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Blood pH, lactate, and ammonia following repeat sprints in trained and untrained men

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ABSTRACT

Purpose: Examine repeat sprint performance along with blood lactate, pH, and plasma ammonia in untrained and recreationally trained men. **Methods:** Participants performed three 30-second Wingate cycling tests separated by five minutes each. Blood pH and lactate were measured pre-exercise and between each sprint, plasma ammonia was measured pre- and post-exercise. Each subject was classified as NO (no exercise), EO (endurance only), RO (resistance only), or RE (resistance and endurance) based upon exercise history. **Results:** There were significant effects for group in repeat sprint performance, as measured by peak power (Mean peak power for NO 532.88 ± 35.32; EO 607.37 ± 27.05; RO 829.01 ± 74.68; RE 731.60 ± 48.56; $F = 6.75$; $p < 0.01$) and mean power (Mean of mean power for NO 378.62 ± 34.06; EO 501.75 ± 35.31; RO 596.48 ± 45.22; RE 550.36 ± 37.51; $F = 6.88$; $p < 0.01$). No significant group differences were found for blood lactate, pH, plasma ammonia values, or fatigue index. **Conclusions:** Similar fatigue responses regardless of training type and that acute physiological responses to repeat sprints, including buffering of blood pH, lactate, and ammonia are not different between groups with different exercise training history.

Keywords: anaerobic, cycling, fatigue, power, Wingate

1. INTRODUCTION

The ability to quickly recover from an acute high-intensity exercise bout, particularly sprints, then repeat that exercise bout several seconds to several minutes later at a similar intensity is a critical component to many sports. This repeat sprint activity closely mimics activity performed during competition. The inability to maintain a desired force output that results from repeated high-intensity activity with short recovery periods is known as fatigue (Brooks et al, 2005). While fatigue is induced by interconnected central and peripheral mechanisms, as reviewed by Amann (2011), the focus of the current study is to examine the effects of repeated sprints on acute peripheral fatigue. The underlying physiological responses associated with acute peripheral fatigue within the muscle and nerve are multifaceted and complex, but the accumulation of blood lactate (Spencer et al., 2005), lower blood pH (Coso et al., 2009), and elevated blood ammonia levels (Wilkinson et al., 2010) are often measured alongside power to determine the extent of fatigue in human trials. While each of these outcomes have been studied in recreationally trained and untrained populations, additional research is needed to determine the interplay of these factors and which of these factors, if any, may have a more significant influence on performance.

It has been shown (Brooks, 2020) that the accumulation of lactate does not cause acidosis, but acidosis may cause ammonia and lactate formation. Specifically, according to Robergs et al. 2006, it is not physiologically possible for lactic acid to exist within human cells and therefore lactate, not lactic acid is formed during high-intensity exercise. In addition, the formation of lactate consumes, rather than produces H+ ions, and therefore cannot be causative for metabolic acidosis, rather acidosis is due to ATP hydrolysis (which forms ADP, Pi, and H+). Rapid ATP hydrolysis, which is necessary during sprinting activity, results in a high concentration of ADP and Pi, which stimulates glycolysis, which in turn forms lactate, even in the presence of oxygen, and thus since both physiological changes occur around the same time, lactate is incorrectly assumed to cause acidosis. Interestingly, however, is that phosphofructokinase (PFK), the rate-limiting enzyme of glycolysis, is inhibited by low pH (Dobson et al., 1986).

Ammonia formation following high-intensity exercise, on the other hand, which occurs due to the deamination of AMP to IMP along with BCAA deamination (Wilkinson et al., 2010), stimulates PFK (Mutch & Banister, 1983), and AMP deaminase itself is stimulated by low pH, increased ADP, and increased AMP concentration (Roussel et al., 2003). Ammonia is alkaline, and is low in concentration (~2 $mmol \cdot kg^{-1}$ wet weight) (Robergs et

al., 2006) relative to lactate (several hundred mmol ·kg^{-1} wet weight) (Böning et al., 2008) and as a result, does not contribute to acidosis. Thus, the rate of muscular contraction, which forms ADP, AMP, Pi, and H+, dictates the formation of lactate and ammonia via overlapping negative feedback loops initiated by rapid ATP hydrolysis.

If this premise of metabolism is accepted, the question becomes how or if recreationally trained athletes are better at countering peripheral fatigue mechanisms during high-intensity sprint exercise and recovery. In both recreationally trained and untrained subjects, muscle pH drops to a similar extent during exhaustive exercise (Coggan et al., 1993). However, recreationally trained athletes presumably are producing greater power output and as a result are hydrolyzing more ATP per unit time and therefore may be more effective in buffering blood pH, though this has not been extensively studied. In some studies, anaerobically trained athletes exhibited higher blood lactate levels following supramaximal exercise (Calbet et al., 2003; Trapp et al., 2007) compared to untrained counterparts. In another study, however, no differences were found in blood lactate levels in trained and untrained subjects (Coso et al., 2009). Previous research has found a significant correlation between blood pH and lactate (Kato et al., 2005) suggesting that either blood pH should be lower in recreationally trained athletes exhibiting higher lactate levels since the formation of lactate is due to greater feedback signaling from greater rates of ATP hydrolysis, or that other mechanisms, perhaps ammonia formation, are working to buffer pH to a greater extent in recreationally trained athletes.

