (1999). High-speed running performance is largely unaffected by hypoxic reductions in aerobic power. *J. Appl. Physiol.* 86, 2059–2064.

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Cardiac autonomic responses after resistance exercise in treated hypertensive subjects

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The aim of this study was to assess and to compare heart rate variability (HRV) after resistance exercise (RE) in treated hypertensive and normotensive subjects. Nine hypertensive men [HT: 58.0 ± 7.7 years, systolic blood pressure (SBP) = 133.6 ± 6.5 mmHg, diastolic blood pressure (DBP) = 87.3 ± 8.1 mmHg; under antihypertensive treatment] and 11 normotensive men (NT: 57.1 ± 6.0 years, SBP = 127 ± 8.5 mmHg, DBP = 82.7 ± 5.5 mmHg) performed a single session of RE (2 sets of 15–20 repetitions, 50% of 1 RM, 120 s interval between sets/exercise) for the following exercises: leg extension, leg press, leg curl, bench press, seated row, triceps push-down, seated calf flexion, seated arm curl. HRV was assessed at resting and during 10 min of recovery period by calculating time (SDNN, RMSSD, pNN50) and frequency domain (LF, HF, LF/HF) indices. Mean values of HRV indices were reduced in the post-exercise period compared to the resting period (HT: lnHF: 4.7 ± 1.4 vs. 2.4 ± 1.2 ms^2; NT: lnHF: 4.8 ± 1.5 vs. 2.2 ± 1.1 ms^2, $p < 0.01$). However, there was no group vs. time interaction in this response ($p = 0.8$). The results indicate that HRV is equally suppressed after RE in normotensive and hypertensive individuals. These findings suggest that a single session of RE does not bring additional cardiac autonomic stress to treated hypertensive subjects.

Keywords: heart rate variability, autonomic nervous system, parasympathetic activity, sympathetic activity, resistance exercise

Introduction

Resistance exercise (RE) has been widely used as an adjunct to aerobic exercise in a comprehensive exercise training program oriented to health (Williams et al., 2007; Garber et al., 2011). Several studies have demonstrated the benefits of RE for increasing muscle mass, strength, balance, and quality of life in older adults or other frail populations (Cheema et al., 2014; Joshua et al., 2014; Silva-Batista et al., 2014; Vechin et al., 2015) and more recent evidence also indicate positive effects of RE in cardiovascular function and regulation (Queiroz et al., 2010; Grizzo Cucato et al., 2011).

Despite these recommendations, a single session of RE promotes a great cardiac autonomic stress, characterized by a reduction in cardiac vagal modulation and an increase in sympathetic

activity that persist during the post-exercise period (Heffernan et al., 2006; Rezk et al., 2006; Kingsley and Figueroa, 2014). This autonomic stress promoted by RE has been claimed to be greater than that promoted by aerobic exercise (Heffernan et al., 2006), a fact that can acutely increase the risks of cardiovascular events after RE, particularly in subjects with cardiovascular diseases, such as hypertension (Thompson et al., 2007).

Hypertension (HTN) is a highly prevalent chronic disease (Mozaffarian et al., 2015), characterized by increased levels of blood pressure, end-organ damage, and increased cardiovascular risks (Chobanian et al., 2003). Autonomic dysfunction is one of the main pathophysiological mechanisms of HTN, since it is related both to the development and also the complications of this disease (Mancia and Grassi, 2014). The autonomic dysfunction of HTN has been mainly demonstrated in rest, either by increased levels of sympathetic nerve firing (Schlaich et al., 2004) or reduced heart rate variability (HRV; Singh et al., 1998), but also during physiological maneuvres, such as exercise (Rondon et al., 2006). Accordingly, some recent studies have demonstrated a slower autonomic recovery after aerobic exercise in hypertensives in comparison with normotensives (Erdogan et al., 2011; Aneni et al., 2014).

Given that a single session of RE promotes significant autonomic disturbances, and that autonomic recovery after aerobic exercise is suggested to be slower in hypertensives compared with normotensives, it seems reasonable to expect that autonomic recovery from RE will be further slowed in hypertensives in comparison with normotensives. Thus, the aim of this study was to assess and to compare the cardiac autonomic recovery, assessed by HRV, after RE in treated hypertensive and normotensive subjects.

Methods

Sample

Eleven normotensive (NT) and nine hypertensive men (HT) under regular antihypertensive treatment (period of treatment = 9.7 ± 4.9 years; **Table 1**) participated in this study. Inclusion criteria were: age greater than 50 years old, no smoking for at least 1 year, no practicing of regular physical exercise (frequency up to one session per week) for at least 1 year. None of the study subjects had a history of musculoskeletal injury or cardiovascular diseases that could affect results, no arrhythmias were detected in the resting electrocardiography and none were using beta blockers. All of the subjects provided written voluntary informed consent, which was approved by the University Human Ethics Review Board and followed the recommendations from the Declaration of Helsinki.

Preliminary Assessment

Preliminary evaluations were performed on non-consecutive days. On the first day, the volunteers performed a clinical evaluation, anamneses (personal data, lifestyle questionnaires, previous diseases history, and cardiovascular risk parameters) and physical measurements were taken, such as height and body mass for subsequent calculation of the body mass index (BMI = mass/height^2; kg/m^2), waist and hip circumferences for the calculation of waist–hip ratio and percentage of body fat. Single measurements of systolic (SBP) and diastolic (DBP) blood pressures were taken by an experienced evaluator using a calibrated sphygmomanometer after 10 and 20 min of supine resting. The average of these two measurements was retained for analysis. Additionally, mean blood pressure (MBP) was calculated through the sum of the DBP and one-third of the pulse pressure. From the second to the fourth day, the subjects underwent three RE sessions to become familiar with the equipment and exercise techniques. On the fifth and sixth day, individuals performed the test and retest of one-repetition maximum (1 RM) to evaluate the maximum dynamic muscle strength. During the 1 RM assessment, participants were allowed to perform up to five attempts to reach the maximal load for each exercise (see experimental protocol), with rest intervals of 5 min between exercises. Differences lower than 5% between tests were accepted and the greatest 1 RM-value was considered for the prescription of exercise sessions.

TABLE 1 | Antihypertensive medication.

Pharmacological class	Number of volunteers	Percentage (%)
1. Not on medication	1	11.1
2. Under medication use	8	88.9
2.1. Monotherapy	5	55.6
Diuretics	1	11.1
Calcium channel blockers	1	11.1
Angiotensin receptor antagonist	3	33.3
2.2. Combination of therapies	3	33.3
Angiotensin receptor antagonist and diuretics	1	11.1
Angiotensin receptor antagonist and calcium channel blockers	1	11.1
Angiotensin receptor antagonist, calcium channel blockers and diuretics	1	11.1

Experimental Protocol

The experimental protocol consisted of three phases: rest, RE session, and recovery. All participants were advised not to ingest caffeinated or alcoholic drinks, and not to practice vigorous physical activity in the 24 h prior to the experiments. Initially, the volunteers remained seated for 10 min (rest stage). Then, they were submitted to a RE session in which they performed two sets of 20 repetitions at 50% of 1 RM on the following exercises: leg extension, leg press, leg curl, bench press, and seated row; and 15 repetitions at: triceps push-down, seated calf flexion, and seated arm curl. The rest interval between the sets and exercises was set to 2 min. Finally, individuals returned to the seated position, remaining in recovery for 10 min. At rest and during the recovery phases, heart rate (HR) was recorded beat-to-beat (RR-intervals) by an HR monitor (Polar® RS800CX, Kempele, Finland, sampling frequency = 1000 Hz; Nunan et al., 2009).

Procedures

Heart Rate Measures

The series of RR-intervals (RRi) recorded during rest and recovery were directed to a microcomputer, by infrared transmission to the Polar Precision Performance software (Polar Inc., Kempele, Finland). After a visual inspection, ectopic beats and artifacts were manually corrected by an expert (J.N.). Then, trend component removal of the time series was carried out according to the "*a priori*" smoothing method (Tarvainen et al., 2002), and interpolation using cubic splines at a frequency of 4 Hz was thus applied to extract equally spaced samples, thereby ensuring series of normal RR-intervals (NN). In sequence, the HR average value of the initial 5 min (rest) and HR-values in each 30-s window during the initial 5 min of recovery were calculated.

Heart Rate Variability Analysis: First 5 Min of Recovery

Since the behavior of the RRi signal in the first 5 min of recovery is non-linear (Goldberger et al., 2006), for the analysis of the HRV in such a period the time-varying vagal-related index RMSSD (square root of the mean of the sum of the squares of differences between adjacent normal R–R intervals) was calculated on subsequent 30 s non-overlapped segments (RMSSD30s), as proposed by Goldberger et al. (2006). To smooth out any transient outliers in the RMSSD30s plots, a median filter operation was applied, where each outlier value was replaced with the median of the value as well as the preceding and following values. The first and last values were not median filtered (Goldberger et al., 2006).

Heart Rate Variability Analysis: Resting and 5–10 Min of Recovery

The resting and late recovery period (5–10 min) HRV was analyzed according to the HRV Task Force (Task-Force, 1996) in the Kubios software (v 2.0, Biomedical Signal Analysis Group, Department of Applied Physics, University of Kuopio, Finland). The following time domain indices were calculated: the standard deviation of NN (SDNN), the square root of the mean square differences of successive NN (RMSSD) and the ratio between the number of times in which the difference between successive NN presented a duration higher than 50 ms in relation to the total number of NN (pNN50). The SDNN reflects the participation of all the rhythmic components responsible for variability, and is related to the joint action of both branches of the autonomous nervous system for the control of heart rate, whereas the RMSSD and pNN50 reflect the contributions of variations in high frequencies, which are related to the vagal action on the sinoatrial node (Task-Force, 1996). The frequency domain indices were calculated by the power spectral density function (PSD) using the Fast Fourier Transform (FFT; Malik and Camm, 1990; Task-Force, 1996). Prior to this transformation, the time series were detrended (smoothing priors) and resampled to 4 Hz sampling rate using cubic spline interpolation. For the spectral analysis, the following indices were calculated: the power of the spectral bands of low frequencies (LF; 0.04–0.15 Hz) in absolute units (ms^2), which represents the set of sympathetic and vagal influences on the sinoatrial node, and in normalized units (nu), which predominantly represents the cardiac sympathetic modulation (Task-Force, 1996); the power of the spectral bands of high frequencies (HF; 0.15–0.4 Hz) in absolute (ms^2) and normalized (nu) units, which represents the cardiac vagal modulation (Task-Force, 1996); and the LF/HF ratio, whose value is interpreted as a sympathetic-vagal balance indicator (Pagani et al., 1986; Task-Force, 1996).

Statistical Analysis

The results of this study are reported as mean ± standard deviation and the Alpha level was set at 5%. Following the use of the Shapiro–Wilk test, the hypothesis of normality was rejected for SDNN, RMSSD30s, RMSSD, HF, and LF indices, so variables were natural log-transformed (ln). The Student-*t*-test for independent samples and the Mann–Whitney tests were used to compare demographic, anthropometric and haemodynamic variables, and the measures of maximum dynamic muscle strength between the groups. A Two-Way ANOVA (group vs. time), followed by Tukey's *post hoc*-test, were employed to compare the HRV variables between the groups.

Results

Table 2 presents the demographic, anthropometric, and haemodynamic characteristics of the experimental groups. There were no differences between groups in any of these variables. There were also no differences in maximum dynamic muscle strength in each exercise between groups (**Table 3**).

The HR and RMSSD30s values in the post-exercise period were, respectively, increased and decreased in comparison to their resting values in both groups (time effect: $p < 0.01$ for all analyses), however, there were no differences in these responses between NT and HT (group vs. time interactions: $p = 0.10$ and 0.83, for HR and RMSSD30s, respectively; **Figure 1**).

The mean values of the HRV time-domain indices were reduced in the post-exercise period compared to the resting

TABLE 2 | Sample characterization: demographic, anthropometric, and haemodynamic variables.

	HT	NT	*p*-value
DEMOGRAPHIC VARIABLES			
Age (years)	58.0 ± 7.7 (50–65 years)	56.5 ± 6.3 (50–74 years)	0.65
ANTHROPOMETRIC VARIABLES			
BMI (kg/m^2)	29.0 ± 3.9	24.8 ± 3.5	0.06
Waist–hip ratio	0.93 ± 0.1	0.88 ± 0.1	0.08
% BF	26.8 ± 7.0	22.7 ± 6.0	0.27
HAEMODYNAMIC VARIABLES			
SBP (mmHg)	133.6 ± 6.5	127.0 ± 8.5	0.09
DBP (mmHg)	87.3 ± 8.1	82.7 ± 5.5	0.10
MBP (mmHg)	102.8 ± 6.9	96.2 ± 7.8	0.08

Values described as mean ± standard deviation.

HT, hypertensive group; NT, normotensive group; BMI, body mass index; % BF, percentage of body fat; SBP, systolic blood pressure; DBP, diastolic blood pressure; MBP, mean blood pressure.

period in both groups (time effect: $p < 0.01$ for all analyses), however there was no difference in this response between NT and HT (group vs. time interactions: $p = 0.2$–0.4 depending on the index analyzed; **Figure 2**). Regarding the frequency domain indices, we observed a significant reduction in the LF (ms^2) and HF (ms^2 and nu) and an increase in the LF (nu) and LF/HF in the post-exercise period in comparison to resting values (time effect: $p < 0.01$ for all analyses), with no differences between groups in these responses (group vs. time interactions: $p = 0.2$–0.8 depending on the index analyzed; **Figure 3**).

Discussion

The main findings of this study were the suppression of the HRV after RE and the lack of influence of hypertension in this response.

It has been demonstrated that an increase in sympathetic and decrease in parasympathetic activity in the post-exercise period underlies the increased risk of acute cardiovascular events in this period (Smith et al., 2005; Thompson et al., 2007). In this context, several studies have been conducted in order to understand which aspects of the exercise could influence the autonomic responses after exercise. Accordingly, the type of exercise seems to play an important role on the post-exercise autonomic recovery. In this regard, Heffernan et al. (2006) compared the HRV after a session of resistance or aerobic exercise, demonstrating a greater reduction of HRV after the RE. Similar results were found by Niemelä et al. (2008), who observed a delayed autonomic recovery after heavy-resistance exercise in comparison with aerobic and light-resistance exercises. Despite the absence of comparisons between resistance and aerobic exercise, the present results are in line with the previous ones (Heffernan et al., 2006) since a significant reduction was observed in parasympathetic activity (reduction in RMSSD and HF) and an increase in sympathetic balance [increase of LF (nu) and LF/HF], leading to an increase in HR and a suppression of HRV during the entire recovery period after the RE session in both groups. These findings spark an alert for the potential risks that could be brought by the practice of RE in subjects more prone to developing cardiovascular abnormalities after exercise, such as individuals with HTN (Thompson et al., 2007).

Hypertension is a chronic highly prevalent disease that is characterized mainly by increased levels of blood pressure, leading to higher rates of cardiovascular morbidity and mortality (Chobanian et al., 2003). The degenerative process of this disease is mediated by some pathophysiological mechanisms and autonomic dysfunction has been advocated as being a crucial one (Mancia and Grassi, 2014). Indeed, studies have already identified an increased sympathetic drive to the kidneys and muscles (Schlaich et al., 2004), and a reduced HRV (Guzzetti et al., 1988; Pagani and Lucini, 2001; Mancia and Grassi, 2014) in hypertensive individuals and it seems that these responses are even worse in more complicated hypertensive states (Grassi et al., 1998a, 2004). Recently, some studies with aerobic exercise have also demonstrated that this autonomic dysfunction in hypertensive subjects is also present in the post-exercise period (Erdogan et al., 2011; Aneni et al., 2014; Best et al., 2014). Accordingly, Erdogan et al. (2011) have demonstrated a slower heart rate recovery after aerobic exercise in hypertensives in comparison to normotensives, and Aneni et al. (2014) observed that this reduction in heart rate recovery accompanies the progression of HTN.

TABLE 3 | Measures of maximum dynamic muscle strength for each exercise.

Exercises	Maximum dynamic muscle strength (kg)		
	HT	NT	*p*-value
Leg extension	127.7 ± 41.2	130.9 ± 33.8	0.72
Leg press	122.2 ± 37.0	148.4 ± 39.7	0.56
Leg curl	78.4 ± 29.2	92.2 ± 18.3	0.28
Bench press	52.4 ± 15.7	59.6 ± 12.8	0.16
Seated row	99.1 ± 18.4	102.1 ± 16.6	0.75
Triceps push-down	51.0 ± 14.0	54.6 ± 10.1	0.71
Seated calf flexion	34.7 ± 9.3	37.5 ± 5.8	0.43
Seated arm curl	50.0 ± 10.0	55.0 ± 7.8	0.30

Values described as mean ± standard deviation.
HT, Hypertensive group; NT, Normotensive group.

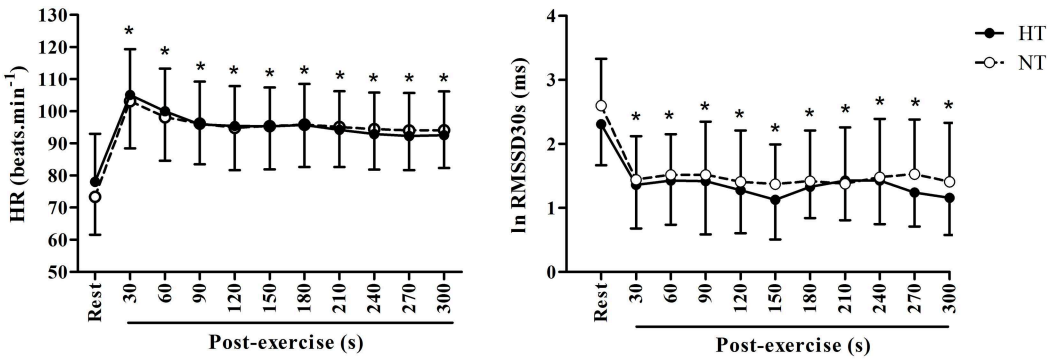

FIGURE 1 | Heart rate (HR) and heart rate variability (RMSSD30s index) at rest and during 5 min of recovery (post-exercise). HT, hypertensive group; NT, normotensive group; * significantly different from rest in both groups ($p < 0.05$).

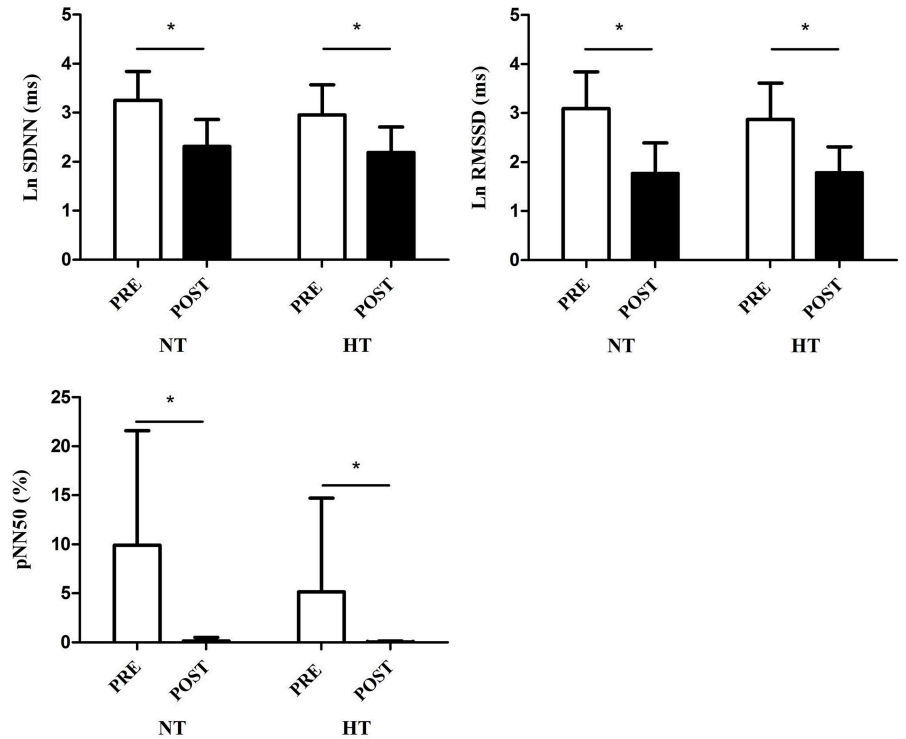

FIGURE 2 | Time-domain heart rate variability indices (SDNN, RMSSD, and pNN50) at rest (PRE) and during 5–10 min of recovery (POST). HT, hypertensive group; NT, normotensive group; * $p < 0.05$.

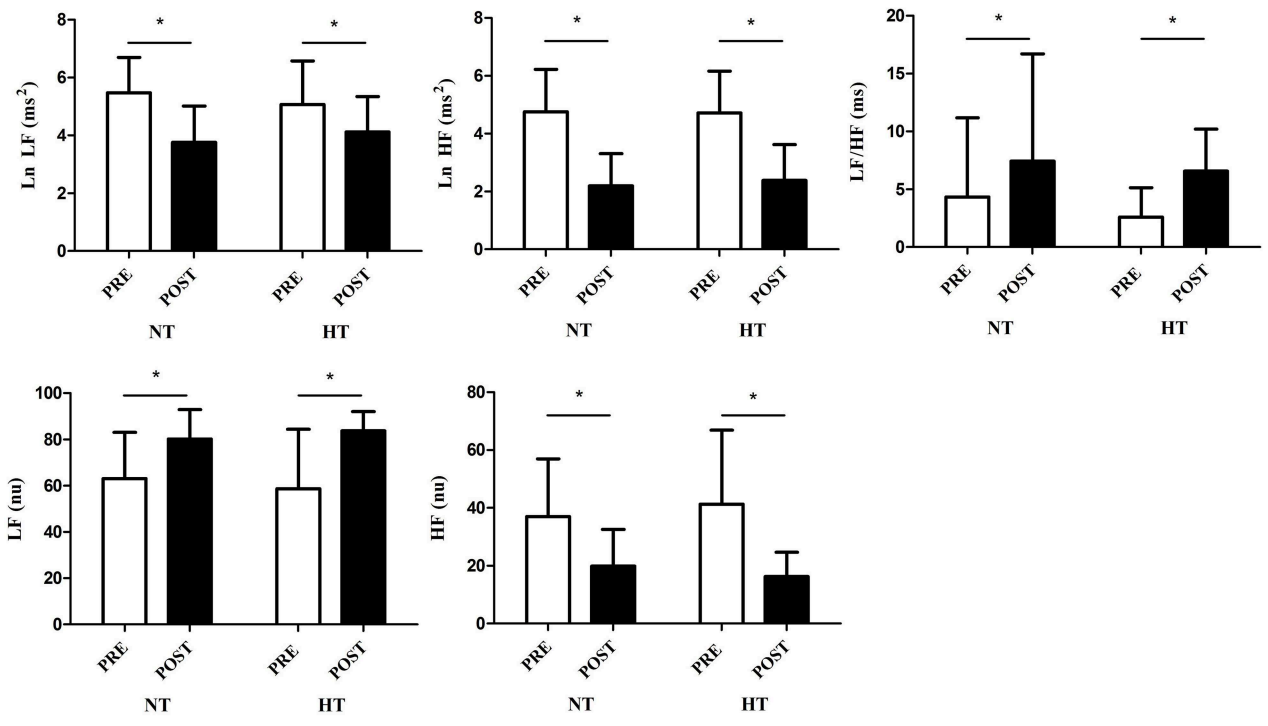

FIGURE 3 | Frequency-domain heart rate variability indices [LF, HF (ms^2 and nu) and LF/HF] at rest (PRE) and during 5–10 min of recovery (POST). HT, hypertensive group; NT, normotensive group. * $p < 0.05$.