By examining the power generated in recreationally trained and untrained populations in addition to blood pH, lactate, and ammonia during and following repeated sprints, it will be possible to potentially pinpoint physiological differences in recreationally trained and untrained populations as it relates to acute peripheral fatigue. Therefore, the primary aim of the current study was to examine changes in blood pH, lactate, and ammonia following repeated sprints in recreationally trained and untrained subjects. The secondary aim of the study was to examine the relationship between peak power, mean power, fatigue index and blood parameters.

2. METHODS

2.1 Subjects

Healthy, young men (N = 33) aged eighteen to thirty-four with no medical conditions, based upon the Physical Activity Readiness Questionnaire, were recruited to participate in the current study. Subjects were screened with an exercise history questionnaire that asked how frequently they engaged in resistance training and endurance training in a typical week. Based upon these responses, subjects were placed into one of four groups based upon the type(s) of exercise in which they had been habitually engaged (average of 5 days per week or more, and least 150 minutes of exercise for the past 6 months or more). Resistance exercise only (RO; N = 9), endurance exercise only (EO; N = 7), resistance and endurance exercise (RE; N = 8), or no exercise (NO; N = 9). Subject characteristics are provided in table 1. Subjects were informed of risks and benefits of the study, informed consent was then obtained from subjects prior to data collection. The study was approved by the University's Institutional Review Board.

2.2 Design

Sprint ability was tested using a standardized and validated Wingate cycling protocol to ensure construct validity and to have comparative data. All exercise was completed on a Monark 894E cycle ergometer which is designed to drop and remove exact external resistance quickly. To effectively examine repeat sprint ability, the protocol was repeated with minimal recovery. The Wingate test was completed a total of three times with five minutes of recovery between trials. These numbers were determined following pilot testing of the protocol in which shorter recovery periods resulted in either minimal effort for subsequent Wingate tests or an inability to complete all three Wingate protocols due to nausea and/or dizziness. Lactate levels begin to decline following five minutes of recovery (Goodwin et al., 2007) and thus, recovery times exceeding five minutes would have compromised results. None of the pilot subjects were able to complete more than three Wingate tests. Baseline blood values were obtained prior to the onset of the exercise protocol. Each subject was verbally encouraged to provide maximal effort for all three Wingate tests. In order to quickly and safely obtain blood samples between Wingate tests, capillary blood was collected via dermal puncture. A larger volume of plasma was required for ammonia assays and as a result, this outcome was analyzed at pre- and post-exercise time-points only.

Table 1. Subject Characteristics.

Characteristic	NO	EO	RO	RE	Statistic	P Value
Age (y)	22.67 ± 0.62	22.00 ± 0.76	24.00 ± 1.50	24.50 ± 1.45	χ^2= 2.38,	0.5
Height (cm)	175.89 ± 1.85	177.00 ± 1.88	177.67 ± 2.17*†‡#	187.00 ± 2.42	F = 5.81	< 0.01
Weight (kg)	81.12 ± 4.06	80.91 ± 5.24	81.83 ± 4.64	90.54 ± 4.10	F = 1.03	0.39
Body Fat (%)	23.84 ± 3.39	20.56 ± 3.47	14.97 ± 1.72	20.48 ± 4.22	Welch's F = 2.12	0.14

*NO No training, EO Endurance only training, RE Resistance and Endurance training, RO Resistance only training. * Significantly different from EO, † Significantly different from RO, ‡ Significantly different from NO, # Significantly different from RE*

2.3 Methodology

Subjects were instructed to abstain from caffeine, alcohol, or intense exercise for 48 hours prior to study participation. Following an overnight fast, subjects came to the laboratory between 0500 and 0800, consent was obtained, and questionnaires were completed. Ten milliliters of blood were then drawn from the cubital vein via venipuncture to obtain resting plasma ammonia levels. A dermal puncture was then performed on the third or fourth digit using a spring-loaded lancet (Becton, Dickinson and Company, Franklin Lakes, NJ, USA). The first drop of blood was wiped away with clean gauze. A drop of blood was collected onto a lactate strip and analyzed using a lactate plus meter (Nova Biomedical, Waltham, MA, USA). Additional blood was collected into a microtube and immediately analyzed using a micro pH probe (ThermoFisher Scientific, Waltham, MA, USA).