Given that RE is known to produce high levels of autonomic stress, and that hypertensive subjects are supposed to present autonomic dysfunction in the post-exercise period, the hypothesis of this study was that treated hypertensives would present a reduced HRV in the post-exercise period in comparison with normotensives. Despite that, this study did not observe any influence of HTN status on post-exercise HR or HRV. This finding suggests that the autonomic stress imposed by the RE is not different between normotensive and treated hypertensive subjects.

It should be highlighted that the hypertensive subjects in this study were under medication treatment and well-controlled, a factor that is known to improve autonomic function (Kailasam et al., 1995; Ye et al., 2002). This could have prevented a greater post-exercise autonomic stress after RE in this group. Accordingly, most of studies that have shown the presence of autonomic dysfunction in hypertensives have used never-treated subjects (Rondon et al., 2006; Erdogan et al., 2011) or employed a wash-out period before the exercise intervention (Schlaich et al., 2004). Indeed, in the present study HRV-values showed no differences between treated hypertensives and normotensives even in the resting state (i.e., pre-exercise values), a fact that suggests that they did not present autonomic dysfunction in their baseline. The hypertensive subjects of the present study were also free of associated comorbidities and HTN-related complications, factors which are known to negatively influence autonomic function (Grassi et al., 1998b, 2004). Therefore, it seems likely that at least in well-controlled hypertensive subjects with no additional comorbidities and complications, the autonomic stress posed by RE is similar to that of normotensive ones.

The absence of standardization of the antihypertensive drugs among the hypertensive subjects could be viewed as a limitation of this study. However, the modification of the antihypertensive drugs, particularly in well-controlled hypertensives may be problematic, since it could worsen their blood pressure control, thus hampering the treatment. For this reason, the decision to maintain their original prescribed medication was taken in order to warrant the best treatment for each subject. It should also be emphasized that despite each individual using a specific antihypertensive drug (**Table 1**), no one was under the use of beta blockers, a class of drugs known to directly influence the autonomic nervous system (Frishman, 2003). Another potential limitation of this study could be the reduced number of subjects. However, a *post hoc* power analysis revealed a power >0.7 in most of the statistical comparisons, indicating that the sample size of the current study was adequate for the analysis performed. Finally, it should be emphasized that HRV provides information regarding the cardiac autonomic modulation rather than cardiac autonomic tone *per se*. This means that the tools of the present study did not allow an assessment of the degree of the sympathetic and parasympathetic drives to the heart, but rather its balanced responses to variations in respiration, blood pressure, and temperature, among other factors (Saul, 1990).

Conclusion

The results of the present study indicate that RE promotes a significant suppression of HRV after exercise, a fact that can potentially increase the cardiovascular risks of the practice of this exercise. However, this response was similar between normotensives and treated hypertensives, suggesting that a controlled hypertension does not bring additional cardiovascular risks to the practice of RE.

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References

Aneni, E., Roberson, L. L., Shaharyar, S., Blaha, M. J., Agatston, A. A., Blumenthal, R. S., et al. (2014). Delayed heart rate recovery is strongly associated with early and late-stage prehypertension during exercise stress testing. *Am. J. Hypertens.* 27, 514–521. doi: 10.1093/ajh/hpt173

Best, S. A., Bivens, T. B., Dean Palmer, M., Boyd, K. N., Galbreath, M. M., Okada, Y., et al. (2014). Heart rate recovery after maximal exercise is blunted in hypertensive seniors. *J. Appl. Physiol.* 117, 1302–1307. doi: 10.1152/japplphysiol.00395.2014

Cheema, B. S., Chan, D., Fahey, P., and Atlantis, E. (2014). Effect of progressive resistance training on measures of skeletal muscle hypertrophy, muscular strength and health-related quality of life in patients with chronic kidney disease: a systematic review and meta-analysis. *Sports Med.* 44, 1125–1138. doi: 10.1007/s40279-014-0176-8

Chobanian, A. V., Bakris, G. L., Black, H. R., Cushman, W. C., Green, L. A., Izzo, J. L. Jr., et al. (2003). The seventh report of the joint national committee on prevention, detection, evaluation, and treatment of high blood pressure: the JNC 7 report. *JAMA* 289, 2560–2571. doi: 10.1001/jama.289.19.2560

Erdogan, D., Gonul, E., Icli, A., Yucel, H., Arslan, A., Akcay, S., et al. (2011). Effects of normal blood pressure, prehypertension, and hypertension on autonomic nervous system function. *Int. J. Cardiol.* 151, 50–53. doi: 10.1016/j.ijcard.2010.04.079

Frishman, W. H. (2003). Beta-adrenergic blockers. *Circulation* 107, e117–e119. doi: 10.1161/01.CIR.0000070983.15903.A2

Garber, C. E., Blissmer, B., Deschenes, M. R., Franklin, B. A., Lamonte, M. J., Lee, I. M., et al. (2011). American College of Sports Medicine position stand. Quantity and quality of exercise for developing and maintaining cardiorespiratory, musculoskeletal, and neuromotor fitness in apparently healthy adults: guidance for prescribing exercise. *Med. Sci. Sports Exerc.* 43, 1334–1359. doi: 10.1249/MSS.0b013e318213fefb

Goldberger, J. J., Le, F. K., Lahiri, M., Kannankeril, P. J., Ng, J., and Kadish, A. H. (2006). Assessment of parasympathetic reactivation after exercise. *Am. J. Physiol. Heart Circ. Physiol.* 290, H2446–H2452. doi: 10.1152/ajpheart.01118.2005

Grassi, G., Cattaneo, B. M., Seravalle, G., Lanfranchi, A., and Mancia, G. (1998a). Baroreflex control of sympathetic nerve activity in essential and secondary hypertension. *Hypertension* 31, 68–72. doi: 10.1161/01.HYP.31.1.68

Grassi, G., Colombo, M., Seravalle, G., Spaziani, D., and Mancia, G. (1998b). Dissociation between muscle and skin sympathetic nerve activity in essential hypertension, obesity, and congestive heart failure. *Hypertension* 31, 64–67. doi: 10.1161/01.HYP.31.1.64

Grassi, G., Seravalle, G., Dell'oro, R., Facchini, A., Ilardo, V., and Mancia, G. (2004). Sympathetic and baroreflex function in hypertensive or heart failure patients with ventricular arrhythmias. *J. Hypertens.* 22, 1747–1753. doi: 10.1097/00004872-200409000-00019

Grizzo Cucato, G., de Moraes Forjaz, C. L., Kanegusuku, H., da Rocha Chehuen, M., Riani Costa, L. A., Wolosker, N., et al. (2011). Effects of walking and strength training on resting and exercise cardiovascular responses in patients with intermittent claudication. *Vasa* 40, 390–397. doi: 10.1024/0301-1526/a000136

Guzzetti, S., Piccaluga, E., Casati, R., Cerutti, S., Lombardi, F., Pagani, M., et al. (1988). Sympathetic predominance in essential hypertension: a study employing spectral analysis of heart rate variability. *J. Hypertens.* 6, 711–717. doi: 10.1097/00004872-198809000-00004

Heffernan, K. S., Kelly, E. E., Collier, S. R., and Fernhall, B. (2006). Cardiac autonomic modulation during recovery from acute endurance versus resistance exercise. *Eur. J. Cardiovasc. Prev. Rehabil.* 13, 80–86. doi: 10.1097/01.hjr.0000197470.74070.46

Joshua, A. M., D'souza, V., Unnikrishnan, B., Mithra, P., Kamath, A., Acharya, V., et al. (2014). Effectiveness of progressive resistance strength training versus traditional balance exercise in improving balance among the elderly - a randomised controlled trial. *J. Clin. Diagn. Res.* 8, 98–102. doi: 10.7860/jcdr/2014/8217.4119

Kailasam, M. T., Parmer, R. J., Cervenka, J. H., Wu, R. A., Ziegler, M. G., Kennedy, B. P., et al. (1995). Divergent effects of dihydropyridine and phenylalkylamine calcium channel antagonist classes on autonomic function in human hypertension. *Hypertension* 26, 143–149.

Kingsley, J. D., and Figueroa, A. (2014). Acute and training effects of resistance exercise on heart rate variability. *Clin. Physiol. Funct. Imaging*. doi: 10.1111/cpf.12223. [Epub ahead of print].

Malik, M., and Camm, A. J. (1990). Heart rate variability. *Clin. Cardiol.* 13, 570–576. doi: 10.1002/clc.4960130811

Mancia, G., and Grassi, G. (2014). The autonomic nervous system and hypertension. *Circ. Res.* 114, 1804–1814. doi: 10.1161/CIRCRESAHA.114.302524

Mozaffarian, D., Benjamin, E. J., Go, A. S., Arnett, D. K., Blaha, M. J., Cushman, M., et al. (2015). Heart disease and stroke statistics-2015 update: a report from the American Heart Association. *Circulation* 131, e29–e322. doi: 10.1161/CIR.0000000000000157

Niemela, T. H., Kiviniemi, A. M., Hautala, A. J., Salmi, J. A., Linnamo, V., and Tulppo, M. P. (2008). Recovery pattern of baroreflex sensitivity after exercise. *Med. Sci. Sports Exerc.* 40, 864–870. doi: 10.1249/MSS.0b013e3181666f08

Nunan, D., Donovan, G., Jakovljevic, D. G., Hodges, L. D., Sandercock, G. R., and Brodie, D. A. (2009). Validity and reliability of short-term heart-rate variability from the Polar S810. *Med. Sci. Sports Exerc.* 41, 243–250. doi: 10.1249/MSS.0b013e318184a4b1

Pagani, M., Lombardi, F., Guzzetti, S., Rimoldi, O., Furlan, R., Pizzinelli, P., et al. (1986). Power spectral analysis of heart rate and arterial pressure variabilities as a marker of sympatho-vagal interaction in man and conscious dog. *Circ. Res.* 59, 178–193. doi: 10.1161/01.RES.59.2.178

Pagani, M., and Lucini, D. (2001). Autonomic dysregulation in essential hypertension: insight from heart rate and arterial pressure variability. *Auton. Neurosci.* 90, 76–82. doi: 10.1016/S1566-0702(01)00270-3

Queiroz, A. C., Kanegusuku, H., and Forjaz, C. L. (2010). Effects of resistance training on blood pressure in the elderly. *Arq. Bras. Cardiol.* 95, 135–140. doi: 10.1590/S0066-782X2010001100020

Rezk, C. C., Marrache, R. C., Tinucci, T., Mion, D. Jr., and Forjaz, C. L. (2006). Post-resistance exercise hypotension, hemodynamics, and heart rate variability: influence of exercise intensity. *Eur. J. Appl. Physiol.* 98, 105–112. doi: 10.1007/s00421-006-0257-y

Rondon, M. U., Laterza, M. C., de Matos, L. D., Trombetta, I. C., Braga, A. M., Roveda, F., et al. (2006). Abnormal muscle metaboreflex control of sympathetic activity in never-treated hypertensive subjects. *Am. J. Hypertens.* 19, 951–957. doi: 10.1016/j.amjhyper.2006.02.001

Saul, J. (1990). Beat-to-Beat variations of heart rate reflect modulation of cardiac autonomic outflow. *Physiology* 5, 32–37.

Schlaich, M. P., Lambert, E., Kaye, D. M., Krozowski, Z., Campbell, D. J., Lambert, G., et al. (2004). Sympathetic augmentation in hypertension: role of nerve firing, norepinephrine reuptake, and angiotensin neuromodulation. *Hypertension* 43, 169–175. doi: 10.1161/01.HYP.0000103160.35395.9E

Silva-Batista, C., Kanegusuku, H., Roschel, H., Souza, E. O., Cunha, T. F., Laurentino, G. C., et al. (2014). Resistance training with instability in multiple system atrophy: a case report. *J. Sports Sci. Med.* 13, 597–603.

Singh, J. P., Larson, M. G., Tsuji, H., Evans, J. C., O'donnell, C. J., and Levy, D. (1998). Reduced heart rate variability and new-onset hypertension: insights into pathogenesis of hypertension: the Framingham Heart Study. *Hypertension* 32, 293–297. doi: 10.1161/01.HYP.32.2.293

Smith, L. L., Kukielka, M., and Billman, G. E. (2005). Heart rate recovery after exercise: a predictor of ventricular fibrillation susceptibility after myocardial infarction. *Am. J. Physiol. Heart Circ. Physiol.* 288, H1763–H1769. doi: 10.1152/ajpheart.00785.2004

Tarvainen, M. P., Ranta-Aho, P. O., and Karjalainen, P. A. (2002). An advanced detrending method with application to HRV analysis. *IEEE Trans. Biomed. Eng.* 49, 172–175. doi: 10.1109/10.979357

Task-Force. (1996). Heart rate variability. Standards of measurement, physiological interpretation, and clinical use. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. *Eur. Heart J.* 17, 354–381. doi: 10.1093/oxfordjournals.eurheartj.a014868

Thompson, P. D., Franklin, B. A., Balady, G. J., Blair, S. N., Corrado, D., Estes, N. A. III., et al. (2007). Exercise and acute cardiovascular events placing the risks into perspective: a scientific statement from the American Heart Association Council on Nutrition, Physical Activity, and Metabolism and the Council on Clinical Cardiology. *Circulation* 115, 2358–2368. doi: 10.1161/CIRCULATIONAHA.107.181485

Vechin, F. C., Libardi, C. A., Conceição, M. S., Damas, F. R., Lixandrão, M. E., Berton, R. P., et al. (2015). Comparisons between low-intensity resistance training with blood flow restriction and high-intensity resistance training on quadriceps muscle mass and strength in elderly. *J. Strength Cond. Res.* 29, 1071–1076. doi: 10.1519/JSC.0000000000000703

Williams, M. A., Haskell, W. L., Ades, P. A., Amsterdam, E. A., Bittner, V., Franklin, B. A., et al. (2007). Resistance exercise in individuals with and without cardiovascular disease: 2007 update: a scientific statement from the American Heart Association Council on Clinical Cardiology and Council on Nutrition, Physical Activity, and Metabolism. *Circulation* 116, 572–584. doi: 10.1161/CIRCULATIONAHA.107.185214

Ye, S., Zhong, H., Duong, V. N., and Campese, V. M. (2002). Losartan reduces central and peripheral sympathetic nerve activity in a rat model of neurogenic hypertension. *Hypertension* 39, 1101–1106. doi: 10.1161/01.HYP.0000018590.26853.C7

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frontiers in Physiology

REVIEW
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Nutritional interventions to augment resistance training-induced skeletal muscle hypertrophy

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Skeletal muscle mass is regulated by a balance between muscle protein synthesis (MPS) and muscle protein breakdown (MPB). In healthy humans, MPS is more sensitive (varying 4–5 times more than MPB) to changes in protein feeding and loading rendering it the primary locus determining gains in muscle mass. Performing resistance exercise (RE) followed by the consumption of protein results in an augmentation of MPS and, over time, can lead to muscle hypertrophy. The magnitude of the RE-induced increase in MPS is dictated by a variety of factors including: the dose of protein, source of protein, and possibly the distribution and timing of post-exercise protein ingestion. In addition, RE variables such as frequency of sessions, time under tension, volume, and training status play roles in regulating MPS. This review provides a brief overview of our current understanding of how RE and protein ingestion can influence gains in skeletal muscle mass in young, healthy individuals. It is the goal of this review to provide nutritional recommendations for optimal skeletal muscle adaptation. Specifically, we will focus on how the manipulation of protein intake during the recovery period following RE augments the adaptive response.

Keywords: muscle protein synthesis, strength, protein balance, leucine, whey, anabolism

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Introduction

Beyond its role in locomotion, skeletal muscle is the largest site of postprandial glucose disposal, a large site of lipid oxidation, and a substantial contributor to resting metabolic rate (for review see Wolfe, 2006). As a result, considerable research using stable isotopic tracers has been conducted that has aimed to understand the biology of muscle protein turnover in response to various stimuli. What this work has shown us is that the size of human muscle mass is dictated by diurnal changes in rates of muscle protein synthesis (MPS) and muscle protein breakdown (MPB) (Phillips, 2004). In the rested, fasted state, rates of MPB exceed those of MPS and thus skeletal muscle is in a state of negative net protein balance (Biolo et al., 1995b). However, in response to amino acid (AA) or protein feeding, there is a significant but transient increase in rates of MPS and no significant change in MPB rendering skeletal muscle in a state of positive net protein balance (Biolo et al., 1997; Phillips, 2004). It is the relative contribution of these fed and fasted periods to overall net protein balance that dictates skeletal muscle mass homeostasis over time (Phillips, 2004).

In addition to the protein feeding-induced increases in MPS, resistance exercise (RE) also imparts a positive impact on skeletal muscle size (Chesley et al., 1992; Yarasheski et al., 1993; Cermak et al., 2012). Indeed, a single bout of RE in the fasted state significantly increases rates

of MPS, however, this rise in MPS is not enough to promote a positive net protein balance (Biolo et al., 1995b). Instead, RE serves to potentiate MPS in response to AA feeding (Biolo et al., 1997), an effect that may persist for up to 24 h (Burd et al., 2011). Therefore, repeated bouts of RE and protein feeding result in skeletal muscle hypertrophy (Cermak et al., 2012). What remains largely unknown is what the most anabolic or sensitizing RE protocol is. Moreover, data pertaining to the optimal dose, timing and quality of protein intake to optimize post-RE muscle anabolism have only recently enabled appropriate recommendations to be made. The aim of this review is to concisely summarize these data as well as discuss new evidence with regards to RE prescription for muscle hypertrophy. We do not provide a comprehensive overview of the cellular and molecular mechanisms regulating cell size but refer the interested reader to other reviews on this topic (Adams and Bamman, 2010; Egan and Zierath, 2013; Blaauw and Reggiani, 2014).

Protein Dose

The first study to examine a protein dose-response relationship with MPS following RE was conducted by Moore et al. (2009). Moore et al. (2009) fed whole-egg proteins after a bout of RE to healthy young men with a wide range of resistance-training experience (4 months to 8 years). The authors found that after a bout of unilateral lower-body RE the MPS response plateaued with ingestion of 20 g of protein such that there was no statistically significant benefit toward MPS with the ingestion of 40 g (Moore et al., 2009). Alternatively phrased, ingestion of 20 g of protein resulted in 89% of the response conferred by ingestion of 40 g. In young, resistance-trained ($\geq$6 months previous weight-lifting experience) men 20 g of whey protein following unilateral RE was also shown to sufficiently stimulate post-absorptive MPS with no further increase ingesting 40 g (Witard et al., 2014a). It appears that 20 g of whey protein (or $\sim$0.25 g protein/kg) is an ample amount of protein to ingest for healthy young men both at rest (Cuthbertson et al., 2005) and after exercise (Moore et al., 2009) regardless of training status (Witard et al., 2014a). Similar results have also been found at rest using whole food (90% lean ground beef) in young men and women where a moderate ($\sim$30 g protein) amount was just as effective as a high ($\sim$90 g protein) amount at stimulating MPS (Symons et al., 2009). Altogether, these results suggest that 20 g is the maximally effective protein dose per meal in healthy, young individuals. Protein consumed beyond this level is oxidized at a higher rate (Moore et al., 2009; Witard et al., 2014a) and results in urea production (Witard et al., 2014a) indicating there is a limit of AAs that can be used for MPS. The theory behind why, with increasing protein doses, there is a ceiling on MPS has been termed the "muscle full effect" (Atherton et al., 2010). It is important to acknowledge that these dose-response studies have been limited to lower limb RE and thus it remains unknown as to whether the absolute dose of protein required to maximally stimulate rates of MPS following whole-body RE is >20 g.

In this respect, we have refined the estimates for protein to a dose that is expressed per kilogram of body mass or even lean body mass (Moore et al., 2015). Using a two-phase linear regression model we reported that the dose of protein beyond which there was no further increase in MPS in young men was 0.25 g/kg/meal (90% confidence interval 0.18–0.3 g/kg/meal). To account for inter-individual variability we propose the addition of two standard deviations to our estimate, yielding a dose of protein that would optimally stimulate MPS at intake of 0.4 g/kg/meal. In our view, ingestion of protein beyond this dose would result in no further stimulation of MPS. The effects of AA ingestion beyond that needed to maximally stimulate MPS may include metabolic feedback regulation (Layman et al., 2015), satiety (Leidy et al., 2015), and thermogenesis (Acheson et al., 2011). Nonetheless, it needs to be appreciated that AA availability at levels beyond the rate at which they can be used for protein synthesis or other AA-requiring processes means that the amino nitrogen will be used for ureagenesis (Price et al., 1994; Witard et al., 2014a).

Changes in MPS are much greater (4–5 times) in response to stimuli such as contraction and feeding than MPB in healthy humans (Phillips et al., 1997, 2009; Rennie et al., 2004). It has been theorized that defining the protein dose that optimally stimulates MPS is insufficient to accurately characterize the true "anabolic potential" of protein-containing meals (Deutz and Wolfe, 2013). Citing data from whole-body protein turnover Deutz and Wolfe made the case that larger doses of protein can still be more anabolic than smaller doses due to a marked suppression of protein breakdown (Deutz and Wolfe, 2013). The problem in translating these findings to skeletal muscle is that non-muscle tissues dominate whole-body measures of protein turnover, with muscle accounting for only 25–30% of whole body protein turnover (Nair et al., 1988). Thus, even if there is increasingly positive whole-body protein balance with protein doses higher than what we are recommending here we propose that those would be predominantly due to suppression of proteolysis in non-muscle tissues. Even if 25–30% of the suppression of whole-body proteolysis with larger protein doses (Deutz and Wolfe, 2013) were from skeletal MPB such potential gains would be, in our estimation, unlikely to impart a marked benefit in terms of stimulating muscular hypertrophy. While such a conclusion awaits experimental confirmation we propose that marked suppression of proteolysis may not be an optimal strategy to pursue for those engaging in RE. In our opinion, given the multiple mechanisms damaging muscle during exercise, a higher rate of protein turnover (and not persistently suppressing proteolysis) would provide a more efficient mechanism for the removal of damaged proteins.

Timing of Protein Ingestion

We have known for some time that RE alone results in a long-lasting elevation in MPS for at least 48 h and MPB for 24 h (Phillips et al., 1997); thus, even in the basal fasted state there is a subsequent increase in the turnover of muscle proteins. RE alone elevating basal MPS will "prime" the muscle to be responsive, in terms of an increased sensitivity of MPS, to aminoacidemia. The duration of this sensitivity is at least 24 h (Burd et al., 2011) and, based on the similar protein dose thresholds (Moore et al., 2009; Witard et al., 2014a), we predict no difference in

sensitivity between untrained and trained individuals. Given the sensitizing effect of RE, we conclude it is most advantageous to ingest protein and generate hyeraminoacidemia in the post-RE period.

Some have postulated that pre-exercise protein ingestion may also "prime" the system and offer some advantage over a post-exercise supplementation strategy. However, ingesting 20 g of whey protein either before or 1 h after 10 sets of leg extension resulted in similar rates of AA uptake (Tipton et al., 2007). In other studies there was no benefit shown with pre-exercise AA feeding (Fujita et al., 2009; Burke et al., 2012a). Considering the synergistic response of aminoacidemia following RE (Biolo et al., 1997; Burd et al., 2011), we see it as being optimal to ingest protein immediately following RE. Moreover, we speculate pre-exercise aminoacidemia may blunt the subsequent post-RE MPS response to AAs due to an overlap in the aminoacidemic responses and a muscle full effect (Atherton et al., 2010).

There is only one study to date that has supplemented with protein during exercise and examined the MPS response (Beelen et al., 2008). Beelen and colleagues supplemented young men during an extended RE workout. The supplements were taken before and every 15 min during exercise providing 0.15 g/kg/h carbohydrate with or without 0.15 g/kg/h casein hydrolysate. There was a greater MPS response with carbohydrate plus protein ingestion, which was most likely due to the protein; however, the extra total energy cannot be discounted as a factor (Beelen et al., 2008). Evidence suggests that during-exercise consumption of protein may be beneficial though once again we counsel caution on this practice as the additional post-exercise hyperaminoacidemia may be less effective due to the muscle full effect.

A recent meta-analysis examining protein timing and hypertrophy concluded that the ingestion of a post-exercise supplement in closer temporal proximity to RE positively influenced hypertrophy (Schoenfeld et al., 2013); however, after adjustment for all covariates, the authors concluded that total protein intake was the strongest predictor of muscular hypertrophy and that protein timing did not influence hypertrophy. Nonetheless, practical advice would dictate that the post-exercise period is a time when rehydration, refueling (carbohydrate), and repair (3R) of damaged tissues should occur. We propose that it is still a pragmatic message to tell athletes to ingest fluid, carbohydrates, and protein to accomplish the goals defined by the 3R.

How protein should be consumed throughout the day is matter of debate. In an acute study, an "intermediate" pattern of whey protein ingestion (4 × 20 g every 3 h) throughout a 12 h recovery period post-RE was found to be more effective than ingestion of large boluses (2 × 40 g every 6 h) or a pulse (8 × 10 g every 1.5 h) protocol at stimulating MPS (Areta et al., 2013). These results are in agreement with the muscle full effect where, when AA delivery is sufficient (∼20 g), AAs are no longer used for MPS and are targeted for oxidation (Moore et al., 2009; Atherton et al., 2010; Witard et al., 2014a). However, many studies examining the impact of protein feeding on MPS either infuse AAs or provide protein in a bolus form. Though these are an efficient and direct way to provide protein in a laboratory setting, it is not how protein is consumed in the applied setting (i.e., a mixed macronutrient meal). The macronutrient composition and form of meal intake may influence both the meal-induced rise in hyperaminoacidemia and protein synthesis (Burke et al., 2012b). It is also important to, when considering the distribution of protein throughout the day, acknowledge that the recommended dietary allowance for the United States and Canada is 0.8 g/kg/day, which, for an 80 kg individual, would equate to only 64 g of protein per day. Future studies should focus on mixed macronutrients meals and rates of muscle protein turnover over a longer period of time.

Pre-sleep feeding is a time when protein provision may provide a marked benefit to remodel muscle proteins. Ingestion of 40 g of casein protein before bed stimulates MPS and improves net protein balance overnight in healthy young men (Res et al., 2012). Recently, a 12 week progressive RE training study showed that a pre-sleep casein beverage (27.5 g protein, 15 g carbohydrate, 0.1 g fat) in comparison with a placebo beverage augmented muscle mass, muscle fiber area, and strength gains (Snijders et al., 2015). However, the control group in this study did not receive a protein supplement resulting in a 0.6 g/kg difference in total protein intakes (1.3 vs. 1.9 g/kg/d), which some would argue would confer an advantage to the supplemented group regardless of when the protein was consumed. This may be the case and we acknowledge that 1.3 g/kg/d does not fall within even our recommendations for a protein intake that appears to be optimal for hypertrophy (Phillips, 2014a). Nonetheless, it is interesting to note that in a meta-analysis done by Cermak et al. (2012) only 3 of the 16 studies she analyzed showed statistically significant gains in lean mass with protein supplementation in young persons. While there were a further 4–5 studies that approached statistical significance, the fact that only 3 (19%) of the studies [one of which was in women in a hypoenergetic state (Josse et al., 2011)] independently reported augmented hypertrophy with protein supplementation shows that protein's effect on hypertrophy is small compared to the stimulus of the exercise itself. The point we make here is that the magnitude of the effects seen by Snijders et al. (2015) are impressive even considering the extra protein ingested and so we propose that the pre-sleep timing of the protein supplement was as, if not more, important as the higher protein intake of the supplemented group.

Altogether, we propose that the timing of protein intake is an important variable to consider in optimizing skeletal muscle recovery and hypertrophy. It appears optimal to ingest protein in the post-exercise period though the purported "anabolic window" for protein ingestion lasts at least 24 h (Burd et al., 2011) and does not have as drastic an effect on outcomes as has been believed (Schoenfeld et al., 2013). It is also important to ingest protein in sufficient doses (∼0.4 g/kg/meal) distributed throughout the day (Areta et al., 2013). Lastly, ingesting AAs in larger doses of protein (40 g casein or up to 0.6 g/kg/meal) pre-sleep appears to augment both acute overnight MPS (Res et al., 2012) and chronic skeletal muscle adaptations (Snijders et al., 2015). We wish to emphasize, however, that the magnitude of gains that are attributable to

protein supplementation compared to the overall gains made as a result of the RE training program itself appear to be relatively small.

Protein Quality

There are inherent differences in quality between the three most commonly consumed isolated protein sources: soy, casein, and whey. Proteins such as whey and soy are digested relatively rapidly, resulting in rapid aminoacidemia, and induce a larger but more transient rise in MPS than casein (Tang et al., 2009; Reitelseder et al., 2011). Whole-body protein synthesis is stimulated more with whey protein whereas whole-body protein breakdown is suppressed with ingestion of casein (Boirie et al., 1997). After ingestion of isolated casein, soy and whey protein (all providing 10 g EAA) the acute (3 h) rise in MPS was found to be greatest with whey protein both at rest and following exercise (Tang et al., 2009). Interestingly, soy protein had higher MPS than casein at both rest and after exercise as well (Tang et al., 2009). It appears that at least up to 3 h post-RE the most effective protein source is whey (Tang et al., 2009). Even for those considering weight loss, after 2 week of being hypocaloric, habitual daily consumption of whey (54 g) is more effective than soy at offsetting the decline in the postprandial MPS response (Hector et al., 2015).

In an effort to elucidate the attenuated anabolic response with casein supplementation, we evaluated the rates of MPS after a bout of RE with either a single bolus (25 g) or small pulses every 20 min (2.5 g) of whey protein (West et al., 2011). The 25 g bolus of whey protein lead to higher MPS between both 1–3 and 3–5 h post-exercise (West et al., 2011). The rapid and immediate bolus may be increasing EAA delivery to the muscle, specifically leucine, to a certain threshold that is triggering a MPS and the associated anabolic pathways. Indeed, blends of protein (1:2:1, whey:casein:soy) were later shown, when leucine content was matched, to be as effective as whey in stimulating MPS (Reidy et al., 2013). Furthermore, participants given 25 g of whey protein or 6.25 g whey with 5 g leucine added showed an increased MPS at rest and after RE to a similar extent despite a four-fold lower protein dose (Churchward-Venne et al., 2014). It appears that the leucinemia (and quite possibly the ensuing intramuscular leucine concentration) is the driver of the MPS response and thus the recovery process. The addition of isoleucine and valine (the other branched-chain AAs) does not improve MPS (Churchward-Venne et al., 2014). This response is an underappreciated result considering many supplements contain combinations of the branched-chain AAs, which, based on our data, would not be advantageous to consume co-temporally because they share the same transporter (Hyde et al., 2003). Thus, as we speculated (Churchward-Venne et al., 2014), consumption of crystalline BCAA resulted in competitive antagonism for uptake from the gut and into the muscle and was actually not as effective as leucine alone in stimulating MPS. Despite the popularity of BCAA supplements we find shockingly little evidence for their efficacy in promoting MPS or lean mass gains and would advise the use of intact proteins as opposed to a purified combination of BCAA that appear to antagonize each other in terms of transport both into circulation and likely in to the muscle (Churchward-Venne et al., 2014).

It appears that post-exercise MPS, measured within 3 h, is optimized by protein ingestion that contains a high leucine content where proteins are rapidly digested (i.e., whey) (Tang et al., 2009). The slower and more protracted aminoacidemia accompanying the ingestion of casein protein (Pennings et al., 2011), shown in pre-sleep protein ingestion studies (Res et al., 2012; Snijders et al., 2015), may be more effective at sustaining MPS and possibly at attenuating negative net protein balance (although all data to date on this mechanism are at the whole-body level) over longer periods of time. We propose the differences between protein sources in their ability to stimulate MPS are a combination of both the delivery (digestion) and AA composition of the protein, in particular leucine content. The AA composition in whey is superior to that of soy likely due to an increased leucine content (Tang et al., 2009). Lastly, there appears to be a leucine "threshold" for stimulation of MPS that is around ∼3 g of leucine per meal (Churchward-Venne et al., 2014), which may be determining the per meal protein recommendation of ∼0.4 g protein/kg.

Protein and Carbohydrate Co-ingestion

The purpose of carbohydrate (CHO) co-ingestion with protein is to stimulate insulin release beyond that seen with AA ingestion alone with the idea that insulin improves net protein balance. Indeed, local insulin infusion at rest increases MPS (Biolo et al., 1995a, 1999; Hillier et al., 1998) and blood flow (Biolo et al., 1999). When insulin is infused along with AAs there is an increase in MPS (Bennet et al., 1990; Hillier et al., 1998) and slight attenuation of MPB (Bennet et al., 1990) beyond that of just AA ingestion (Bennet et al., 1990) or insulin infusion (Hillier et al., 1998). However, following RE, insulin infusion has no effect on blood flow or MPS, though the slight suppression of MPB remains (Biolo et al., 1999). Coinciding with the previous finding (Biolo et al., 1999), in response to a single bout of RE, the ingestion of CHO alone has no effect on MPS, but attenuates MPB (Roy et al., 1997; Børsheim et al., 2004). However, co-ingesting CHO with AA/protein following RE has no further stimulatory effects on MPS and does not suppress MPB so long as protein is adequate (∼25 g) (Koopman et al., 2007; Glynn et al., 2010; Staples et al., 2011). These results indicate that when performing RE and providing adequate protein there is no benefit of co-ingesting CHO on stimulating MPS. This is most likely because the level of insulin required for optimal stimulation of MPS is remarkably low (Greenhaff et al., 2008; Trommelen et al., 2015) (i.e., 10–15 IU/ml), only 2–3 times basal resting levels for most healthy persons, which is easily reached with even a small dose of protein. With lower doses of protein (i.e., <0.25 g protein/kg), however, CHO ingestion may impact net protein balance via the ability to increase systemic insulin and suppress MPB and/or enhance AA delivery to the muscle, but we need to experimentally test this thesis. We conclude that while ingestion of CHO post-exercise would be necessary to replenish depleted glycogen stores we do not see a strong need to recommend CHO on top of protein to be consumed post-exercise. It appears

that even in a glycogen-depleted state protein is still effective at stimulating MPS following resistance exercise (Camera et al., 2012) and that only a minimal level of insulin is required to achieve optimal rates of MPS (Greenhaff et al., 2008).

Training Status

Training "age" may be an important variable impacting the quantity and duration of the anabolic response following RE. Compared to untrained participants, trained individuals have attenuated post-RE MPS and MPB resulting in less total muscle protein turnover (Phillips et al., 1999). A study by Tang et al. (2008) had participants train one leg for 8 week while the other served as the control. After the 8 week intervention, an acute bout of exercise stimulated a longer MPS response in the untrained or control leg relative to the trained leg suggesting an attenuation of the duration (but not magnitude) of MPS with training (Tang et al., 2008). Following a similar study design, after 8 weeks Kim et al. (2005) found an attenuation in mixed MPS in the trained leg, though myofibrillar protein synthesis remained the same. This finding is similar to that of Wilkinson et al. (2008) indicating a training-induced refinement, and perhaps efficiency, of post-exercise MPS. For a comprehensive review on the topic of training status and how it affects the MPS response and time course see Damas et al. (2015). The general conclusion from this review is that RE training reduces not the amplitude but the duration of the MPS response (Damas et al., 2015). This may in fact highlight that maximizing hypertrophic potential in the trained state may require greater focus on the post-exercise period for protein provision.

Despite the wealth of studies relating to the role of protein in augmenting the adaptive response to resistance exercise, relatively little has been conducted to identify whether resistance-trained individuals require greater relative post-exercise or daily protein consumption compared to those who are untrained. Data exist to suggest that athletes performing intensive periods of training may benefit from increased protein intake from the perspective of supporting immune function (Witard et al., 2014b). Moreover, those who engage in weight-categorized competition or sport may benefit from increased dietary protein intake (Mettler et al., 2010; Areta et al., 2014; Phillips, 2014b). However, as mentioned above, the post-RE MPS response reaches a maximum at 20 g or ∼0.25 g/kg in both untrained (Moore et al., 2009) and trained (Witard et al., 2014a) young men. Whether or not these results hold true when performing whole-body RE has yet to be determined. We direct the interested reader to the following papers for more discussion on the topic: (Phillips and van Loon, 2011; Phillips, 2012, 2014b). The opinions of these reviews suggest that resistance-training athletes may benefit from larger protein intakes higher than the recommended dietary allowance in the range of 1.3–1.8 g/kg/day (Phillips and van Loon, 2011; Phillips, 2012, 2014b). Nonetheless, the training regimens of the modern athlete are often interdisciplinary in nature and it is therefore critical to appreciate the context of the research, athlete, and training paradigm before making recommendations regarding "optimal" protein intake. Regardless, consideration for the "3R" approach should be common practice.

Resistance Exercise Program Variables and Training

Different skeletal muscle adaptations are induced by RE training than endurance training (Egan and Zierath, 2013). In this regard, we have shown that after 10 week of RE training, performing a single bout of RE increases myofibrillar, but not mitochondrial, protein synthesis whereas synthesis in both protein pools were acutely stimulated by RE in the pre-trained state (Wilkinson et al., 2008). Furthermore, with resistance training mixed MPS may decrease but fraction-specific adaptations (in this case myofibrillar MPS) may actually be enhanced (Kim et al., 2005). Indeed, it appears that the remodeling process following exercise is specific to the type of exercise performed (Wilkinson et al., 2008) and is tailored with training (Kim et al., 2005).

Manipulating different RE variables impacts both the acute and chronic anabolic response. For example, when young, resistance-trained (recreationally weight-training ≥2 times per week for ≥2 years) men received 20 g of whey protein after exercise, those who lifted with increased time under tensions (12 s per repetition) had elevated MPS compared to a repetition-matched control (2 s per repetition) (Burd et al., 2012). Specifically, Burd et al. (2012) found that sarcoplasmic MPS between 0 and 6 h, mitochondrial protein synthesis between 0–6 and 24–30 h, and myofibrillar protein synthesis between 24 and 30 h were all elevated with a longer time under tension beyond that of the repetition-matched group. It is worth noting that the repetition-matched group performing less time under tension per repetition lifted the same relative load. Indeed, the electromyography of the vastus lateralis indicated that the group exercising with a longer time under tension had increased muscle activity, and presumably muscle fatigue, toward the end of set completion (Burd et al., 2012). We speculate that the elevated MPS response to the longer time under tension is a result of increased motor unit recruitment which may be linked to muscle damage/remodeling (Proske and Morgan, 2001); however, we acknowledge we do not have experimental support for our proposed mechanisms. Interestingly, we have reported that when recreationally-active participants performed leg extensions at either 30 or 90% of their one-repetition max (1RM) to contractile failure there was an equal increase in mixed MPS (Burd et al., 2010). Additionally, 24 h after the RE bouts there was elevated myofibrillar MPS in only the 30% group (Burd et al., 2010). Not surprisingly, the 30% group had to perform more repetitions to achieve contractile failure and thus accumulated significantly more time under tension. Another study from our laboratory investigated this same principle over a 10 week period of training (Mitchell et al., 2012) in healthy but untrained young men and showed that the acute changes in MPS (Burd et al., 2010) mirrored those seen with training (i.e., equivalent hypertrophy). Though time under tension was not measured, it was concluded that regardless of the load lifted, performing RE to volitional failure results in hypertrophy (Mitchell et al., 2012). It appears that reaching contractile failure is required for optimal skeletal muscle growth. This can be achieved regardless of the repetition load. Manipulating variables such as time under tension or repetition-load may accelerate the time it takes

to reach contractile failure by increasing muscle fatigue and enhancing the rate of motor unit recruitment, but they do not likely individually enhance MPS.

In contrast to current recommendations (American College of Sports Medicine, 2009), we propose that an important variable to consider in regards to the optimization of MPS and the subsequent hypertrophic response is to ensure, regardless of the load lifted, that loads are lifted to the point of contractile failure. Contractile failure, particularly when lifting lighter loads, often occurs when there is significant muscle fatigue and motor unit activation. Motor unit activation refers to the size and quantity of motor units recruited. The term "muscle fatigue" is frequently misinterpreted. Fatigue is the inability to produce maximal force; thus, muscle fatigue is the inability of recruited motor units to generate their maximal force output (Stephens and Taylor, 1972; Dorfman et al., 1990). Significant muscle fatigue is reached by activating and exhausting a full cadre of motor units (and thus fiber types) and, regardless of any RE variable, requires a high degree of effort. From a broad prescriptive standpoint, we have emphasized the need for a high degree of effort in performing RE (Phillips and Winett, 2010). We propose that the manipulation of a multitude of RE variables may mean much less in terms of stimulating hypertrophy than simply exerting a high degree of effort to achieve contractile failure.

Relatively high (70–100% 1RM) training loads have been proposed to induce greater muscle hypertrophy (Campos et al., 2002; American College of Sports Medicine, 2009) than lower loads due to the increased mechanical loading and demand for fiber recruitment. However, as muscle fibers fatigue their motor units drop out and cease firing; a process that necessitates different motor units to be recruited to preserve the required force (Dorfman et al., 1990; Moritani et al., 1992). This is, at least partially, why surface electromyography and motor unit activation increase with muscular fatigue (Dorfman et al., 1990) and why similar hypertrophic adaptations are seen with varying external loads (Schoenfeld et al., 2014). Though lower loads may not initially need to recruit the larger motor units (innervating fast-twitch fibers) like higher loads may, with significant muscle fatigue there is an accompanied "dropout" of the smaller motor units (innervating slow-twitch fibers) such that subsequent contractions will be obliged to recruit additional (larger) motor units. If comparable motor units are activated and both groups are exercising until contractile failure it seems reasonable that similar adaptations are seen between low- and high-load RE training (Schoenfeld et al., 2014). However, we hypothesize that muscle fatigue (inability to generate maximal force) is not as important as motor unit activation in inducing muscle hypertrophy. For example, to reach contractile failure exercising at ∼30% 1RM one would have to achieve ∼70% muscle fatigue. In contrast, to reach contractile failure at ∼70% 1RM, an individual would only achieve ∼30% muscle fatigue. Thus muscle fatigue, albeit rendering an increase in motor unit activation, cannot be the most important determinant of the skeletal muscle response to RE if low- and high-load RE are inducing similar MPS (Burd et al., 2010) and hypertrophy (Mitchell et al., 2012). Instead, we hold on to the hypothesis that reaching contractile failure is what drives skeletal muscle adaptation (see **Figure 1**). We emphasize that it is naïve to prescribe moderate-heavy loads as the only way to induce muscle hypertrophy (American College of Sports Medicine, 2009). We also acknowledge that, as Mitchell et al. (2012) has shown, there may be a neuromuscular effect where the practice of lifting heavier loads over longer durations stimulates greater improvements of muscular strength. This is potentially due to a lack of inhibition on afferent feedback (Amann et al., 2009), but future research is required to be certain.

A number of meta-analyses on the impact of different RE program variables on muscle strength and hypertrophy are available (Peterson et al., 2005; Krieger, 2010; Schoenfeld et al.,

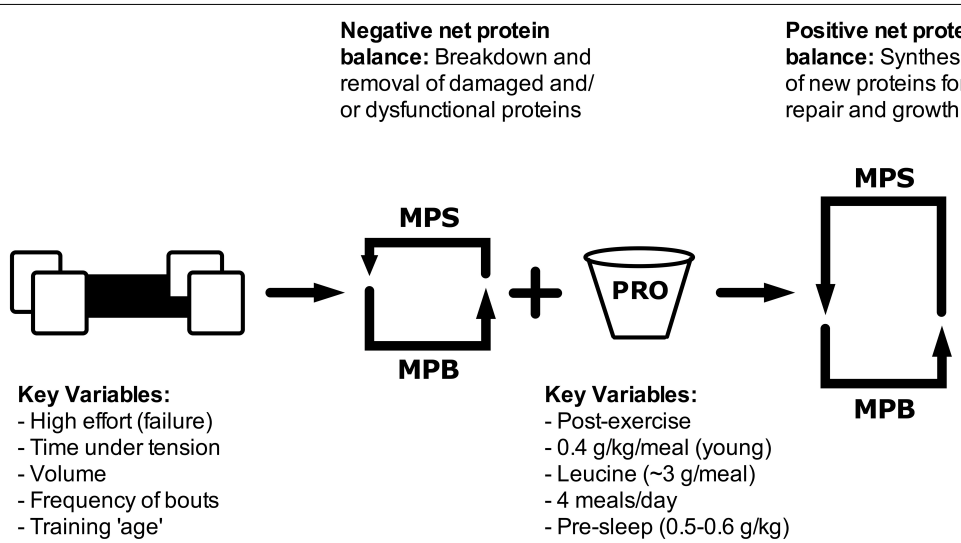

FIGURE 1 | Schematic showing how resistance exercise variables and protein ingestion can impact muscle protein turnover. MPS, muscle protein synthesis; MPB, muscle protein breakdown; PRO, protein.

2014, 2015). The conclusion, on examination of these analyses (Peterson et al., 2005; Krieger, 2010; Schoenfeld et al., 2014, 2015), would be that exercise volume (load × sets × reps) and training frequency (sessions per week) are important variables that affect the hypertrophic response and to this list we would propose the addition of effort. Contrary to popular belief, muscle hypertrophy may not be significantly influenced by resistance exercise load (Schoenfeld et al., 2014). This is despite 7 out of the 11 studies being volume equated, essentially suggesting the participants in the low-load groups did not train until contractile failure (Schoenfeld et al., 2014). We recognize there are many other variables that are manipulated to maximize changes in muscle mass, however, we hypothesize that these are largely moot when contractile failure is reached. Instead of any particular medley of RE variables, we propose that muscular hypertrophy is fundamentally driven by maximal motor unit recruitment and exercising until contractile failure.

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References

Acheson, K. J., Blondel-Lubrano, A., Oguey-Araymon, S., Beaumont, M., Emady-Azar, S., Ammon-Zufferey, C., et al. (2011). Protein choices targeting thermogenesis and metabolism. *Am. J. Clin. Nutr.* 93, 525–534. doi: 10.3945/ajcn.110.005850

Adams, G. R., and Bamman, M. M. (2010). Characterization and regulation of mechanical loading-induced compensatory muscle muscle hypertrophy. *Compr. Physiol.* 2, 2829–2870. doi: 10.1002/cphy.cp040251

Amann, M., Proctor, L. T., Sebranek, J. J., Pegelow, D. F., and Dempsey, J. A. (2009). Opioid-mediated muscle afferents inhibit central motor drive and limit peripheral muscle fatigue development in humans. *J. Physiol.* 587, 271–283. doi: 10.1113/jphysiol.2008.163303

American College of Sports Medicine. (2009). Progression models in resistance training for healthy adults. *Med. Sci. Sports Exerc.* 41, 687–708. doi: 10.1249/MSS.0b013e3181915670

Areta, J. L., Burke, L. M., Camera, D. M., West, D. W. D., Crawshay, S., Moore, D. R., et al. (2014). Reduced resting skeletal muscle protein synthesis is rescued by resistance exercise and protein ingestion following short-term energy deficit. *Am. J. Physiol. Endocrinol. Metab.* 306, E989–E997. doi: 10.1152/ajpendo.00590.2013

Areta, J. L., Burke, L. M., Ross, M. L., Camera, D. M., West, D. W. D., Broad, E. M., et al. (2013). Timing and distribution of protein ingestion during prolonged recovery from resistance exercise alters myofibrillar protein synthesis. *J. Physiol.* 591, 2319–2331. doi: 10.1113/jphysiol.2012.244897

Atherton, P. J., Etheridge, T., Watt, P. W., Wilkinson, D., Selby, A., Rankin, D., et al. (2010). Muscle full effect after oral protein: time-dependent concordance and discordance between human muscle protein synthesis and mTORC1 signaling. *Am. J. Clin. Nutr.* 92, 1080–1088. doi: 10.3945/ajcn.2010.29819

Beelen, M., Koopman, R., Gijsen, A. P., Vandereyt, H., Kies, A. K., Kuipers, H., et al. (2008). Protein coingestion stimulates muscle protein synthesis during resistance-type exercise. *Am. J. Physiol. Endocrinol. Metab.* 295, E70–E77. doi: 10.1152/ajpendo.00774.2007

Bennet, W. M., Connacher, A. A., Scrimgeour, C. M., Jung, R. T., and Rennie, M. J. (1990). Euglycemic hyperinsulinemia augments amino acid uptake by human leg tissues during hyperaminoacidemia. *Am. J. Physiol.* 259, E185–E194.

Biolo, G., Fleming, R. Y. D., and Wolfe, R. R. (1995a). Physiologic hyperinsulinemia stimulates protein synthesis and enhances transport of selected amino acids in human skeletal muscle. *J. Clin. Invest.* 95, 811–819. doi: 10.1172/JCI117731

Biolo, G., Maggi, S. P., Williams, B. D., Tipton, K. D., Wolf, R. R., and Wolfe, R. R. (1995b). Increased rates of muscle protein turnover and amino acid transport after resistance exercise in humans. *Am. J. Physiol.* 268, E514–E520.

Biolo, G., Tipton, K. D., Klein, S., and Wolfe, R. R. (1997). An abundant supply of amino acids enhances the metabolic effect of exercise on muscle protein. *Am. J. Physiol.* 273, E122–E129.

Biolo, G., Williams, B. D., Fleming, R. Y. D., and Wolfe, R. R. (1999). Insulin action on muscle protein kinetics and amino acid transport during recovery after resistance exercise. *Diabetes* 48, 949–957. doi: 10.2337/diabetes.48.5.949

Blaauw, B., and Reggiani, C. (2014). The role of satellite cells in muscle hypertrophy. *J. Muscle Res. Cell Motil.* 35, 3–10. doi: 10.1007/s10974-014-9376-y

Boirie, Y., Dangin, M., Gachon, P., Vasson, M. P., Maubois, J. L., Beaufrère, B. et al. (1997). Slow and fast dietary proteins differently modulate postprandial protein accretion. *Proc. Natl. Acad. Sci. U.S.A.* 94, 14930–14935. doi: 10.1073/pnas.94.26.14930

Børsheim, E., Cree, M. G., Tipton, K. D., Elliott, T. A., Aarsland, A., and Wolfe, R. R. (2004). Effect of carbohydrate intake on net muscle protein synthesis during recovery from resistance exercise. *J. Appl. Physiol.* 96, 674–678. doi: 10.1152/japplphysiol.00333.2003

Burd, N. A., Andrews, R. J., West, D. W. D., Little, J. P., Cochran, A. J. R., Hector, A. J., et al. (2012). Muscle time under tension during resistance exercise stimulates differential muscle protein sub-fractional synthetic responses in men. *J. Physiol.* 590, 351–362. doi: 10.1113/jphysiol.2011.221200

Burd, N. A., West, D. W. D., Moore, D. R., Atherton, P. J., Staples, A. W., Prior, T., et al. (2011). Enhanced amino acid sensitivity of myofibrillar protein synthesis persists for up to 24 h after resistance exercise in young men. *J. Nutr.* 141, 568–573. doi: 10.3945/jn.110.135038

Burd, N. A., West, D. W. D., Staples, A. W., Atherton, P. J., Baker, J. M., Moore, D. R., et al. (2010). Low-load high volume resistance exercise stimulates muscle protein synthesis more than high-load low volume resistance exercise in young men. *PLoS ONE* 5:e12033. doi: 10.1371/journal.pone.0012033

Burke, L. M., Hawley, J. A., Ross, M. L., Moore, D. R., Phillips, S. M., Slater, G. R., et al. (2012a). Preexercise aminoacidemia and muscle protein synthesis after resistance exercise. *Med. Sci. Sports Exerc.* 44, 1968–1977. doi: 10.1249/MSS.0b013e31825d28fa

Burke, L. M., Winter, J. A., Cameron-Smith, D., Enslen, M., Farnfield, M., and Decombaz, J. (2012b). Effect of intake of different dietary protein sources on plasma amino acid profiles at rest and after exercise. *Int. J. Sport Nutr. Exerc. Metab.* 22, 452–462.

Camera, D. M., West, D. W. D., Burd, N. A., Phillips, S. M., Garnham, A. P., Hawley, J. A., et al. (2012). Low muscle glycogen concentration does not suppress the anabolic response to resistance exercise. *J. Appl. Physiol.* 113, 206–214. doi: 10.1152/japplphysiol.00395.2012

Campos, G. E. R., Luecke, T. J., Wendeln, H. K., Toma, K., Hagerman, F. C., Murray, T. F., et al. (2002). Muscular adaptations in response to three different resistance-training regimens: specificity of repetition maximum training zones. *Eur. J. Appl. Physiol.* 88, 50–60. doi: 10.1007/s00421-002-0681-6

Cermak, N. M., Res, P. T., Groot, L. C., De Saris, W. H. M., and Van Loon, L. J. C. (2012). Protein supplementation augments the adaptive response of skeletal muscle to resistance-type exercise training: a meta-analysis 1 – 3. *Am. J. Clin. Nutr.* 96, 1454–1464. doi: 10.3945/ajcn.112.037556

Chesley, A., MacDougall, J. D., Tarnopolsky, M. A., Atkinson, S. A., and Smith, K. (1992). Changes in human muscle protein synthesis after resistance exercise. *J. Appl. Physiol.* 73, 1383–1388.

Churchward-Venne, T. A., Breen, L., Di Donato, D. M., Hector, A. J., Mitchell, C. J., Moore, D. R., et al. (2014). Leucine supplementation of a low-protein mixed macronutrient beverage enhances myofibrillar protein synthesis in young men:

a double-blind, randomized trial1-3. *Am. J. Clin. Nutr.* 99, 276–286. doi: 10.3945/ajcn.113.068775

Cuthbertson, D., Smith, K., Babraj, J., Leese, G., Waddell, T., Atherton, P., et al. (2005). Anabolic signaling deficits underlie amino acid resistance of wasting, aging muscle. *FASEB J.* 19, 422–424. doi: 10.1096/fj.04-2640fje

Damas, F., Phillips, S., Vechin, F. C., and Ugrinowitsch, C. (2015). A review of resistance training-induced changes in skeletal muscle protein synthesis and their contribution to hypertrophy. *Sports. Med.* 45, 801–807. doi: 10.1007/s40279-015-0320-0

Deutz, N. E., and Wolfe, R. R. (2013). Is there a maximal anabolic response to protein intake with a meal? *Clin. Nutr.* 32, 309–313. doi: 10.1016/j.clnu.2012.11.018

Dorfman, L. J., Howard, J. E., and McGill, K. C. (1990). Triphasic behavioral response of motor units to submaximal fatiguing exercise. *Muscle Nerve* 13, 621–628. doi: 10.1002/mus.880130711

Egan, B., and Zierath, J. R. (2013). Exercise metabolism and the molecular regulation of skeletal muscle adaptation. *Cell Metab.* 17, 162–184. doi: 10.1016/j.cmet.2012.12.012

Fujita, S., Dreyer, H. C., Drummond, M. J., Glynn, E. L., Volpi, E., and Rasmussen, B. B. (2009). Essential amino acid and carbohydrate ingestion before resistance exercise does not enhance postexercise muscle protein synthesis. *J. Appl. Physiol.* 106, 1730–1739. doi: 10.1152/japplphysiol.90395.2008

Glynn, E. L., Fry, C. S., Drummond, M. J., Dreyer, H. C., Dhanani, S., Volpi, E., et al. (2010). Muscle protein breakdown has a minor role in the protein anabolic response to essential amino acid and carbohydrate intake following resistance exercise. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 299, R533–R540. doi: 10.1152/ajpregu.00077.2010

Greenhaff, P. L., Karagounis, L. G., Peirce, N., Simpson, E. J., Hazell, M., Layfield, R., et al. (2008). Disassociation between the effects of amino acids and insulin on signaling, ubiquitin ligases, and protein turnover in human muscle. *Am. J. Physiol. Endocrinol. Metab.* 295, E595–E604. doi: 10.1152/ajpendo.90411.2008

Hector, A. J., Marcotte, G. R., Churchward-venne, T. A., Murphy, C. H., Breen, L., von Allmen, M., et al. (2015). Whey protein supplementation preserves postprandial myofibrillar protein synthesis during short-term energy restriction in overweight and obese adults. *J. Nutr.* 145, 246–252. doi: 10.3945/jn.114.200832

Hillier, T. A., Fryburg, D. A., Jahn, L. A., and Barrett, E. J. (1998). Extreme hyperinsulinemia unmasks insulin's effect to stimulate protein synthesis in the human forearm. *Am. J. Physiol.* 274, E1067–E1074.

Hyde, R., Taylor, P. M., and Hundal, H. S. (2003). Amino acid transporters: roles in amino acid sensing and signalling in animal cells. *Biochem. J.* 373, 1–18. doi: 10.1042/bj20030405

Josse, A. R., Atkinson, S. A., Tarnopolsky, M. A., and Phillips, S. M. (2011). Increased consumption of dairy foods and protein during diet-and exercise-induced weight loss promotes fat mass loss and lean mass gain in overweight and obese premenopausal women. *J. Nutr.* 141, 1626–1634. doi: 10.3945/jn.111.141028

Kim, P. L., Staron, R. S., and Phillips, S. M. (2005). Fasted-state skeletal muscle protein synthesis after resistance exercise is altered with training. *J. Physiol.* 568, 283–290. doi: 10.1113/jphysiol.2005.093708

Koopman, R., Beelen, M., Stellingwerff, T., Pennings, B., Saris, W. H. M., Kies, A. K., et al. (2007). Coingestion of carbohydrate with protein does not further augment postexercise muscle protein synthesis. *Am. J. Physiol. Endocrinol. Metab.* 293, E833–E842. doi: 10.1152/ajpendo.00135.2007

Krieger, J. W. (2010). Single vs. multiple sets of resistance exercise for muscle hypertrophy: a meta-analysis. *J. Strength Cond. Res.* 24, 1150–1159. doi: 10.1519/JSC.0b013e3181d4d436

Layman, D. K., Anthony, T. G., Rasmussen, B. B., Adams, S. H., Lynch, C. J., Brinkworth, G. D., et al. (2015). Defining meal requirements for protein to optimize metabolic roles of amino acids. *Am. J. Clin. Nutr.* 101, 1330–1338. doi: 10.3945/ajcn.114.084053

Leidy, H. J., Clifton, P. M., Astrup, A., Wycherley, T. P., Westerterp-plantenga, M. S., Luscombe-marsh, N. D., et al. (2015). The role of protein in weight loss and maintenance 1–5. *Am. J. Clin. Nutr.* 101, 1320–1329. doi: 10.3945/ajcn.114.084038

Mettler, S., Mitchell, N., and Tipton, K. D. (2010). Increased protein intake reduces lean body mass loss during weight loss in athletes. *Med. Sci. Sports Exerc.* 42, 326–337. doi: 10.1249/MSS.0b013e3181b2ef8e

Mitchell, C. J., Churchward-venne, T. A., West, D. W. D., Burd, A., Breen, L., Baker, S. K., et al. (2012). Resistance exercise load does not determine training-mediated hypertrophic gains in young men. *J. Apply Physiol.* 113, 71–77. doi: 10.1152/japplphysiol.00307.2012

Moore, D. R., Churchward-Venne, T. A., Witard, O., Breen, L., Burd, N. A., Tipton, K. D., et al. (2015). Protein ingestion to stimulate myofibrillar protein synthesis requires greater relative protein intakes in healthy older versus younger men. *J. Gerontol. Ser. A Biol. Sci. Med. Sci.* 70, 57–62. doi: 10.1093/gerona/glu103

Moore, D. R., Robinson, M. J., Fry, J. L., Tang, J. E., Glover, E. I., Wilkinson, S. B., et al. (2009). Ingested protein dose response of muscle and albumin protein synthesis after resistance exercise in young men 1–3. *Am. J. Clin. Nutr.* 89, 161–168. doi: 10.3945/ajcn.2008.26401

Moritani, T., Sherman, W. M., Shibata, M., Matsumoto, T., and Shinohara, M. (1992). Oxygen availability and motor unit activity in humans. *Eur. J. Appl. Physiol. Occup. Physiol.* 64, 552–556. doi: 10.1007/BF00843767

Nair, K. S., Halliday, D., and Griggs, R. C. (1988). Leucine incorporation into mixed skeletal muscle protein in humans. *Am. J. Physiol.* 254, E208–E213.

Pennings, B., Boirie, Y., Senden, J. M. G., Gijsen, A. P., Kuipers, H., van Loon, L. J. C. et al. (2011). Whey protein stimulates postprandial muscle protein accretion more effectively than do casein and casein hydrolysate in older men. *Am. J. Clin. Nutr.* 93, 997–1005. doi: 10.3945/ajcn.110.008102

Peterson, M. D., Rhea, M. R., and Alvar, B. A. (2005). Applications of the dose-response for muscular strength development: a review of meta-analytic efficacy and reliability for designing training prescription. *J. Strength Cond. Res.* 19, 950–958. doi: 10.1519/R-16874.1

Phillips, S. M. (2004). Protein requirements and supplementation in strength sports. *Nutrition* 20, 689–695. doi: 10.1016/j.nut.2004.04.009

Phillips, S. M. (2012). Dietary protein requirements and adaptive advantages in athletes. *Br. J. Nutr.* 108, S158–S167. doi: 10.1017/s0007114512002516

Phillips, S. M. (2014a). A brief review of critical processes in exercise-induced muscular hypertrophy. *Sport. Med.* 44, 71–77. doi: 10.1007/s40279-014-0152-3

Phillips, S. M. (2014b). A brief review of higher dietary protein diets in weight loss: a focus on athletes. *Sport. Med.* 44, 149–153. doi: 10.1007/s40279-014-0254-y

Phillips, S. M., Glover, E. I., and Rennie, M. J. (2009). Alterations of protein turnover underlying disuse atrophy in human skeletal muscle. *J. Appl. Physiol.* 107, 645–654. doi: 10.1152/japplphysiol.00452.2009

Phillips, S. M., Tipton, K. D., Ferrando, A. A., and Wolfe, R. R. (1999). Resistance training reduces the acute exercise-induced increase in muscle protein turnover. *Am. J. Physiol.* 276, E118–E124.

Phillips, S. M., Tipton, K. D., Aarsland, A., Wolf, S. E., and Wolfe, R. R. (1997). Mixed muscle protein synthesis and breakdown after resistance exercise in humans. *Am. J. Physiol.* 273, E99–E107.

Phillips, S. M., and van Loon, L. J. C. (2011). Dietary protein for athletes: from requirements to optimum adaptation. *J. Sports Sci.* 29, S29–S38. doi: 10.1080/02640414.2011.619204

Phillips, S. M., and Winett, R. A. (2010). Uncomplicated resistance training and health-related outcomes: evidence for a public health mandate. *Curr. Sports Med. Rep.* 9, 208–213. doi: 10.1249/JSR.0b013e3181e7da73

Price, G. M., Halliday, D., Pacy, P. J., Quevedo, M. R., and Millward, D. J. (1994). Nitrogen homeostasis in man: influence of protein intake on the amplitude of diurnal cycling of body nitrogen. *Clin. Sci. (Lond).* 86, 91–102. doi: 10.1042/cs0860091

Proske, U., and Morgan, D. L. (2001). Muscle damage from eccentric exercise: mechanism, mechanical signs, adaptation and clinical applications. *J. Physiol.* 537, 333–345. doi: 10.1111/j.1469-7793.2001.00333.x

Reidy, P. T., Walker, D. K., Dickinson, J. M., Gundermann, D. M., Drummond, M. J., Timmerman, K. L., et al. (2013). Protein blend ingestion following resistance exercise promotes human muscle protein synthesis. *J. Nutr.* 143, 410–416. doi: 10.3945/jn.112.168021

Reitelseder, S., Agergaard, J., Doessing, S., Helmark, I. C., Lund, P., Kristensen, N. B., et al. (2011). Whey and casein labeled with L - [1- 13 C] leucine and muscle protein synthesis: effect of resistance exercise and protein ingestion. *Am. J. Physiol. Endocrinol. Metab.* 300, E231–E242. doi: 10.1152/ajpendo.00513.2010

Rennie, M. J., Wackerhage, H., Spangenburg, E. E., and Booth, F. W. (2004). Control of the size of the human muscle mass. *Annu. Rev. Physiol.* 66, 799–828. doi: 10.1146/annurev.physiol.66.052102.134444

Res, P. T., Groen, B., Pennings, B., Beelen, M., Wallis, G. A., Gijsen, A. P., et al. (2012). Protein ingestion before sleep improves postexercise overnight recovery. *Med. Sci. Sports Exerc.* 44, 1560–1569. doi: 10.1249/MSS.0b013e31824cc363

Roy, B. D., Tarnopolsky, M. A., MacDougall, J. D., Fowles, J., and Yarasheski, K. E. (1997). Effect of glucose supplement timing on protein metabolism after resistance training. *J. Appl. Physiol.* 82, 1882–1888.

Schoenfeld, B. J., Aragon, A. A., and Krieger, J. W. (2013). The effect of protein timing on muscle strength and hypertrophy: a meta-analysis. *J. Int. Soc. Sports Nutr.* 10:53. doi: 10.1186/1550-2783-10-53

Schoenfeld, B. J., Ogborn, D. I., and Krieger, J. W. (2015). Effect of repetition duration during resistance training on muscle hypertrophy: a systematic review and meta-analysis. *Sport. Med.* 45, 577–585. doi: 10.1007/s40279-015-0304-0

Schoenfeld, B. J., Wilson, J. M., Lowery, R. P., and Krieger, J. W. (2014). Muscular adaptations in low- versus high-load resistance training: a meta-analysis. *Eur. J. Sport Sci.* doi: 10.1080/17461391.2014.989922. [Epub ahead of print].

Snijders, T., Res, P. T., Smeets, J. S. J., Vliet, S., Van Kranenburg, J., Van Maase, K., et al. (2015). Protein ingestion before sleep increases muscle mass and strength gains during prolonged resistance-type exercise training in healthy young men. *J. Nutr.* 145, 1178–1784. doi: 10.3945/jn.114.208371

Staples, A. W., Burd, N. A., West, D. W. D., Currie, K. D., Atherton, P. J., Moore, D. R., et al. (2011). Carbohydrate does not augment exercise-induced protein accretion versus protein alone. *Med. Sci. Sports Exerc.* 43, 1154–1161. doi: 10.1249/MSS.0b013e31820751cb

Stephens, J. A., and Taylor, A. (1972). Fatigue of maintained voluntary muscle contraction in man. *J. Physiol.* 220, 1–18. doi: 10.1113/jphysiol.1972.sp009691

Symons, T. B., Sheffield-Moore, M., Wolfe, R. R., and Paddon-Jones, D. (2009). A moderate serving of high-quality protein maximally stimulates skeletal muscle protein synthesis in young and elderly subjects. *J. Am. Diet. Assoc.* 109, 1582–1586. doi: 10.1016/j.jada.2009.06.369

Tang, J. E., Moore, D. R., Kujbida, G. W., Tarnopolsky, M. A., and Phillips, S. M. (2009). Ingestion of whey hydrolysate, casein, or soy protein isolate: effects on mixed muscle protein synthesis at rest and following resistance exercise in young men. *J. Appl. Physiol.* 107, 987–992. doi: 10.1152/japplphysiol.00076.2009

Tang, J. E., Perco, J. G., Moore, D. R., Wilkinson, S. B., and Phillips, S. M. (2008). Resistance training alters the response of fed state mixed muscle protein synthesis in young men. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 294, R172–R178. doi: 10.1152/ajpregu.00636.2007

Tipton, K. D., Elliott, T. A., Cree, M. G., Aarsland, A. A., Sanford, A. P., and Wolfe, R. R. (2007). Stimulation of net muscle protein synthesis by whey protein ingestion before and after exercise. *Am. J. Physiol. Endocrinol. Metab.* 292, E71–E76. doi: 10.1152/ajpendo.00166.2006

Trommelen, J., Groen, B., Hamer, H., de Groot, L. C. P. G. M., and van Loon, L. J. (2015). Mechanisms in endocrinology: exogenous insulin does not increase muscle protein synthesis rate when administrated systemically: a systematic review. *Eur. J. Endocrinol.* 173, R25–R34. doi: 10.1530/eje-14-0902

West, D. W. D., Burd, N. A., Coffey, V. G., Baker, S. K., Burke, L. M., Hawley, J. A., et al. (2011). Rapid aminoacidemia enhances myofibrillar protein synthesis and anabolic intramuscular signaling responses after resistance exercise. *Am. J. Clin. Nutr.* 94, 795–803. doi: 10.3945/ajcn.111.013722

Wilkinson, S. B., Phillips, S. M., Atherton, P. J., Patel, R., Yarasheski, K. E., Tarnopolsky, M. A., et al. (2008). Differential effects of resistance and endurance exercise in the fed state on signalling molecule phosphorylation and protein synthesis in human muscle. *J. Physiol.* 586, 3701–3717. doi: 10.1113/jphysiol.2008.153916

Witard, O. C., Jackman, S. R., Breen, L., Smith, K., Selby, A., and Tipton, K. D. (2014a). Myofibrillar muscle protein synthesis rates subsequent to a meal in response to increasing doses of whey protein at rest and after resistance exercise. *Am. J. Clin. Nutr.* 99, 86–95. doi: 10.3945/ajcn.112.055517

Witard, O. C., Turner, J. E., Jackman, S. R., Kies, A. K., Jeukendrup, A. E., Bosch, J. A., et al. (2014b). High dietary protein restores overreaching induced impairments in leukocyte trafficking and reduces the incidence of upper respiratory tract infection in elite cyclists. *Brain Behav. Immun.* 39, 211–219. doi: 10.1016/j.bbi.2013.10.002

Wolfe, R. R. (2006). The underappreciated role of muscle in health and disease. *Am. J. Clin. Nutr.* 84, 475–482.

Yarasheski, K. E., Zachwieja, J. J., and Bier, D. M. (1993). Acute effects of resistance exercise on muscle protein synthesis rate in young and elderly men and women. *Am. J. Physiol. Endocrinol. Metab.* 265, E210–E214.

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Recovery from exercise: vulnerable state, window of opportunity, or crystal ball?

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Why should we study the recovery from exercise as a discrete phenomenon from exercise itself? We identify three distinct (but not mutually exclusive) rationales that drive the need to investigate the physiology of recovery from exercise. (1) Some individuals are at a heightened risk of clinical outcomes in the immediate post-exercise period; thus the potential negative outcomes of this "vulnerable state" must be weighed against the numerous benefits of exercise training, and may be mitigated to reduce risk. (2) Many of the signaling mechanisms responsible for the beneficial effects of exercise training remain amplified during the exercise recovery period, and may present a "window of opportunity" that can be exploited by interventions to enhance the beneficial adaptations to exercise training, especially in clinical populations. (3) On an individual level, exercise recovery responses may provide investigators with a "crystal ball" ability to predict future clinical outcomes even in apparently healthy individuals. In short, the physiology of recovery is a multi-faceted and complex process, likely involving systems and pathways that are distinct from the physiology of exercise itself. For these reasons, it merits ongoing study.

Keywords: exercise, recovery, athletic performance, regional blood flow, post-exercise, post-exercise hypotension

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Introduction

Traditionally, the field of exercise physiology has been devoted to researching the physiological changes that occur during an acute bout of exercise, and the long-term adaptations to exercise training. More recently, the "physiology of recovery" has emerged as a sub-discipline focused on the time period between the end of a bout of exercise and the subsequent return to what is considered a "resting" or "recovered" state.

Precisely defining "recovery from exercise" is a challenging task due to the varied meanings of recovery. Recovery can refer to a distinct time frame. Depending on the physiological system or pathway of interest, this temporal definition of recovery may range from minutes (e.g., the return of heart rate to near-resting levels) to weeks (e.g., restoration of force-generating capacity after muscle damaging exercise). Additionally, these time frames vary with individual phenotype; for example, trained athletes and individuals with chronic diseases often display altered recovery time courses relative to healthy individuals. Recovery can also refer to specific physiological processes or states, which are distinct from exercise itself and resting physiological states. As a representation of what occurs during the transition from an exercising state to a resting state, we may ask, how do we enhance recovery in athletes? Lastly, recovery can refer to an end-point, e.g., having reached a state of recovery after a bout of exercise, or a starting-point, e.g., an athlete has recovered from

prior training and is physiologically ready for additional training stress, or an injured athlete has recovered and can return to play.

This emerging research area, the physiology of recovery, encompasses multiple physiological systems, and is ripe for rigorous study by integrative physiologists with an interest in generating novel insights related to exercise and physical activity. Translating the basic science of recovery from exercise into practical applications related to human health and performance drives much of the interest in pursuing this intriguing (but often overlooked) aspect of exercise physiology. In this perspective, we identify three distinct (but not mutually exclusive) paradigms that drive the need to investigate the human physiology of recovery from exercise.

Recovery from Exercise: A Vulnerable State?

There is substantial evidence that regular endurance and resistance exercise training reduces vulnerability to a number of chronic diseases and conditions (Booth et al., 2000). However, despite the countless beneficial effects of exercise on health and well-being, some individuals may be vulnerable to negative health outcomes (ranging from minor to life threatening) during recovery from exercise.

A dramatic example of this is the significant risk of sudden cardiac death in the 30 min following a bout of vigorous activity in men free from overt cardiovascular disease, as reported in the Physicians' Health Study (Albert et al., 2000). While this data appears alarming, these authors also note that the overall absolute risk of sudden cardiac death after exercise is quite low, with estimates of 1 death occurring for every 1.5 million bouts of exercise in men, and is even more rare in women, with 1 death for every 36.5 million hours (the difference between bouts of exercise in men and hours of exercise in women are reflective of the measurements reported in the respective studies) (Albert et al., 2000; Whang et al., 2006). The physiological mechanisms underpinning these adverse events are varied, but are likely due to cardiac abnormalities in structure or function in young individuals that lead to fatal arrhythmias, and to the disruption of unstable atherosclerotic plaques resulting in myocardial infarction in adults, particularly in previously sedentary individuals (Thompson et al., 2007). Recommended screening that includes information about previous episodes of exercise-related syncope and screening for cardiac abnormalities can identify individuals at high risk of exercise-related sudden cardiac death, especially in young athletes who are unlikely to have atherosclerotic cardiovascular disease (Maron et al., 1996; Bille et al., 2006).

Although it can be a predictor of sudden cardiac death, post-exercise syncope in the absence of structural or functional cardiac abnormalities is most often benign. An obvious example is that of prolonged dynamic exercise in warm weather, which generates a combination of blood volume loss/dehydration and elevated cutaneous blood flow that contribute to reduced venous return, predisposing individuals to orthostatic intolerance (heat syncope) during or after exercise (Hayes et al., 2000; González-Alonso, 2007). However, even in the absence of heat stress and hypovolemia, between 50 and 80% of otherwise healthy adults develop pre-syncopal signs and symptoms when subjected to head-up tilt following exercise, as recently reviewed (Halliwill et al., 2014). Both prolonged endurance and brief intense exercise predispose individuals to syncope or pre-syncopal symptoms by reducing orthostatic tolerance, a manifestation of an altered physiological state which is distinct from both the exercising state and the resting state (Bjurstedt et al., 1983; Halliwill, 2001; Halliwill et al., 2013). Briefly, post-exercise syncope (in individuals without underlying cardiac or vascular dysfunction, and in the absence of heat stress and hypovolemia) is multifactorial, involving centrally mediated sympatho-inhibition, local sustained release of a post-exercise vasodilator substance within the previously active skeletal muscle, loss of the muscle pump, and in some cases, hyperventilation induced cerebral vasoconstriction (Halliwill et al., 1996; VanNess et al., 1996; Carter et al., 1999; Kulics et al., 1999; MacDonald, 2002; Moynes et al., 2013). Research into this post-exercise phenomenon has provided insight into effective countermeasures against pre-syncopal symptoms (McCord et al., 2008; Lacewell et al., 2014). Wieling et al. (2015) have identified physical countermeasures, such as bending and contracting lower body muscles, that engage the skeletal muscle pump to augment venous return after exercise. External countermeasures, such as the impedance threshold device, which generate negative intrathoracic pressure to enhance venous return, also protect against pre-syncopal symptoms post-exercise (Lacewell et al., 2014). Lower limb compression garments, which have recently become popular among elite and recreational athletes, may also reduce pre-syncopal signs and symptoms after exercise (Privett et al., 2010).

Another vulnerable state is that of delayed-onset muscle soreness (DOMS), a common occurrence among individuals performing unfamiliar or strenuous exercise that can occur with either endurance or resistance exercise (Armstrong, 1984; Cheung et al., 2003). The muscle fiber and connective tissue damage caused by novel exercise results in a temporary decrement in muscle force development, in addition to the pain and muscle tenderness that is characteristic of this condition. The inflammatory process occurring over the following 48 h after the damaging exercise bout results in macrophage infiltration and edema which is implicated as critical in resolving the muscle damage associated with DOMS, but it is also responsible for the pain and discomfort associated with this condition (Armstrong, 1984; Smith, 1991). Complete recovery from DOMS may take weeks, but this phenomenon is complex and specific components of recovery may vary in duration. Whether this vulnerable state can be mitigated by interventions during recovery is a vibrant area of research, particularly among coaches and athletic training staff who are concerned about returning athletes to full capacity for training and competition. A number of recent articles have investigated the impact of common treatments used to prevent or attenuate soreness, including post-exercise cryotherapy and non-steroidal anti-inflammatory drugs (NSAIDs), on exercise recovery characteristics. The current consensus appears to be that cryotherapy has a negligible impact on alleviating discomfort,

but may hinder the skeletal muscle repair and recovery process (Isabell et al., 1992; Paddon-Jones and Quigley, 1997; Sellwood et al., 2007). Likewise, NSAIDs may also hinder the repair and recovery, but can alleviate some of the discomfort (Urso, 2013). Support for alternative modalities such as massage or light exercise on DOMS-associated pain remains largely inconclusive, but they potentially exert a mild analgesic effect without hindering repair and recovery.

Recovery from Exercise: A Window of Opportunity?

For many physiological systems, recovery from exercise provides a window of opportunity to maximize or even exploit the altered physiology of the recovery period. Many of the responses we discuss here occur anywhere from 2 to 3 h immediately following exercise (e.g., post-exercise hypotension), but may last up to 48 h or more (e.g., altered blood lipids). Athletes have been taking advantage of the physiology of recovery to improve training and athletic performance during competition, by strategically consuming macronutrients during recovery. In the context of clinical populations, recovery from exercise can be exploited to mitigate the negative effects of some chronic diseases. In this section, we discuss just a few situations where recovery from exercise provides a window of opportunity to maximize the benefits of exercise.

Exercise training is a common intervention for many chronic diseases and conditions, both for the long-term training benefits, but also for the acute effects of a single bout of exercise. A bout of dynamic exercise transiently increases insulin sensitivity, decreases blood lipid levels, and reduces blood pressure after exercise, making exercise and the subsequent recovery period an ideal time for therapeutic intervention in individuals with these cardiovascular risk factors (Braun et al., 1995; Crouse et al., 1997, 1995; Grandjean et al., 2000; Holloszy, 2005; Halliwill et al., 2013). In fact, repeated bouts of exercise at least every other day have been suggested as a treatment for high cholesterol (Crouse et al., 1997). The post-exercise "window of opportunity" could be used to exploit these transient changes associated with exercise; for example, this may be a time when pharmacological interventions may act synergistically with enhanced insulin sensitivity and blunted blood lipid levels. Ideally, these interventions would slow or reverse the progression of chronic diseases, thus reducing the need for pharmacological interventions and improving quality of life in these individuals.

Our research group and others have documented the phenomenon of sustained post-exercise hypotension in young healthy adults, and the exaggerated post-exercise hypotensive response in individuals with hypertension (Rueckert et al., 1996; Pescatello et al., 1999; Forjaz et al., 2000; Halliwill, 2001; Halliwill et al., 2013). As with hypercholesterolemia, exercise training (and a single bout of dynamic exercise in particular) induced post-exercise hypotension is a proposed therapy to treat hypertension in some individuals (Hamer, 2006). Evidence from hypertensive animals models suggests that this effect may be at least partially mediated by altered gamma-aminobutyric acid (GABA) signaling in the rostral ventrolateral medulla (RVLM) and nucleus tractus solitarii (NTS), ultimately reducing the gain and range of baroreceptor activity post-exercise (Kajekar et al., 2002; Chen et al., 2009). Further exploiting this mechanism with additional interventions, pharmacological or otherwise, may be a viable treatment option in hypertensive individuals who are resistant to exercise training alone. Pro-angiogenic factors (including vascular endothelial growth factor-a, angiopoietin-2, matrix metalloproteinases) are transiently elevated after an acute exercise bout (Breen et al., 1996; Gustafsson and Kraus, 2001; Hoier et al., 2012), and may also be of therapeutic interest for patients with limited exercise capacity or impaired limb blood flow (e.g., peripheral artery disease, spinal cord injury, or muscular dystrophies). Hypoxic and blood flow restricted exercise have both been hypothesized to enhance the angiogenic adaptation to endurance exercise, although little is known about their effects on angiogenic factors when applied in the post-exercise time period (Esbjörnsson et al., 1993; Minchenko et al., 1994; Richardson et al., 1999; Olfert et al., 2001).The recovery period, when angiogenic factors are already increased, may be a window in which additional therapeutic interventions could prove more potent. It may be possible that reducing blood flow or oxygen delivery post-exercise can have an additive effect on angiogenic signaling induced by exercise alone, although to our knowledge, this has not been experimentally tested in humans.

For athletes concerned with optimizing training and performance, macronutrient intake during recovery may be a key component of their training regimen. The metabolic changes associated with both endurance and resistance exercise and recovery may be enhanced with appropriate nutrient timing strategies. In endurance athletes, maximizing skeletal muscle glycogen storage by ingesting carbohydrates in recovery has a significant effect on subsequent performance. This is taking advantage of the physiology of recovery related to glucose transporters, insulin sensitivity, and perhaps elevations in blood flow (Emhoff et al., 2011). For power and strength athletes, as well as endurance athletes, there is an analogous window of opportunity based on the elevated rate of protein synthesis in recovery, so that this time period is ripe for protein ingestion (Levenhagen et al., 2001; Areta et al., 2013). Optimization of macronutrient intake during recovery is a large area of research related to human performance, and may translate to clinical populations and older adults (Esmarck et al., 2001).

Recent evidence also suggests that interventions such as muscle cooling, applied during recovery from exercise, can enhance skeletal muscle expression of transcription factor PGC-1α, potentially promoting mitochondrial biogenesis beyond levels observed without this intervention (Ihsan et al., 2014). This finding provides a contrast to the use of cryotherapy for mitigating muscle soreness and inflammation, which was discussed above, and leads to the interesting possibility that common interventions may have divergent effects on muscle recovery, depending on the outcome variable of interest (i.e., acute inflammation vs. skeletal muscle mitogenesis). Obviously, more research focused on unraveling these complex and interconnected pathways is necessary, and may provide valuable

insight into the unique physiology of recovery from exercise and how it can be exploited to improve athletic or clinical outcomes.

Recovery from Exercise: A Crystal Ball?

In clinical settings, exercise testing has clear and proven prognostic value, providing insight into future disease risk. Among apparently healthy and clinical populations, the physiology of recovery can also predict an individual's risk of an adverse health outcome. For example, heart rate recovery between 1 and 5 min after a moderate intensity bout of dynamic exercise is an independent predictor of all-cause mortality (Johnson and Goldberger, 2012). Both heart rate and blood pressure recovery can provide non-invasive clinical indicators related to autonomic function, making these simple measurements highly informative (Terziotti et al., 2001; Buchheit et al., 2007; Cahalin et al., 2013). In fact, measurements in recovery can non-invasively be used to assess future clinical risks that would otherwise not be apparent in a typical health screening (Cole et al., 2000; Shetler et al., 2001).

Beyond general screening functions, blood pressure recovery and post-exercise hypotension after a single bout of exercise are predictive of an individual's blood pressure response to chronic exercise training (Liu et al., 2012; Hecksteden et al., 2013). This simple, minimally invasive test could make effective use of resources in a clinical setting to identify individuals who are responsive to blood pressure reductions with training, and individuals who may require additional pharmacological intervention. In these cases, recovery from exercise provides researchers and clinicians clues to patient cardiovascular health. This concept aligns with current interest in identifying "responders" and "nonresponders" to exercise and exercise training (Karavirta et al., 2011; Timmons, 2011). With additional research in this area, it may soon be possible to identify individuals who may reap more health or performance benefits from one type of training (e.g., endurance vs. resistance training), or may also identify individuals for whom exercise would be contra-indicated (e.g., hypertrophic cardiomyopathy) (Keller et al., 2011). To our knowledge, there are currently no studies that have identified recovery from exercise variables as a means to identify responders vs. non-responders to specific exercise interventions, but this may be an interesting future direction for the physiology of recovery.

Can these notions be generalized beyond the realm of the cardiovascular and autonomic nervous systems? Relatively little is currently known about how other major organ systems recover from exercise, or how other recovery phenotypes could provide clues about either future health or even future athletic performance. For example, are there individual differences in recovery of skeletal muscle function and force development that could predict development or loss of muscle strength or function with age or training? Or could metabolic recovery predict adaptations in substrate utilization that may identify individuals for whom exercise training may prevent insulin resistance? These questions may appear far-fetched, but given what we now understand about individual responses to exercise and exercise training, it would not be surprising to discover that individual recovery from exercise is heterogeneous, phenotype-sensitive, and can be exploited for prediction of health or athletic performance benefits. It is also conceivable that the results of a simple recovery test which predicts an individual's mortality risk could provide sufficient motivation for some individuals to make lifestyle improvements to mitigate this risk. Given the relative ease of tracking heart rate and blood pressure, these recovery measurements could be made on an individual level to monitor exercise effectiveness or track health outcomes. Ideally, this information will inform future patient care through personalized medication and exercise prescription (i.e., precision exercise training). As research on the physiology of recovery expands, there is great potential for studies to find new "crystal ball" forecasters of future health.

The Trifecta of Recovery

As evidenced by the diversity of systems engaged during recovery from exercise, this field of research has implications for both human health and athletic performance, and can be useful to researchers and healthcare professionals alike. From what is currently known about the physiology of recovery, the three paradigms we have outlined in this perspective all likely overlap within an individual after a single bout of exercise. For example, our personal interest in the sustained post-exercise vasodilation crosses all three paradigms, creating vulnerabilities and opportunities, and providing prognostic implications, as depicted in **Figure 1**. Such responses allow for potentially different pathways of intervention, depending on the health and goals of the individual. For example, an athlete vulnerable to post-exercise syncope may choose

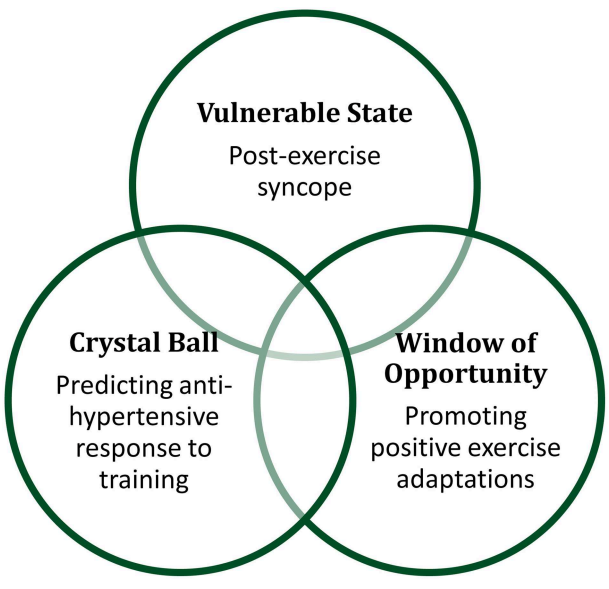

FIGURE 1 | Recovery from exercise can be conceptualized as creating vulnerable states, windows of opportunity, and providing crystal ball prognostic value.

to perform physical counter-maneuvers to prevent syncopal symptoms, rather than pursue a pharmacological intervention that may close the window of opportunity after exercise for beneficial exercise training effects. By conceptualizing the physiology of recovery as this balance of vulnerable state, window of opportunity, and crystal ball paradigms provides a way to frame lines of inquiry and help broaden the field of exercise physiology in exciting directions for the benefit of the clinical patient, elite athlete, and weekend warrior alike.

References

Albert, C. M., Mittleman, M. A., Chae, C. U., Lee, I. M., Hennekens, C. H., and Manson, J. E. (2000). Triggering of sudden death from cardiac causes by vigorous exertion. *N. Engl. J. Med.* 343, 1355–1361. doi: 10.1056/NEJM200011093431902

Areta, J. L., Burke, L. M., Ross, M. L., Camera, D. M., West, D. W. D., Broad, E. M., et al. (2013). Timing and distribution of protein ingestion during prolonged recovery from resistance exercise alters myofibrillar protein synthesis. *J. Physiol.* 591, 2319–2331. doi: 10.1113/jphysiol.2012.244897

Armstrong, R. B. (1984). Mechanisms of exercise-induced delayed onset muscular soreness: a brief review. *Med. Sci. Sport. Exerc.* 16, 529–538. doi: 10.1249/00005768-198412000-00002

Bille, K., Figueiras, D., Schamasch, P., Kappenberger, L., Brenner, J. I., Meijboom, F. J., et al. (2006). Sudden cardiac death in athletes: the Lausanne Recommendations. *Eur. J. Cardiovasc. Prev. Rehabil.* 13, 859–875. doi: 10.1097/01.hjr.0000238397.50341.4a

Bjurstedt, H., Rosenhamer, G., Balldin, U., and Katkov, V. (1983). Orthostatic reactions during recovery from exhaustive exercise of short duration. *Acta Physiol. Scand.* 119, 25–31. doi: 10.1111/j.1748-1716.1983.tb07301.x

Booth, F. W., Gordon, S. E., Carlson, C. J., and Hamilton, M. T. (2000). Waging war on modern chronic diseases: primary prevention through exercise biology. *J. Appl. Physiol.* 88, 774–787.

Braun, B., Zimmermann, M. B., and Kretchmer, N. (1995). Effects of exercise intensity on insulin sensitivity in women with non-insulin-dependent diabetes mellitus. *J. Appl. Physiol.* 78, 300–306.

Breen, E. C., Johnson, E. C., Wagner, H., Tseng, H. M., Sung, L. A., and Wagner, P. D. (1996). Angiogenic growth factor mRNA responses in muscle to a single bout of exercise. *J. Appl. Physiol.* 81, 355–361.

Buchheit, M., Papelier, Y., Laursen, P. B., and Ahmaidi, S. (2007). Noninvasive assessment of cardiac parasympathetic function: postexercise heart rate recovery or heart rate variability? *Am. J. Physiol. Heart Circ. Physiol.* 293, H8–H10. doi: 10.1152/ajpheart.00335.2007

Cahalin, L. P., Forman, D. E., Chase, P., Guazzi, M., Myers, J., Bensimhon, D., et al. (2013). The prognostic significance of heart rate recovery is not dependent upon maximal effort in patients with heart failure. *Int. J. Cardiol.* 168, 1496–1501. doi: 10.1016/j.ijcard.2012.12.102

Carter, R. I., Watenpaugh, D. E., Wasmund, W. L., Wasmund, S. L., and Smith, M. L. (1999). Muscle pump and central command during recovery from exercise in humans. *J. Appl. Physiol.* 87, 1463–1469.

Chen, C.-Y., Bechtold, A. G., Tabor, J., and Bonham, A. C. (2009). Exercise reduces GABA synaptic input onto nucleus tractus solitarii baroreceptor second-order neurons via NK1 receptor internalization in spontaneously hypertensive rats. *J. Neurosci.* 29, 2754–2761. doi: 10.1523/JNEUROSCI.4413-08.2009

Cheung, K., Hume, P. A., and Maxwell, L. (2003). Delayed onset muscle soreness. *Sport. Med.* 33, 145–164. doi: 10.2165/00007256-200333020-00005

Cole, C. R., Foody, J. M., Blackstone, E. H., and Lauer, M. S. (2000). Heart rate recovery after submaximal exercise testing as a predictor of mortality in a cardiovascularly healthy cohort. *Ann. Intern. Med.* 132, 552–555. doi: 10.7326/0003-4819-132-7-200004040-00007

Crouse, S. F., O'Brien, B. C., Grandjean, P. W., Lowe, R. C., Rohack, J. J., and Green, J. S. (1997). Effects of training and a single session of exercise on lipids and apolipoproteins in hypercholesterolemic men. *J. Appl. Physiol.* 83, 2019–2028.

Crouse, S. F., O'Brien, B. C., Rohack, J. J., Lowe, R. C., Green, J. S., Tolson, H., et al. (1995). Changes in serum lipids and apolipoproteins after exercise in men with high cholesterol: influence of intensity. *J. Appl. Physiol.* 79, 279–286.

Emhoff, C. A., Barrett-O'Keefe, Z., Padgett, R. C., Hawn, J. A., and Halliwill, J. R. (2011). Histamine-receptor blockade reduces blood flow but not muscle glucose uptake during postexercise recovery in humans. *Exp. Physiol.* 96, 664–673. doi: 10.1113/expphysiol.2010.056150

Esbjörnsson, M., Jansson, E., Sundberg, C. J., Sylvén, C., Eiken, O., Nygren, A., et al. (1993). Muscle fibre types and enzyme activities after training with local leg ischaemia in man. *Acta Physiol. Scand.* 148, 233–241. doi: 10.1111/j.1748-1716.1993.tb09554.x

Esmarck, B., Andersen, J. L., Olsen, S., Richter, E. A., Mizuno, M., and Kjaer, M. (2001). Timing of postexercise protein intake is important for muscle hypertrophy with resistance training in elderly humans. *J. Physiol.* 535, 301–311. doi: 10.1111/j.1469-7793.2001.00301.x

Forjaz, C. L., Tinucci, T., Ortega, K. C., Santaella, D. F., Mion, D., and Negrão, C. E. (2000). Factors affecting post-exercise hypotension in normotensive and hypertensive humans. *Blood Press. Monit.* 5, 255–262. doi: 10.1097/00126097-200010000-00002

González-Alonso, J. (2007). Separate and combined influences of dehydration and hyperthermia on cardiovascular responses to exercise. *Int. J. Sports Med.* 19, S111–S114. doi: 10.1055/s-2007-971972

Grandjean, P. W., Crouse, S. F., and Rohack, J. J. (2000). Influence of cholesterol status on blood lipid and lipoprotein enzyme responses to aerobic exercise. *J. Appl. Physiol.* 89, 472–480.

Gustafsson, T., and Kraus, W. E. (2001). Exercise-induced angiogenesis-related growth and transcription factors in skeletal muscle, and their modification in muscle pathology. *Front. Biosci.* 6, D75–D89. doi: 10.2741/gustafss

Halliwill, J. R. (2001). Mechanisms and clinical implications of post-exercise hypotension in humans. *Exerc. Sport Sci. Rev.* 29, 65–70. doi: 10.1097/00003677-200104000-00005

Halliwill, J. R., Buck, T. M., Lacewell, A. N., and Romero, S. A. (2013). Postexercise hypotension and sustained postexercise vasodilation: what happens after we exercise? *Exp. Physiol.* 98, 7–18. doi: 10.1113/expphysiol.2011.058065

Halliwill, J. R., Sieck, D. C., Romero, S. A., Buck, T. M., and Ely, M. R. (2014). Blood pressure regulation X: what happens when the muscle pump is lost? Post-exercise hypotension and syncope. *Eur. J. Appl. Physiol.* 114, 561–578. doi: 10.1007/s00421-013-2761-1

Halliwill, J. R., Taylor, J. A., and Eckberg, D. L. (1996). Impaired sympathetic vascular regulation in humans after acute dynamic exercise. *J. Physiol.* 495, 279–288. doi: 10.1113/jphysiol.1996.sp021592

Hamer, M. (2006). The anti-hypertensive effects of exercise: integrating acute and chronic mechanisms. *Sport. Med* 36, 109–116. doi: 10.2165/00007256-200636020-00002

Hayes, P. M., Lucas, J. C., and Shi, X. (2000). Importance of post-exercise hypotension in plasma volume restoration. *Acta Physiol. Scand.* 169, 115–124. doi: 10.1046/j.1365-201x.2000.00728.x

Hecksteden, A., Grütters, T., and Meyer, T. (2013). Association between postexercise hypotension and long-term training-induced blood pressure reduction: a pilot study. *Clin. J. Sport Med.* 23, 58–63. doi: 10.1097/JSM.0b013e31825b6974

Hoier, B., Nordsborg, N., Andersen, S., Jensen, L., Nybo, L., Bangsbo, J., et al. (2012). Pro- and anti-angiogenic factors in human skeletal muscle in response to acute exercise and training. *J. Physiol.* 590, 595–606. doi: 10.1113/jphysiol.2011.216135

Holloszy, J. O. (2005). Exercise-induced increase in muscle insulin sensitivity. *J. Appl. Physiol.* 99, 338–343. doi: 10.1152/japplphysiol.00123.2005

Ihsan, M., Watson, G., Choo, H. C., Lewandowski, P., Papazzo, A., Cameron-Smith, D., et al. (2014). Postexercise muscle cooling enhances gene expression of PGC-1α. *Med. Sci. Sport. Exerc.* 46, 1900–1907. doi: 10.1249/MSS.0000000000000308

Isabell, W. K., Durrant, E., Myrer, W., and Anderson, S. (1992). The effects of ice massage, ice massage with exercise, and exercise on the prevention and treatment of delayed onset muscle soreness. *J. Athl. Train.* 27, 208–217.

Johnson, N. P., and Goldberger, J. J. (2012). Prognostic value of late heart rate recovery after treadmill exercise. *Am. J. Cardiol.* 110, 45–49. doi: 10.1016/j.amjcard.2012.02.046

Kajekar, R., Chen, C.-Y., Mutoh, T., and Bonham, A. C. (2002). GABA(A) receptor activation at medullary sympathetic neurons contributes to postexercise hypotension. *Am. J. Physiol. Heart Circ. Physiol.* 282, H1615–H1624. doi: 10.1152/ajpheart.00725.2001

Karavirta, L., Häkkinen, K., Kauhanen, A., Arija-Blázquez, A., Sillanpää, E., Rinkinen, N., et al. (2011). Individual responses to combined endurance and strength training in older adults. *Med. Sci. Sports Exerc.* 43, 484–490. doi: 10.1249/MSS.0b013e3181f1bf0d

Keller, P., Vollaard, N. B. J., Gustafsson, T., Gallagher, I. J., Sundberg, C. J., Rankinen, T., et al. (2011). A transcriptional map of the impact of endurance exercise training on skeletal muscle phenotype. *J. Appl. Physiol.* 110, 46–59. doi: 10.1152/japplphysiol.00634.2010

Kulics, J. M., Collins, H. L., and DiCarlo, S. E. (1999). Postexercise hypotension is mediated by reductions in sympathetic nerve activity. *Am. J. Physiol. Hear. Circ Physiol.* 276, H27–H32.

Lacewell, A. N., Buck, T. M., Romero, S. A., and Halliwill, J. R. (2014). Post-exercise syncope: wingate syncope test and effective countermeasure. *Exp. Physiol.* 99, 172–186. doi: 10.1113/expphysiol.2013.075333

Levenhagen, D. K., Gresham, J. D., Carlson, M. G., Maron, D. J., Borel, M. J., and Flakoll, P. J. (2001). Postexercise nutrient intake timing in humans is critical to recovery of leg glucose and protein homeostasis. *Am. J. Physiol. Endocrinol. Metab.* 280, E982–E993.

Liu, S., Goodman, J., Nolan, R., Lacombe, S., and Thomas, S. G. (2012). Blood pressure responses to acute and chronic exercise are related in prehypertension. *Med. Sci. Sport. Exerc.* 44, 1644–1652. doi: 10.1249/MSS.0b013e318 25408fb

MacDonald, J. R. (2002). Potential causes, mechanisms, and implications of post exercise hypotension. *J. Hum. Hypertens.* 16, 225–236. doi: 10.1038/sj.jhh.1001377

Maron, B. J., Thompson, P. D., Puffer, J. C., McGrew, C. A., Strong, W. B., Douglas, P. S., et al. (1996). Cardiovascular preparticipation screening of competitive athletes: a statement for health professionals from the Sudden Death Committee (clinical cardiology) and Congenital Cardiac Defects Committee (cardiovascular disease in the young), American Heart As. *Circulation* 94, 850–856. doi: 10.1161/01.CIR.94.4.850

McCord, J. L., Pellinger, T. K., Lynn, B. M., and Halliwill, J. R. (2008). Potential benefit from an H1-receptor antagonist on postexercise syncope in the heat. *Med. Sci. Sport. Exerc.* 40, 1953–1961. doi: 10.1249/MSS.0b013e31817f1970

Minchenko, A., Bauer, T., Salceda, S., and Caro, J. (1994). Hypoxic stimulation of vascular endothelial growth factor expression *in vitro* and *in vivo*. *Lab. Invest.* 71, 374–379.

Moynes, J., Bentley, R. F., Bravo, M., Kellawan, J. M., and Tschakovsky, M. E. (2013). Persistence of functional sympatholysis post-exercise in human skeletal muscle. *Front. Physiol.* 4:131. doi: 10.3389/fphys.2013.00131

Olfert, I. M., Breen, E. C., Mathieu-Costello, O., and Wagner, P. D. (2001). Skeletal muscle capillarity and angiogenic mRNA levels after exercise training in normoxia and chronic hypoxia. *J. Appl. Physiol.* 91, 1176–1184.

Paddon-Jones, D. J., and Quigley, B. M. (1997). Effect of cryotherapy on muscle soreness and strength following eccentric exercise. *Int. J. Sports Med.* 18, 588–593. doi: 10.1055/s-2007-972686

Pescatello, L. S., Miller, B., Danias, P. G., Werner, M., Hess, M., Baker, C., et al. (1999). Dynamic exercise normalizes resting blood pressure in mildly hypertensive premenopausal women. *Am. Heart J.* 138, 916–921. doi: 10.1016/S0002-8703(99)70017-7

Privett, S. E., George, K. P., Whyte, G. P., and Cable, N. T. (2010). The effectiveness of compression garments and lower limb exercise on post-exercise blood pressure regulation in orthostatically intolerant athletes. *Clin. J. Sport Med.* 20, 362–367. doi: 10.1097/JSM.0b013e3181f20292

Richardson, R. S., Wagner, H., Mudaliar, S. R. D., Henry, R., Noyszewski, E. A., and Wagner, P. D. (1999). Human VEGF gene expression in skeletal muscle: effect of acute normoxic and hypoxic exercise. *Am. J. Physiol. Hear. Circ Physiol.* 277, H2247–H2252.

Rueckert, P. A., Slane, P. R., lillis, D. L., and Hanson, P. (1996). Hemodynamic patterns and duration of post-dynamic exercise hypotension in hypertensive humans. *Med. Sci. Sport. Exerc.* 28, 24–32. doi: 10.1097/00005768-199601000-00010

Sellwood, K. L., Brukner, P., Williams, D., Nicol, A., and Hinman, R. (2007). Ice-water immersion and delayed-onset muscle soreness: a randomised controlled trial. *Br. J. Sports Med.* 41, 392–397. doi: 10.1136/bjsm.2006.033985

Shetler, K., Marcus, R., Froelicher, V. F., Vora, S., Kalisetti, D., Prakash, M., et al. (2001). Heart rate recovery: validation and methodologic issues. *J. Am. Coll. Cardiol.* 38, 1980–1987. doi: 10.1016/S0735-1097(01)01652-7

Smith, L. L. (1991). Acute inflammation: the underlying mechanism in delayed onset muscle soreness? *Med. Sci. Sport. Exerc.* 23, 542–551. doi: 10.1249/00005768-199105000-00006

Terziotti, P., Schena, F., Gulli, G., and Cevese, A. (2001). Post-exercise recovery of autonomic cardiovascular control: a study by spectrum and cross-spectrum analysis in humans. *Eur. J. Appl. Physiol.* 84, 187–194. doi: 10.1007/s004210170003

Thompson, P. D., Franklin, B. A., Balady, G. J., Blair, S. N., Corrado, D., Estes, N. A. M., et al. (2007). Exercise and acute cardiovascular events placing the risks into perspective: a scientific statement from the American Heart Association Council on Nutrition, Physical Activity, and Metabolism and the Council on Clinical Cardiology. *Circulation* 115, 2358–2368. doi: 10.1161/CIRCULATIONAHA.107.181485

Timmons, J. A. (2011). Variability in training-induced skeletal muscle adaptation. *J. Appl. Physiol.* 110, 846–853. doi: 10.1152/japplphysiol.00934.2010

Urso, M. L. (2013). Anti-inflammatory interventions and skeletal muscle injury: benefit or detriment? *J. Appl. Physiol.* 115, 920–928. doi: 10.1152/japplphysiol.00036.2013

VanNess, J. M., Takata, H. J., and Overton, J. M. (1996). Attenuated blood pressure responsiveness during post-exercise hypotension. *Clin. Exp. Hypertens.* 18, 891–900. doi: 10.3109/10641969609097906

Whang, W., Manson, J. E., Hu, F. B., Chae, C. U., Rexrode, K. M., Willett, W. C., et al. (2006). Physical exertion, exercise, and sudden cardiac death in women. *JAMA* 295, 1399–1403. doi: 10.1001/jama.295.12.1399

Wieling, W., van Dijk, N., Thijs, R. D., de Lange, F. J., Krediet, C. T. P., and Halliwill, J. R. (2015). Physical countermeasures to increase orthostatic tolerance. *J. Int. Med.* 277, 69–82. doi: 10.1111/joim.12249

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OPINION
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Specificity and context in post-exercise recovery: it is not a one-size-fits-all approach

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Keywords: muscle damage, fatigue, cryotherapy, cold water immersion, training adaptation

The concept of specificity of exercise prescription and training is a longstanding and widely accepted foundation of the exercise sciences. Simply, the principle holds that training adaptations are achieved relative to the stimulus applied. That is, the manipulation of training variables (e.g., intensity or loading, mode, volume, and frequency) directly influences the acute training stimulus, and so the long-term adaptive response (Young et al., 2001; Bird et al., 2005). Translating this concept to practice then recommends that exercise be prescribed specific to the desired outcomes, and the more closely this is achieved, the greater the performance gain is likely to be. However, the cardiovascular and metabolic adaptations traditionally associated with long, slow distance training types, similarly achieved using high-intensity training methods (for a review see Gibala et al., 2012), highlights understanding of underlying physiology as paramount for effective training program design. Various other factors including illness, sleep, and psychology also impact on the training stimulus (Halson, 2014) and must be managed collectively with appropriate post-exercise recovery to continue performance improvements and reduce overtraining and injury risks (Kenttä and Hassmén, 1998).

Despite the emphasis that is placed on specificity in the application of the desired training stimulus, it is noteworthy that this concept receives less attention within the post-exercise recovery literature. Indeed, most recovery strategies are intended to treat only symptoms of exercise-induced muscle damage by blunting inflammatory responses associated with disturbances to the structural integrity of the exercised musculature (Minett and Duffield, 2014). Be it through lifestyle (e.g., active recovery, sleep), physiological (e.g., post-exercise cooling, massage, compression), or nutritional and pharmacological interventions (e.g., supplements, anti-inflammatory medications), these common recovery techniques aim to hasten regenerative processes below the neuromuscular junction with limited consideration for other causative mechanisms (Minett and Duffield, 2014). Compounded by the use of different exercise tasks under varying environmental conditions (e.g., hot vs. thermo-neutral temperatures) and contexts (e.g., isolated vs. repeated exercise bouts, pre-season vs. competition), this tendency for a *one-size-fits-all* approach to post-exercise recovery is likely to contribute to contrasting reports of the efficacy of many techniques. While these concerns could indicate the need for greater understanding of the mechanistic demands of specific exercise tasks and post-exercise recovery protocols so to be suitably matched, they are of great consequence in applied settings where maladaptation may be the result of inappropriate recovery practices (Kenttä and Hassmén, 1998).

The research narrative surrounding post-exercise cooling for recovery reflects this point. A derivative of the use of ice as a therapeutic treatment of soft tissue injuries, the proposed benefits of acute cooling interventions on recovery after exercise relate to peripheral vasoconstriction centralizing blood volume away from exercised musculature (Bleakley and Davison, 2009). This is proposed to benefit metabolite removal, biochemical expression of damage and inflammation,

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swelling and soreness (Bleakley and Davison, 2009; Costello et al., 2013). While a series of meta-analyses show considerable variance in the effectiveness of post-exercise cooling in optimizing performance return (e.g., strength, jump, and sprint variables) (Bleakley et al., 2012; Leeder et al., 2012; Poppendieck et al., 2013), evidence for a disconnect between the rise in blood-based muscle damage markers and the recovery of neuromuscular force production is noteworthy (Pointon et al., 2012; Minett et al., 2014). It could be reasoned that the indirect nature of biochemical time course expressions of common variables reported during recovery (e.g., creatine kinase) may not necessarily directly reflect concurrent neuromuscular function, though it does at least question the traditional rationale for using post-exercise cooling and how it influences performance recovery. Further, such reports give strength to the argument that physiological rationale for post-exercise cooling is limited and that any ergogenic influences reflect a perceptual or placebo effect (Broatch et al., 2014).

Irrespective of whether post-exercise cooling benefits recovery through the treatment of exercise-induced muscle damage or through other means, the specificity of administration of this intervention becomes key. Most pertinent, changes in circulatory dynamics and muscle metabolism as a result of post-exercise cooling (Vaile et al., 2011; Ihsan et al., 2013) seemingly contrast the blood flow needs required for muscle protein synthesis and training adaptation to occur (Yamane et al., 2006; Fröhlich et al., 2014). Roberts et al. (2014) recently suggested that the greater work capacity achieved using cold-water immersion recovery after resistance training could facilitate advantageous chronic adaptations, with similar conclusion drawn after the maintenance of force output following intermittent-sprint performance (Pointon et al., 2012; Minett et al., 2014). Contrastingly, however, interactions between the use of post-exercise cooling recoveries and training adaptation may be exercise specific, with both positive (Halson et al., 2014) and negative findings (Fröhlich et al., 2014) reported in cyclists and strength trained individuals, respectively. For example, while highly speculative, it might be hypothesized that the upregulation of PGC-1α expression and nitric oxide production after post-exercise cooling could stimulate GLUT4 translocation and muscle glucose uptake (Ihsan et al., 2014), thus augmenting mRNA expressions of genes associated with cellular metabolism and mitochondrial biogenesis achieved through endurance exercise (Mahoney et al., 2005). Equally, as resistance training stresses different pathways, maladaptation reported could be resultant of delayed amino acid delivery with blood flow changes (Biolo et al., 1995), or altered macrophage activity and a lesser growth factor concentrations as a result of cold application (Takagi et al., 2011). Regardless, this does point to the need for matching of training stimulus and recovery mechanisms to avoid unfavorable outcomes.

The topic of post-exercise recovery from training has been the focus of recent attention in both narrative (e.g., Nédélec et al., 2013; Minett and Duffield, 2014) and systematic reviews (e.g., Bleakley et al., 2012; Leeder et al., 2012; Bieuzen et al., 2013; Costello et al., 2013; Poppendieck et al., 2013). While these literature detail the physiological, perceptual, and performance effects during recovery, discussion as to the specificity and context within which interventions are best applied is limited. Emphasis should be placed on the matching of the recovery needs (e.g., cellular vs. specific systems, or both) with those affected by any particular recovery approach. As seen with the recent training studies focused on the use of post-exercise cooling recoveries (Fröhlich et al., 2014; Halson et al., 2014), chronic adaptations are affected by recovery choices. In the case of the elite sporting environment where small changes often represent a meaningful difference for performance outcomes, informed decisions surrounding the context of post-exercise recovery (e.g., timing, frequency, exercise mode) are of utmost importance. Areas for future research include consideration for the individual responses to specific recovery methods, influence of athlete preference or perception, and the need to link this to applied practices where sport-specific skill performance, psychology and usability are as valuable as physiological change.

References

Bieuzen, F., Bleakley, C. M., and Costello, J. T. (2013). Contrast water therapy and exercise induced muscle damage: a systematic review and meta-analysis. *PLoS ONE* 8:e62356. doi: 10.1371/journal.pone.0062356

Biolo, G., Maggi, S. P., Williams, B. D., Tipton, K. D., and Wolfe, R. R. (1995). Increased rates of muscle protein turnover and amino acid transport after resistance exercise in humans. *Am. J. Physiol.* 268, E514–E520.

Bird, S. P., Tarpenning, K. M., and Marino, F. E. (2005). Designing resistance training programmes to enhance muscular fitness. *Sports Med.* 35, 841–851. doi: 10.2165/00007256-200535100-00002

Bleakley, C. M., and Davison, G. W. (2009). What is the biochemical and physiological rationale for using cold-water immersion in sports recovery? A systematic review. *Br. J. Sports Med.* 44, 179–187. doi: 10.1136/bjsm.2009.065565

Bleakley, C., McDonough, S., Gardner, E., Baxter, G. D., Hopkins, J. T., and Davison, G. W. (2012). Cold-water immersion (cryotherapy) for preventing and treating muscle soreness after exercise. *Cochrane Database Syst. Rev.* 2:CD008262. doi: 10.1002/14651858.CD008262.pub2

Broatch, J. R., Petersen, A., and Bishop, D. J. (2014). Postexercise cold water immersion benefits are not greater than the placebo effect. *Med. Sci. Sports Exerc.* 46, 2139–2147. doi: 10.1249/MSS.0000000000000348

Costello, J. T., Baker, P. R. A., Minett, G. M., Bieuzen, F., Stewart, I. B., and Bleakley, C. (2013). Whole-body cryotherapy (extreme cold air exposure) for preventing and treating muscle soreness after exercise in adults (Protocol). *Cochrane Database Syst. Rev.* 2013, Issue 10. Art. No.: CD010789. doi: 10.1002/14651858.CD010789

Fröhlich, M., Faude, O., Klein, M., Pieter, A., Emrich, E., and Meyer, T. (2014). Strength training adaptations after cold-water immersion. *J. Strength Cond. Res.* 28, 2628–2633. doi: 10.1519/JSC.0000000000000434

Gibala, M. J., Little, J. P., MacDonald, M. J., and Hawley, J. A. (2012). Physiological adaptations to low-volume, high-intensity interval training in health and disease. *J. Physiol.* 590, 1077–1084. doi: 10.1113/jphysiol.2011.224725

Halson, S. L. (2014). Monitoring training load to understand fatigue in athletes. *Sports Med.* 44, 139–147. doi: 10.1007/s40279-014-0253-z

Halson, S. L., Bartram, J., West, N., Stephens, J., Argus, C. K., Driller, M. W., et al. (2014). Does hydrotherapy help or hinder adaptation to training in competitive cyclists? *Med. Sci. Sports Exerc.* 46, 1631–1639. doi: 10.1249/MSS.0000000000000268

Ihsan, M., Watson, G., Choo, H. C., Lewandowski, P., Papazzo, A., Cameron-Smith, D., et al. (2014). Postexercise muscle cooling enhances gene expression of PGC-1α. *Med. Sci. Sports Exerc.* 46, 1900–1907. doi: 10.1249/MSS.0000000000000308

Ihsan, M., Watson, G., Lipski, M., and Abbiss, C. R. (2013). Influence of postexercise cooling on muscle oxygenation and blood volume changes. *Med. Sci. Sports Exerc*. 45, 876–882. doi: 10.1249/MSS.0b013e31827e13a2

Kenttä, G., and Hassmén, P. (1998). Overtraining and recovery: a conceptual model. *Sports Med*. 26, 1–16. doi: 10.2165/00007256-199826010-00001

Leeder, J., Gissane, C., van Someren, K., Gregson, W., and Howatson, G. (2012). Cold water immersion and recovery from strenuous exercise: a meta-analysis. *Br. J. Sports Med*. 46, 233–240. doi: 10.1136/bjsports-2011-090061

Mahoney, D. J., Parise, G., Melov, S., Safdar, A., and Tarnopolsky, M. A. (2005). Analysis of global mRNA expression in human skeletal muscle during recovery from endurance exercise. *FASEB J*. 19, 1498–1500. doi: 10.1096/fj.04-3149fje

Minett, G. M., and Duffield, R. (2014). Is recovery driven by central or peripheral factors? A role for the brain in recovery following intermittent-sprint exercise. *Front. Physiol*. 5:24. doi: 10.3389/fphys.2014.00024

Minett, G. M., Duffield, R., Billaut, F., Cannon, J., Portus, M. R., and Marino, F. E. (2014). Cold-water immersion decreases cerebral oxygenation but improves recovery after intermittent-sprint exercise in the heat. *Scand. J. Med. Sci. Sports* 24, 656–666. doi: 10.1111/sms.12060.

Nédélec, M., McCall, A., Carling, C., Legall, F., Berthoin, S., and Dupont, G. (2013). Recovery in soccer: part II-recovery strategies. *Sports Med*. 43, 9–22. doi: 10.1007/s40279-012-0002-0

Pointon, M., Duffield, R., Cannon, J., and Marino, F. E. (2012). Cold water immersion recovery following intermittent-sprint exercise in the heat. *Eur. J. Appl. Physiol*. 112, 2483–2494. doi: 10.1007/s00421-011-2218-3

Poppendieck, W., Faude, O., Wegmann, M., and Meyer, T. (2013). Cooling and performance recovery of trained athletes: a meta-analytical review. *Int. J. Sports Physiol. Perform*. 8, 227–241.

Roberts, L. A., Nosaka, K., Coombes, J. S., and Peake, J. M. (2014). Cold water immersion enhances recovery of submaximal muscle function after resistance exercise. *Am. J. Physiol. Regul. Integr. Comp. Physiol*. 307, R998–R1008. doi: 10.1152/ajpregu.00180.2014.

Takagi, R., Fujita, N., Arakawa, T., Kawada, S., Ishii, N., and Miki, A. (2011). Influence of icing on muscle regeneration after crush injury to skeletal muscles in rats. *J. Appl. Physiol*. 110, 382–388. doi: 10.1152/japplphysiol.01187.2010

Vaile, J., O'Hagan, C., Stefanovic, B., Walker, M., Gill, N., and Askew, C. D. (2011). Effect of cold water immersion on repeated cycling performance and limb blood flow. *Br. J. Sports Med*. 45, 825–829. doi: 10.1136/bjsm.2009.067272

Yamane, M., Teruya, H., Nakano, M., Ogai, R., Ohnishi, N., and Kosaka, M. (2006). Post-exercise leg and forearm flexor muscle cooling in humans attenuates endurance and resistance training effects on muscle performance and on circulatory adaptation. *Eur. J. Appl. Physiol*. 96, 572–580. doi: 10.1007/s00421-005-0095-3

Young, W. B., McDowell, M. H., and Scarlett, B. J. (2001). Specificity of sprint and agility training methods. *J. Strength Cond. Res*. 15, 315–319. doi: 10.1519/00124278-200108000-00009

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TNF-α and TNFR1 responses to recovery therapies following acute resistance exercise

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The purpose of this investigation was to compare the effect of two commonly used therapeutic modalities (a) neuromuscular electrical stimulation (NMES) and (b) cold water immersion (CWI) on circulating tumor necrosis factor alpha (TNF-α) and monocyte TNF-α receptor (TNFR1) expression following intense acute resistance exercise and subsequent recovery. Thirty ($n = 30$) resistance trained men (22.5 ± 2.7 y) performed an acute heavy resistance exercise protocol on three consecutive days followed by one of three recovery methods (CON, NMES, and CWI). Circulating TNF-α levels were assayed and TNFR1 expression on CD14+ monocytes was measured by flow cytometry measured PRE, immediately post (IP), 30-min post (30M), 24 h post (24H), and 48 h post (48H) exercise. Circulating TNF-α was elevated at IP ($p = 0.001$) and 30M ($p = 0.005$) and decreased at 24H and 48H recovery from IP in CON ($p = 0.015$) and CWI ($p = 0.011$). TNF-α did not significantly decrease from IP during recovery in NMES. TNFR1 expression was elevated ($p < 0.001$) at 30M compared to PRE and all other time points. No significant differences between groups were observed in TNFR1 expression. During recovery (24H, 48H) from muscle damaging exercise, NMES treatment appears to prevent the decline in circulating TNF-α observed during recovery in those receiving no treatment or CWI.

Keywords: muscle damage, immune function, cytokines, athletic training, TNF-α

INTRODUCTION

Tumor necrosis factor-α (TNF-α) is a multifunctional cytokine involved in the regulation of inflammation and tissue injury. Among its various functions, it is most noted for its pro-inflammatory role in tissue degradation (Li and Reid, 2001; Peterson et al., 2006; Zaldivar et al., 2006). As an early mediator in muscle damage, TNF-α can be synthesized by several immune and nervous cells and is rapidly released in the blood by circulating monocytes and in skeletal muscle by invading macrophages after exercise-induced muscle damage (Hirose et al., 2004; Zaldivar et al., 2006). Furthermore, TNF-α can induce both necrosis and apoptosis of myocytes through intracellular signaling pathways (Hardin et al., 2008) which accompanies a decline in muscle contractile properties and diminished performance (Cassatella, 1995; Hardin et al., 2008).

Strenuous exercise often results in considerable muscle damage that may impact subsequent athletic performance (Crameri et al., 2007). Thus, various recovery modalities are often used by athletes as a means to counteract the inflammatory response which accompanies the potentially damaging effects of intense exercise. Cold water immersion (CWI) is one of the most common recovery modalities used by athletes to expedite muscle repair and recovery (Barnett, 2006; Rice et al., 2008; Bleakley et al., 2012).

While previous literature has shown beneficial results of CWI such as decreasing indirect markers of muscle damage as well as attenuating pro-inflammatory cytokine release, some studies show little to no effect on blood markers and immune responses (Bleakley et al., 2012; Leeder et al., 2012; Tseng et al., 2013). While there is evidence that CWI is effective in decreasing leukocytosis (Tseng et al., 2013), to date, research on the effects of CWI on inflammatory cells following intense exercise is limited (Leeder et al., 2012; Tseng et al., 2013).

Neuromuscular electrical stimulation (NMES) is another therapeutic modality used to facilitate rehabilitation and recovery after muscular trauma (Peake et al., 2008; Babault et al., 2011). Current literature examining the benefit NMES is equivocal, revealing both positive and neutral effects on measures of muscle strength and power, as well as pain modulation (Miller et al., 2000; Peake et al., 2008; Babault et al., 2011). One possible mechanism for the benefit of NMES is an increase in blood flow (Babault et al., 2011), which could possibly lead to an increase in recruitment of leukocytes to damaged tissue, potentially accelerating the muscle regeneration process. While currently unknown, it is possible that NMES may also elicit unique effects on specific immune cells and cytokines.

The purpose of the present investigation was to investigate the efficacy of CWI and NMES on the cellular interaction between circulating TNF-α levels and its receptor expression (TNFR1) on human monocytes in response to heavy resistance exercise. Since circulating concentrations of cytokines exert their effects through binding to a receptor, TNFR1 was included in our measurement. The assessment of how recovery modalities mediate the inflammatory response may provide additional

insight to muscle recovery processes and subsequent performance. We hypothesized that CWI would mitigate circulating TNF-α and TNFR1 expression while NMES would not attenuate TNF-α and TNFR1 on circulating monocytes following the heavy resistance training protocol and subsequent bouts of resistance exercise.

METHODS

PARTICIPANTS AND DESIGN

Thirty resistance-trained men (22.5 ± 2.7 y, 1.74 ± 0.12 m, 83.4 ± 6.9 kg) participated in this study and were randomly divided into one of three groups: Control (CON), neuromuscular electrical stimulation (NMES), and cold water immersion (CWI), by use of a random number generator. Participants were required to have a minimum of 1 year of resistance training experience to participate, particularly in the squat exercise, and were not permitted to use additional nutritional supplements or recovery strategies while enrolled in the study. The University Institutional Review Board approved the research protocol which was in accordance with the Declaration of Helsinki and each participant gave his informed consent.

METHODOLOGY

Participants reported to the lab on four separate occasions. On the first visit (T1), participants were tested for maximal strength [one repetition-maximum (1-RM)] on the barbell back squat, dead lift, and barbell split squat exercises. Prior to the second visit (T2), participants were instructed to refrain from exercising for 72 h. The 1RM session was completed no more than a week before the T2 visit. At T2, participants performed four sets of no more than 10 repetitions for each set until failure of the squat (80% 1-RM), dead lift (70% 1-RM), and barbell split squat (70% 1-RM) exercises with 90 s rest intervals between each set and exercise. At T3 and T4, squats (4 sets of 10 repetitions at 80% 1RM) were performed (**Figure 1**).

NEUROMUSCULAR ELECTRIC STIMULATION THERAPY (NMES)

Participants randomly assigned to NMES were provided with 24 min of electrical stimulation immediately following the post-exercise blood draw (T2) or post-exercise (T3) using a commercially available product (Compex Performance US, DJO, Vista, CA). Participants were asked to lay supine with three electrodes placed on the quadriceps muscles of each leg. Specifically, one large electrode with a negative charge was placed at the most proximal point of the upper leg, while two small electrodes with positive charge were placed on the belly of the vastus lateralis and vastus medialis. The unit was set to the manufacturer's established recovery mode. The investigator adjusted the stimulation intensity to a level as high as possible before the participant felt unwarranted discomfort. The intensity administered to each participant was noted by the research investigator, and was used for the subsequent recovery intervention period (T3), similar to previously published methods (Vanderthommen et al., 2007). The treatment protocol consisted of nine sequences, with the first three stages lasting for 2 min, and the remaining six for 3 min. Frequency of contraction started at 9 Hz, stepping down 1 Hz per stage to 1 Hz.

COLD WATER IMMERSION

Immediately post-exercise participants in the CWI group were provided a 22.9 cm high ice bath with a temperature that was maintained at 10–12°C immediately following a post-exercise blood draw (T2) or immediately post-exercise (T3). Participants were required to fully submerge their lower body into the water up to their umbilicus for 10-min.

BLOOD MEASUREMENTS

During the T2 experimental session, blood samples were obtained pre-exercise for baseline measurement (PRE), immediately post-exercise (IP), and 30 min post-exercise (30M). During the T3 and T4 experimental sessions, blood samples were obtained pre-exercise marking 24 h post-exercise (24H), and 48 h post-exercise (48H). During T2, all blood samples were obtained using a 20-gauge Teflon cannula placed in a superficial forearm vein kept patent with isotonic saline. PRE blood samples were drawn following a 15-min equilibration period prior to exercise. IP blood samples were taken within 1 min of exercise cessation. Each participant's blood samples were obtained at the same time of day during each session.

Blood samples were collected into two Vacutainer® tubes, one containing no anti-clotting agent and the second containing K_2 EDTA. The blood in the first tube was allowed to clot at room temperature for 30 min and subsequently centrifuged at 3000 × g for 15 min along with the remaining whole blood from the second tube. The resulting plasma and serum was placed into separate 1.8-mL microcentrifuge tubes and frozen at −80°C for later analysis.

BIOCHEMICAL ANALYSIS

Creatine Kinase (CK) was analyzed with the use of a spectrophotometer and a commercially available enzymatic kit (Sekisui Diagnostics, Charlottetown, PE, Canada) per manufacturer's instructions. Myoglobin concentrations were determined using enzyme-linked immunosorbent assays (ELISA) (Calbiotech, Spring Valley, CA). Determination of serum immunoreactivity values was determined using a BioTek Eon spectrophotometer (BioTek, Winooski, VT, USA). All samples were run in duplicate with a mean intra-assay variance of 2.6% for CK and 5.73% for myoglobin. Coefficient of variation for each hemoglobin assay was 3.73% for and 0.65% for hematocrit.

Serum samples were assayed for concentrations of TNF-α, using a cytokine assay (Millipore Milliplex, cat no. HCYTOMAG-60K; Billerica, MA) on a MAGPIX instrument (Luminex Technologies; Luminex, Austin, TX) according to the manufacturer's instructions. All samples were run in duplicate with a mean intra-assay variance of 8.36% for TNF-α.

CELL STAINING

Erythrocytes were lysed from 350 µl of whole blood (Pharm Lyse; BD Biosciences, Franklin Lakes, NJ) within 30 min of collection. Samples were then washed in staining buffer containing 1 × phosphate-buffered saline containing fetal bovine serum (FBS) by centrifugation and aspiration. This process was repeated three times. Leukocytes were then resuspended in 100 µl Pharminigen stain buffer (BD Biosciences, Franklin Lakes, NJ).

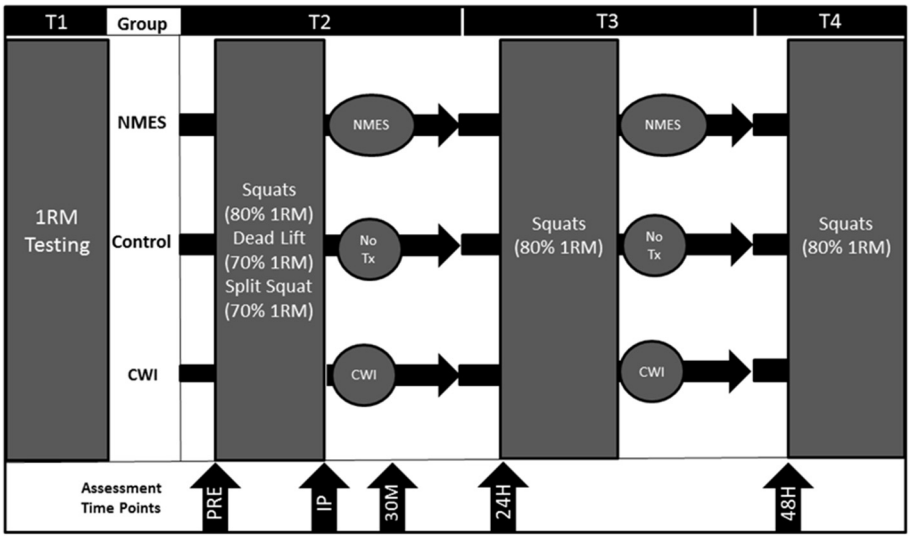

FIGURE 1 | Study design for control (CON), neuromuscular electrical stimulation (NMES), and cold water immersion (CWI). PRE = Pre-exercise. IP = Immediately post-exercise. 30M = 30 min post-exercise. 24H = 24 h post-exercise. 48H = 48 h post-exercise. 1RM = 1-repetiton maximum. Tx = Treatment.

Direct staining methods were used to label CD14 and CD120a (TNFR1). Fluorescein isothiocyanate (FITC) conjugated to anti-CD120a (H398, IgG2 α; AbDSerotec, Raleigh, NC) and PerCP Cy5.5 conjugated anti-CD14 (M5E2; BD Pharminigen) were used in the receptor labeling process. Surface staining was performed by adding 10 μl of directly conjugated FITC-anti-CD120a, 5 μl of directly conjugated PerCP Cy5.5-anti-CD14 to the cell suspension and incubating in the dark for 30 min at 20°C. Cells were resuspended in 1.0 ml of stain buffer for flow cytometry analysis.

FLOW CYTOMETRY

Flow cytometry analysis of stained cells was performed on a C6 Accuri Flow Cytometer (BD Biosciences); equipped with BD Accuri analysis software (BD Biosciences). Forward and side scatter along with four fluorescent channels of data were collected using two lasers providing excitation at 488 and 640 nm. Monocytes were determined by initial gating based on forward and side scatter, followed by gating for CD14+ cells as also described by Tallone et al. (2011). A minimum of 10,000 events, defined as CD14+ monocytes, were obtained with each sample. Analysis of monocyte subpopulations was completed by quadrant analyses, in which CD14 was compared with CD120a. Mean fluorescence of CD120a was recorded, representing the mean density of receptors per cell.

STATISTICAL ANALYSIS

A repeated measures analysis of variance (ANOVA) was used to analyze both TNF-α and TNFR1 data. If an interaction was found, follow-up analyses included One-Way repeated measures ANOVAs and Tukey *post-hoc* comparisons. Prior to analysis all data was assessed to ensure normal distribution, homogeneity of variance and sphericity. Dietary compositions between groups were analyzed using an unpaired *t*-test. Results were considered significant at an alpha level of $p = 0.05$. All data are reported as mean ± SD. Data were analyzed using SPSS v20 software (SPSS Inc., Chicago, IL, USA).

RESULTS

Participants assigned to the CWI, NMES, and CON groups did not differ in in any physical characteristics. Participants were experienced, resistance trained individuals having an average resistance training history of 7.9 ± 3.3 years and an average squat 1RM of 150.2 ± 22.4 kg. Additionally, the exercise load presented a similar stress to all groups in terms of total volume and number of repetitions performed. Muscle damage corresponded with significant elevations in CK from PRE (103.4–166.3 U/L) to 24H (290%, 468.9–677.1 U/L) and 48H (271% 424.6–612.5 U/L) post-T2 and myoglobin from PRE (23.3–35.5 ng/mL) to IP (192%, 63.8–114.8 ng/mL) and 30P (357%, 89.3–180.6 ng/mL), as reported previously (Jajtner et al., 2014).

CK and myoglobin responses to the protocol did not appear to be affected by NMES or CWI treatments. Analysis of the participants' dietary recall revealed no differences in total caloric or protein intake (g and g•kg^{-1} body mass) between groups on days of T2 and T3.

CIRCULATING TNF-α LEVELS

No between-group differences were noted in TNF-α at PRE (8.3 ± 4.2 pg/mL). The Two-Way time × group repeated measures ANOVA for TNF-α indicated a significant time effect ($F = 8.68$, $p < 0.001$), but no significant main effect for group ($p > 0.05$), or time × group interaction ($p > 0.05$). With all groups combined, circulating TNF-α was elevated at IP (10.8 ± 5.3 pg/mL; $p = 0.001$) and at 30M (9.6 ± 4.3 pg/mL; $p = 0.005$) compared to PRE concentrations (**Figure 2**). TNF-α concentrations were in similar physiological range to other resistance trained males (Kraemer et al., 2014).

Since treatments were not administered until after IP, a separate repeated measures was run from IP to determine the effect of

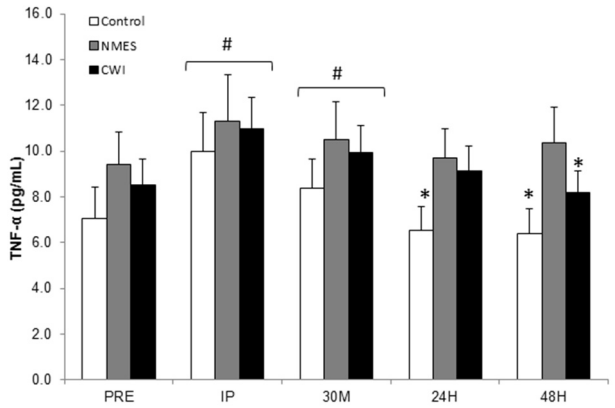

FIGURE 2 | Circulating TNF-α response to acute heavy resistance exercise and subsequent recovery in control (CON), neuromuscular electro stimulation (NMES) and cold water immersion (CWI), groups at Pre-exercise (PRE), Immediately post-exercise (IP), 30-min post-exercise (30M), 24 h post-exercise (24H), and 48 h post-exercise (48H) # Significantly elevated from PRE ($p < 0.05$). * Significantly ($p \leq 0.05$) lower than IP.

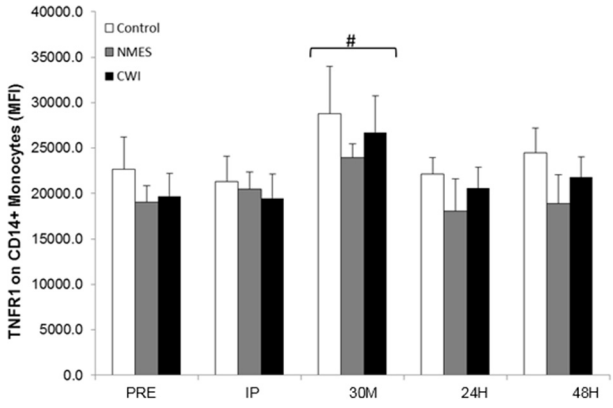

FIGURE 3 | TNFR1 [expressed as Mean Fluorescence Intensity (MFI)] on CD14+ monocytes response to acute heavy resistance exercise and subsequent recovery in control (CON), neuromuscular electro stimulation (NMES) and cold water immersion (CWI), groups at Pre-exercise (PRE), Immediately post-exercise (IP), 30-min post-exercise (30M), 24 h post-exercise (24H), and 48 h post-exercise (48H) # Significantly elevated from PRE ($p < 0.05$).

each treatment. When compared to IP values, TNF-α was significantly lower at 24H ($p = 0.015$) and 48H ($p = 0.001$) in CON. CWI was significantly lower ($p = 0.011$) at 48H compared to IP values.

TNFR1

One hundred percent of CD+14 monocytes expressed TNFR1. The Two-Way × group repeated measures ANOVA for TNFR1 density (expressed as relative fluorescence) indicated a significant time effect ($F = 4.25$, $p = 0.023$), but no main effect for group ($p > 0.05$), or time × group interactions ($p > 0.05$). TNFR1 levels were significantly elevated ($p < 0.01$) at 30M compared to all other time points (**Figure 3**).

DISCUSSION

The main findings of this investigation were that TNF-α was elevated immediately following acute heavy resistance exercise, and decreased at 24 h and 48 h into recovery in those receiving no treatments. Treatment with NMES appeared to prevent the recovery decline in circulating TNF-α compared to the CON group. In addition, TNFR1 expression on CD14+ monocytes appears to be elevated 30-min following acute heavy resistance exercise, and did not appear to be affected by treatment during the recovery period.

The increase in circulating TNF-α following heavy resistance exercise is consistent with findings reported by others. Brenner et al., found that resistance exercise increased TNF-α to a greater extent than moderate aerobic or maximal cycling (Brenner et al., 1999). Conversely, some investigations have found no change in TNF-α, Smith et al. (2000), Peake et al. (2006), and in one study a decrease in TNF-α was observed (Hirose et al., 2004). However, studies which saw no elevation in TNF-α recruited individuals without prior resistance training experience and employed only eccentric movements (Smith et al., 2000; Buford et al., 2009). Another study, which observed a decrease in circulating TNF-α, used only eccentric elbow flexion (Hirose et al., 2004). Therefore, it is likely that resistance-trained individuals performing heavy dynamic resistance exercise experience a more pronounced TNF-α response following an acute exercise bout than non-trained individuals. The elevated circulating TNF-α levels in response to heavy resistance exercise is an important pro-inflammatory event in the tissue repair process (Freidenreich and Volek, 2012). Resistance-trained individuals possibly elicit an increased immunological response to exercise and when coupled with large amount of muscle mass involved in this type of exercise, acutely produce more pro-inflammatory cytokines (Leeder et al., 2012).

Without treatment, circulating TNF-α returned to baseline at 24H and 48H into recovery. Treatment with NMES resulted in circulating TNF-α concentrations that did not return to baseline values at 24H and 48H into recovery while. To the authors' knowledge, this is the first study to examine the effects of NMES on the immune response to heavy resistance training. While used commonly in therapy settings for pain modulation, there is little evidence in the literature that NMES is an effective means of recovery following strenuous exercise (Vanderthommen et al., 2007; Peake et al., 2008).

NMES is believed to function by initiating muscular contractions that accelerate the blood flow to the stimulation site, which increases the rate of lactate and other metabolic byproduct removal (Babault et al., 2011). Therefore, in the present study, NMES treatment may have provided a constant stimulus for tissue breakdown and removal. The nature of NMES stimulating muscle contractions may have evoked a continuous elevation of pro-inflammatory cytokines during the recovery period as TNF-α has been shown to be expressed from injured skeletal muscle (Tseng et al., 2013). The CWI group was elevated at IP and returned to baseline at 48 h post-exercise. Since there is evidence that CWI is effective in decreasing leukocytosis (Tseng et al., 2013), it is possible that cryotherapy may prevent or even delay the repair process.

Our data demonstrate an exercise-induced elevation in TNFR1 on CD14+ monocytes at 30M following heavy resistance exercise which is supports previously published data (Townsend et al., 2013). However, TNFR1 expression on CD14+ monocytes was not significantly different between any of the interventions. While not significant, TNFR1 expression on CD14+ monocytes in NMES was lower on average than expression in CON and CWI during recovery. This lower expression corresponded to time points where circulating TNF-α was elevated in the NMES group in our study. Previous studies have also reported that receptor expression and serum concentration cytokine patterns may not coincide (Peake et al., 2006; Zaldivar et al., 2006). Potentially, elevations in circulating cytokines may be saturating their receptors and thus reducing subsequent expression (Fragala et al., 2011). Furthermore, secretion of TNF-α cannot solely be attributed to immune cells (Li and Reid, 2001), so it is possible that NMES modulates the presence of TNFR1 on monocytes differently than serum concentration TNF-α and the observed increase in circulating TNF-α may have been attributed to muscle cytokine release. Nevertheless, there is currently little research on TNFR1 expression on leukocytes in response to strenuous resistance exercise protocols, and further investigations are needed aimed at determining how exercise modulates specific immune cells and the implications of their response.

Although inflammation has been commonly associated with detrimental consequences, pro-inflammatory processes are essential for subsequent muscle repair and regeneration (Li and Reid, 2001; Peterson et al., 2006; Crameri et al., 2007; Urso, 2013). In the tissue repair cascade, early responding inflammatory cells, mainly circulating monocytes (Peake et al., 2005) and tissue-embedded macrophages secrete several pro-inflammatory cytokines which stimulate satellite cell activation (Mackey et al., 2007). Thus, interventions that attenuate or block inflammation may actually hinder or delay the repair processes (Mackey et al., 2007; Urso, 2013). Nevertheless, prior studies have shown that acute anti-inflammatory treatments may allow for improved subsequent muscular contractile and structural function (Grounds and Torrisi, 2004; Piers et al., 2011). Thus, it appears that there may be specific time points following acute damage in which anti-inflammatory interventions are most beneficial to muscle contractile function and repair, however these time frames have yet to be identified (Urso, 2013).

In the present study, therapies were implemented immediately following exercise, which resulted in elevated circulating TNF-α concentration during recovery compared to controls. While this is the first study to report the effect of NMES on circulating TNF-α and TNFR1 expression following heavy resistance exercise, it is important to consider that the cytokine response to exercise stress and subsequent muscle disruption is complex and in constant flux (Cassatella, 1995; Peake et al., 2005; Bleakley et al., 2012). Whereas the present study examined TNF-α and its receptor as a possible mechanism to explain the efficacy of recovery modalities, it is important to note that several additional pro- and anti-inflammatory cytokines and immune markers (IL-1β, IL-6, IL-10) contribute to the tissue repair processes. However, the aim of the current investigation was to provide additional insight to an important subset of immune cells in response to acute resistance exercise. Further study of additional cells and cytokines are needed to fully elucidate the mechanisms behind therapeutic modalities in tissue recovery.

In conclusion, it appears that CWI and NMES therapies do not attenuate circulating TNF-α during recovery compared to control conditions, as had been hypothesized. However, it seems that NMES is potentially attenuates TNFR1 expression more than CWI. These data contribute new insights on how recovery modalities affect the immune response to muscle recovery from resistance exercise.

AUTHOR CONTRIBUTIONS

Conception and design of research: JT, JH, MF, AJ, AG, JS. Acquisition of data: JT, AJ, AG, AW. Data analysis and interpretation: JT, JH, MF, AJ, AG, GM, AW, JS, DH. Drafting of manuscript and manuscript approval: JT, JH, MF, AJ, AG, GM, JS, DH.

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REFERENCES

Babault, N., Cometti, C., Maffiuletti, N. A., and Deley, G. (2011). Does electrical stimulation enhance post-exercise performance recovery? *Eur. J. Appl. Physiol.* 111, 2501–2507. doi: 10.1007/s00421-011-2117-7

Barnett, A. (2006). Using recovery modalities between training sessions in elite athletes. *Sports Med.* 36, 781–796. doi: 10.2165/00007256-200636090-00005

Bleakley, C., Glasgow, P., and Webb, M. (2012). Cooling an acute muscle injury: can basic scientific theory translate into the clinical setting? *Br. J. Sports Med.* 46, 296–298. doi: 10.1136/bjsm.2011.086116

Brenner, I., Natale, V., Vasiliou, P., Moldoveanu, A., Shek, P., and Shephard, R. (1999). Impact of three different types of exercise on components of the inflammatory response. *Eur. J. Appl. Physiol. Occup. Physiol.* 80, 452–460. doi: 10.1007/s004210050617

Buford, T. W., Cooke, M. B., and Willoughby, D. S. (2009). Resistance exercise-induced changes of inflammatory gene expression within human skeletal muscle. *Eur. J. Appl. Physiol.* 107, 463–471. doi: 10.1007/s00421-009-1145-z

Cassatella, M. A. (1995). The production of cytokines by polymorphonuclear neutrophils. *Immunol. Today.* 16, 21–26. doi: 10.1016/0167-5699(95)80066-2

Crameri, R., Aagaard, P., Qvortrup, K., Langberg, H., Olesen, J., and Kjær, M. (2007). Myofibre damage in human skeletal muscle: effects of electrical stimulation versus voluntary contraction. *J. Physiol.* 583, 365–380. doi: 10.1113/jphysiol.2007.128827

Fragala, M. S., Kraemer, W. J., Mastro, A. M., Denegar, C. R., Volek, J. S., Hakkinen, K., et al. (2011). Leukocyte β2-adrenergic receptor expression in response to resistance exercise. *Med. Sci. Sports Exerc.* 43, 1422–1432. doi: 10.1249/MSS.0b013e31820b88bc

Freidenreich, D. J., and Volek, J. S. (2012). Immune responses to resistance exercise. *Exerc. Immunol. Rev.* 18, 8.

Grounds, M. D., and Torrisi, J. (2004). Anti-TNFα (Remicade®) therapy protects dystrophic skeletal muscle from necrosis. *FASEB J.* 18, 676–682. doi: 10.1096/fj.03-1024com

Hardin, B. J., Campbell, K. S., Smith, J. D., Arbogast, S., Smith, J., Moylan, J. S., et al. (2008). TNF-α acts via TNFR1 and muscle-derived oxidants to depress myofibrillar force in murine skeletal muscle. *J. Appl. Physiol.* 104, 694–699. doi: 10.1152/japplphysiol.00898.2007

Hirose, L., Nosaka, K., Newton, M., Laveder, A., Kano, M., Peake, J., et al. (2004). Changes in inflammatory mediators following eccentric exercise of the elbow flexors. *Exerc. Immunol. Rev.* 10, 20.

Jajtner, A. R., Hoffman, J. R., Gonzalez, A. M., Worts, P., Fragala, M. S., and Stout, J. R. (2014). Comparison of electrical stimulation versus cold water immersion treatment on muscle soreness following resistance exercise. *J. Sport Rehabil.* doi: 10.1123/jsr.2013-0113. [Epub ahead of print].

Kraemer, W. J., Hatfield, D. L., Comstock, B. A., Fragala, M. S., Davitt, P. M., Cortis, C., et al. (2014). Influence of HMB supplementation and resistance training on

cytokine responses to resistance exercise. *J. Am. Coll. Nutr.* 33, 247–255. doi: 10.1080/07315724.2014.911669

Leeder, J., Gissane, C., van Someren, K., Gregson, W., and Howatson, G. (2012). Cold water immersion and recovery from strenuous exercise: a meta-analysis. *Br. J. Sports Med.* 46, 233–240. doi: 10.1136/bjsports-2011-090061

Li, Y., and Reid, M. B. (2001). Effect of tumor necrosis factor-[alpha] on skeletal muscle metabolism. *Curr. Opin. Rheumatol.* 13, 483–487. doi: 10.1097/00002281-200111000-00005

Mackey, A. L., Kjaer, M., Dandanell, S., Mikkelsen, K. H., Holm, L., Døssing, S., et al. (2007). The influence of anti-inflammatory medication on exercise-induced myogenic precursor cell responses in humans. *J. Appl. Physiol.* 103, 425–431. doi: 10.1152/japplphysiol.00157.2007

Miller, B. F., Gruben, K. G., and Morgan, B. J. (2000). Circulatory responses to voluntary and electrically induced muscle contractions in humans. *Phys. Ther.* 80, 53–60.

Peake, J., Nosaka, K. K., Muthalib, M., and Suzuki, K. (2006). Systemic inflammatory responses to maximal versus submaximal lengthening contractions of the elbow flexors. *Exerc. Immunol. Rev.* 12, 72–85.

Peake, J., Nosaka, K. K., and Suzuki, K. (2005). Characterization of inflammatory responses to eccentric exercise in humans. *Exerc. Immunol. Rev.* 11, 64–85.

Peake, J., Peiffer, J. J., Abbiss, C. R., Nosaka, K., Okutsu, M., Laursen, P. B., et al. (2008). Body temperature and its effect on leukocyte mobilization, cytokines and markers of neutrophil activation during and after exercise. *Eur. J. Appl. Physiol.* 102, 391–401. doi: 10.1007/s00421-007-0598-1

Peterson, J. M., Feeback, K. D., Baas, J. H., and Pizza, F. X. (2006). Tumor necrosis factor-α promotes the accumulation of neutrophils and macrophages in skeletal muscle. *J. Appl. Physiol.* 101, 1394–1399. doi: 10.1152/japplphysiol.01453.2005

Piers, A., Lavin, T., Radley-Crabb, H., Bakker, A., Grounds, M., and Pinniger, G. (2011). Blockade of TNF-α *in vivo* using cV1q antibody reduces contractile dysfunction of skeletal muscle in response to eccentric exercise in dystrophic mdx and normal mice. *Neuromuscul. Disord.* 21, 132–141. doi: 10.1016/j.nmd.2010.09.013

Rice, T., Chantler, I., and Loram, L. C. (2008). Neutralisation of muscle tumour necrosis factor alpha does not attenuate exercise-induced muscle pain but does improve muscle strength in healthy male volunteers. *Br. J. Sports Med.* 42, 758–762. doi: 10.1136/bjsm.2007.038067

Smith, L., Anwar, A., Fragen, M., Rananto, C., Johnson, R., and Holbert, D. (2000). Cytokines and cell adhesion molecules associated with high-intensity eccentric exercise. *Eur. J. Appl. Physiol.* 82, 61–67. doi: 10.1007/s004210050652

Tallone, T., Turconi, G., Soldati, G., Pedrazzini, G., Moccetti, T., and Vassalli, G. (2011). Heterogeneity of human monocytes: an optimized four-color flow cytometry protocol for analysis of monocyte subsets. *J. Cardiovasc. Transl. Res.* 4, 211–219. doi: 10.1007/s12265-011-9256-4

Townsend, J. R., Fragala, M. S., Jajtner, A. R., Gonzalez, A. M., Wells, A. J., Mangine, G. T., et al. (2013). β-Hydroxy-beta-methylbutyrate (HMB)-free acid attenuates circulating TNF-alpha and TNFR1 expression postresistance exercise. *J. Appl. Physiol.* 115, 1173–1182. doi: 10.1152/japplphysiol.00738.2013

Tseng, C. Y., Lee, J. P., Tsai, Y. S., Lee, S. D., Kao, C. L., Lai, C., et al. (2013). Topical cooling (icing) delays recovery from eccentric exercise-induced muscle damage. *J. Strength Cond. Res.* 27, 1354–1361. doi: 10.1519/JSC.0b013e318267a22c

Urso, M. L. (2013). Anti-inflammatory interventions and skeletal muscle injury: benefit or detriment? *J. Appl. Physiol.* 115, 920–928. doi: 10.1152/japplphysiol.00036.2013

Vanderthommen, M., Soltani, K., Maquet, D., Crielaard, J., and Croisier, J. (2007). Does neuromuscular electrical stimulation influence muscle recovery after maximal isokinetic exercise? *Isokinet. Exerc. Sci.* 15, 143–149.

Zaldivar, F., Wang-Rodriguez, J., Nemet, D., Schwindt, C., Galassetti, P., Mills, P. J., et al. (2006). Constitutive pro-and anti-inflammatory cytokine and growth factor response to exercise in leukocytes. *J. Appl. Physiol.* 100, 1124–1133. doi: 10.1152/japplphysiol.00562.2005

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Can analysis of performance and neuromuscular recoveries from repeated sprints shed more light on its fatigue-causing mechanisms?

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In team sports, game decisive events are often reliant on transient repeated-sprint ability (RSA), which refers to the ability to produce the best possible average sprint performance over a series of sprints (<10 s), separated by short (<60 s) recovery periods (Bishop et al., 2011). Researches on RSA, particularly focusing on factors contributing to fatigue (Girard et al., 2011) and interventions (e.g., training, ergogenic aids, altitude) likely to improve this fitness component (Bishop et al., 2011; Billaut et al., 2013), are undergoing unprecedented popularity. Although differences exist in terms of sprint duration (4–10 s) or distance (10–40 m) and recovery time (10–30 s) or nature (passive or active) between RSA protocols, a single set of 5–15 maximal "all-out" efforts (i.e., close-loop design) is generally used to assess performance or fatigue resistance. Compared to time trials (i.e., possibility to constantly adjust mechanical performance) or time to exhaustion tasks (i.e., option of voluntarily ending exercise; open-loop design), one advantage of the RSA test model is to circumvent the confounding effects associated with pacing.

With the repetition of maximal efforts, muscle fatigue develops (i.e., reversible decline in muscle force production), arising from a complex interaction between muscular perturbations and neural adjustments so that no singular isolated factor likely represents a direct causative mechanism explaining the rate of decline in peak sprint speed (running) or peak/mean power output (cycling) during RSA protocols (Girard et al., 2011). In addition to large perturbations in peripheral physiological state with repeated sprinting, when substantial fatigue levels are incurred (i.e., sprint decrement score >10%), reductions in mechanical performance and in the amplitude of quadriceps EMG signals [Root Mean Square (RMS) activity] often coincide, implying that motor unit activity (i.e., a decrease in recruitment; firing rate; or both) may also become suboptimal (Mendez-Villanueva et al., 2008; Girard et al., 2011; Brocherie et al., 2014). Very recently, RSA investigations have been conducted under elevated environmental stress (heat or hypoxia) or where the degree of fatigue at exercise start was manipulated to more thoroughly understand the nature of the underlying mechanisms. The consistent finding was that acute moderate hypoxia (i.e., a fraction of inspired oxygen of 13.8%; Billaut et al., 2013) or the induction of pre-existing locomotor muscle fatigue (i.e., following a 10-min neuromuscular electrical stimulation protocol of the quadriceps; Hureau et al., 2014) caused significant parallel reductions in RMS activity of the active musculature and in power output with cycle-sprint repetitions (i.e., their magnitudes exceeded those of control situations), while the amount of peripheral quadriceps fatigue incurred at exercise termination was similar. The interpretation was that feedback from fatiguing muscles plays an important role in the determination of central motor drive and force output, so that the development of peripheral muscle fatigue is confined to a certain level (also referred as a "critical" threshold) so as not to surpass a sensory tolerance limit.

Because the modifications in muscle recruitment patterns are highly influenced by changes in RSA performance, it can be argued that muscle "de-recruitment" with sprint repetitions may not be the cause but rather the consequence of progressive decreases in velocity or power production. In an effort to resolve this issue, innovative approaches have emerged, either based on the determination of the power-EMG relationship during warm-up sprints that are subsequently compared to EMG changes during a RSA test (Bishop, 2012) or based on the comparison of fatigue responses during two sets of repeated sprints separated by a recovery period (i.e., few minutes) and matched for initial mechanical output (Mendez-Villanueva et al., 2008). The rationale is to determine whether a disproportionate decrease in neural drive over mechanical performance (sprint time/power output) actually occurs during RSA tests.

To delineate the neural and muscular factors driving performance recovery following repeated sprints a sprint-matching paradigm was introduced, where exercise responses during two sets of repeated cycling sprints (10 × 6-s "all out" sprints with 30 s recovery followed after 6 min of passive recovery by five 6-s sprints), matched for initial mechanical output in a "non-fatigued" (sprints 4–8) and a "fatigued" state (sprints 11–15), were actually compared (Mendez-Villanueva et al.,

2007). Results indicated that there was a greater fatigability in the five repetitions of the second vs. first set, despite mechanical output produced for the initial bout of both sets (i.e., sprints 4 and 11) being similar. Furthermore, muscle activation was lower (∼12%) in sprint 11 than 4, while the rate of decrease in net EMG activity was similar for the two sets of repeated sprints. Taken as a whole, this highlights that the short-term activation history of the active musculature alters the muscle recruitment pattern and fatigability during sets of repeated sprints matched for initial power output. Using the same data set, with the addition of muscle biopsies of the *vastus lateralis* obtained at rest, immediately after the 10 first sprints and after 6 min of recovery it was further demonstrated that phosphocreatine resynthesis was associated with total work done in sprint 11 ($r = 0.79$, $P < 0.05$) and total work done during sprints 11–15 ($r = 0.67$, $P < 0.05$), while EMG amplitude remained depressed (Mendez-Villanueva et al., 2013). The lower performance maintenance during subsequent repeated sprints was mostly mediated by intramuscular factors probably related to limitations in metabolic supply, as also evidenced by the disproportionate ∼2-fold greater decrease in total work in relation to RMS in the second set of sprints (sprint 11–15) than in the first five sprints (sprint 1–5).

In an effort to improve our understanding of fatigue-causing mechanisms during repeated sprinting, we invite multiple-sets RSA studies to carefully evaluate the ensuing recovery rate of single- and multiple-sprint performance and return of neuromuscular markers, with special reference to restoration of central nervous system functioning and of peripheral physiological state. Furthermore, quantifying whether disproportionate decreases in neural drive or in muscle contractility occur over mechanical performance during successive sets of repeated sprints, may help to determine if the attenuation of the EMG amplitude is actually the consequence, or the cause, of slower sprint times or reduced power production. Further investigation where environmental stressing conditions could vary across successive sets of repeated sprints and/or during the between-sets intervening recovery periods may assist in clarifying this contention, accepting the premise that an increase in hypoxia severity would alter exercise-induced demands (and thereby recovery requirements) on the neuromuscular system. Under this framework, our recent comparison of the effects of an initial set of exhaustive intermittent cycling under normoxia, moderate or severe hypoxia on locomotor performance and quadriceps fatigability, and how recovery from this first exercise bout influence subsequent normoxic performance during the completion of a second set using a similar exercise mode may lead the way (Christian et al., 2012). In doing so particular attention should be paid to study perceptual recovery as well, as it may interact with feed-forward/feedback mechanisms to influence athlete preparedness for ensuing exercise bouts (Minett and Duffield, 2014).

REFERENCES

Billaut, F., Kerris, J. P., Rodriguez, R. F., Martin, D. T., Gore, C. J., and Bishop, D. J. (2013). Interactions of central and peripheral factors using repeated sprints at different levels of arterial O_2 saturation. *PLoS ONE* 8:e77297. doi: 10.1371/journal.pone.0077297

Bishop, D. J. (2012). "Fatigue during intermittent-sprint exercise," in *Proceedings of the Australian Physiological Society*, Vol. 43 (Sydney), 9–15.

Bishop, D. J., Girard, O., and Mendez-Villanueva, A. (2011). Repeated-sprint ability – Part II. Recommendations for training. *Sports Med.* 9, 741–756. doi: 10.2165/11590560-000000000-00000

Brocherie, F., Millet, G. P., and Girard, O. (2014). Neuro-mechanical and metabolic adjustments to the repeated anaerobic sprint test in professional football players. *Eur. J. Appl. Physiol.* doi: 10.1007/s00421-014-3070-z. [Epub ahead of print].

Christian, R. J., Racinais, S., Buchheit, M., and Girard, O. (2012). Effect of hypoxia severity on peripheral fatigue development and subsequent exercise performance during exhaustive intermittent cycling. *Proc. Physiol. Soc.* 26, PC89.

Girard, O., Mendez-Villanueva, A., and Bishop, D. J. (2011). Repeated-sprint ability – Part I. *Sports Med.* 41, 673–694. doi: 10.2165/11590550-000000000-00000

Hureau, T. J., Olivier, N., Millet, G. Y., Meste, O., and Blain, G. M. (2014). Exercise performance is regulated during repeated sprints to limit the development of peripheral fatigue beyond a critical threshold. *Exp. Physiol.* 99, 951–963. doi: 10.1113/expphysiol.2014.077974

Mendez-Villanueva, A., Hamer, P., and Bishop, D. J. (2007). Fatigue responses during repeated sprints matched for initial mechanical output. *Med. Sci. Sports Exerc.* 39, 2219–2225. doi: 10.1249/mss.0b013e31815669dc

Mendez-Villanueva, A., Hamer, P., and Bishop, D. J. (2008). Fatigue in repeated-sprint exercise is related to muscle power factors and reduced neuromuscular activity. *Eur. J. Appl. Physiol.* 103, 411–419. doi: 10.1007/s00421-008-0723-9

Mendez-Villanueva, A., Edge, J., Suriano, R., Hamer, P., and Bishop, D. J. (2013). The recovery of repeated-sprint exercise is associated with PCr resynthesis, while muscle pH and EMG amplitude remain depressed. *PLoS ONE* 7:e51977. doi: 10.1371/journal.pone.0051977

Minett, G. M., and Duffield, R. (2014). Is recovery driven by central or peripheral factors? A role for the brain in recovery following intermittent-sprint exercise. *Front. Physiol.* 5:24. doi: 10.3389/fphys.2014.00024

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