2.4 Exercise Protocol

All exercises were completed on a Monark 894E cycle ergometer (Monark, Vansboro, Sweeden). Following a self-paced five-minute aerobic warm-up on the cycle ergometer, subjects were provided with specific instructions to pedal as fast as possible for thirty seconds and to keep the gluteus maximus in contact with the seat at all times, which were identical to those provided during a standard Wingate test (Pescatello, 2014). Subjects completed three (3) 30-second Wingate tests with five (5) minutes of rest between each test. All subjects were verbally encouraged throughout each Wingate test. Power output data were automatically recorded every second utilizing Monark Anaerobic Test Software. The peak power was determined as the highest power output achieved throughout the test, which always occurred within

the first five seconds and mean power was calculated as the average power output achieved throughout the duration of the thirty-second test. During the recovery periods, subjects were provided with water and instructed to stay on the bike and keep pedaling with no resistance to allow for optimal recovery. Blood from the original resting dermal puncture was collected during minute four of recovery to re-analyze pH and lactate. Following the last Wingate test, a second venipuncture was performed within two (2) minutes to re-assess blood ammonia values. Blood pH and lactate were assessed during the fourth minute of recovery.

2.5 Blood sample processing and analysis

Blood from the venipunctures were collected into a heparin vacutainer (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) and immediately centrifuged at 1250 rotations per minute (RPM) at 4 degrees Celsius for 15 minutes. Plasma was separated into a sterile 1.5 ml tube (ThermoFisher Scientific, Waltham, MA, USA) and was analyzed via Evolution 60s UV-Visible spectrophotometry (ThermoFisher Scientific, Waltham, MA, USA) using a commercially available assay kit (Sigma-Aldrich, St. Louis, MO, USA).

2.6 Statistical analysis

All data were checked for normality using a Shapiro Wilk test. Unequal variances were checked using a Levene test. A one-way ANOVA was used to examine group characteristics as well as differences is group peak power, mean power, fatigue index, lactate, and pH after each Wingate test and ammonia after the final Wingate test. Welch's test was used for data with unequal variances. A Tukey post hoc test was used to analyze significant group differences for normally distributed data, the Wilcoxon method was used to compare significant non-normally distributed data. A repeated measures ANOVA was used to compare ammonia, pH, and lactate changes over time within and between groups. A contrast analysis was used to compare significant effects between groups. An alpha level of 0.05 was used to determine statistical significance. Effect size was calculated using partial η^2 for all statistically significant results. All values are presented as mean ± SEM. All statistical analyses were completed using JMP version 14.0 (SAS Institute Inc., Cary, NC, USA).

3. RESULTS

Baseline levels of blood lactate, pH, and plasma ammonia were not significantly different and are provided in table 2.

Table 2. Baseline Group Comparisons.

Outcome	NO	EO	RO	RE	Statistic	P Value
Pre-Ammonia (μM)	24.53 ± 5.33	16.93 ± 7.54	26.67 ± 8.43	32.28 ± 8.49	$\chi^2 = 2.66$	0.63
Pre-pH	7.51 ± 0.03	7.50 ± 0.03	7.50 ± 0.04	7.53 ± 0.01	F = 0.27	0.85
Pre-Lactate (mM)	1.10 ± 0.13	1.63 ± 0.28	1.61 ± 0.23	1.24 ± 0.26	F = 1.41	0.26

NO No training, EO Endurance only training, RE Resistance and Endurance training, RO Resistance only training.

There was a significant group effect for peak power ($F = 6.75$; $p < 0.01$). Peak power significantly decreased between each trial ($F = 17.17$; $p < 0.01$), but there was no significant group by time interaction effect ($F = 1.76$; $p = 0.13$). Contrast analysis revealed a significant difference between the NO and RO group ($F = 16.99$; $p < 0.01$), between the NO and RE group ($F = 8.62$; $p < 0.01$), and between the RO and EO group ($F = 8.47$; $p < 0.01$).

Similarly, there was a significant effect group for mean power ($F = 6.88$; $p < 0.01$). Mean power significantly decreased between each trial ($F = 34.76$; $p < 0.01$), but there was no significant group by time interaction effect ($F = 2.16$; $p = 0.06$). Contrast analysis revealed a significant difference between the NO and EO group ($F = 5.40$; $p = 0.03$), NO and RO ($F = 17.44$; $p < 0.01$), and NO and RE ($F = 12.78$; $p < 0.01$). The η^2 for peak and mean power between groups following Wingate 1, 2, and 3 were 0.46, 0.49, 0.31, 0.31, 0.32, and 0.33, respectively. Data for peak and mean power are provided in Figure 1. There was no significant group effect for fatigue index ($F = 1.41$; $p = 0.26$). Fatigue index did not significantly decrease between trials ($F = 0.05$; $p = 0.95$) and there was no significant group by time interaction effect ($F = 0.53$; $p = 0.78$).

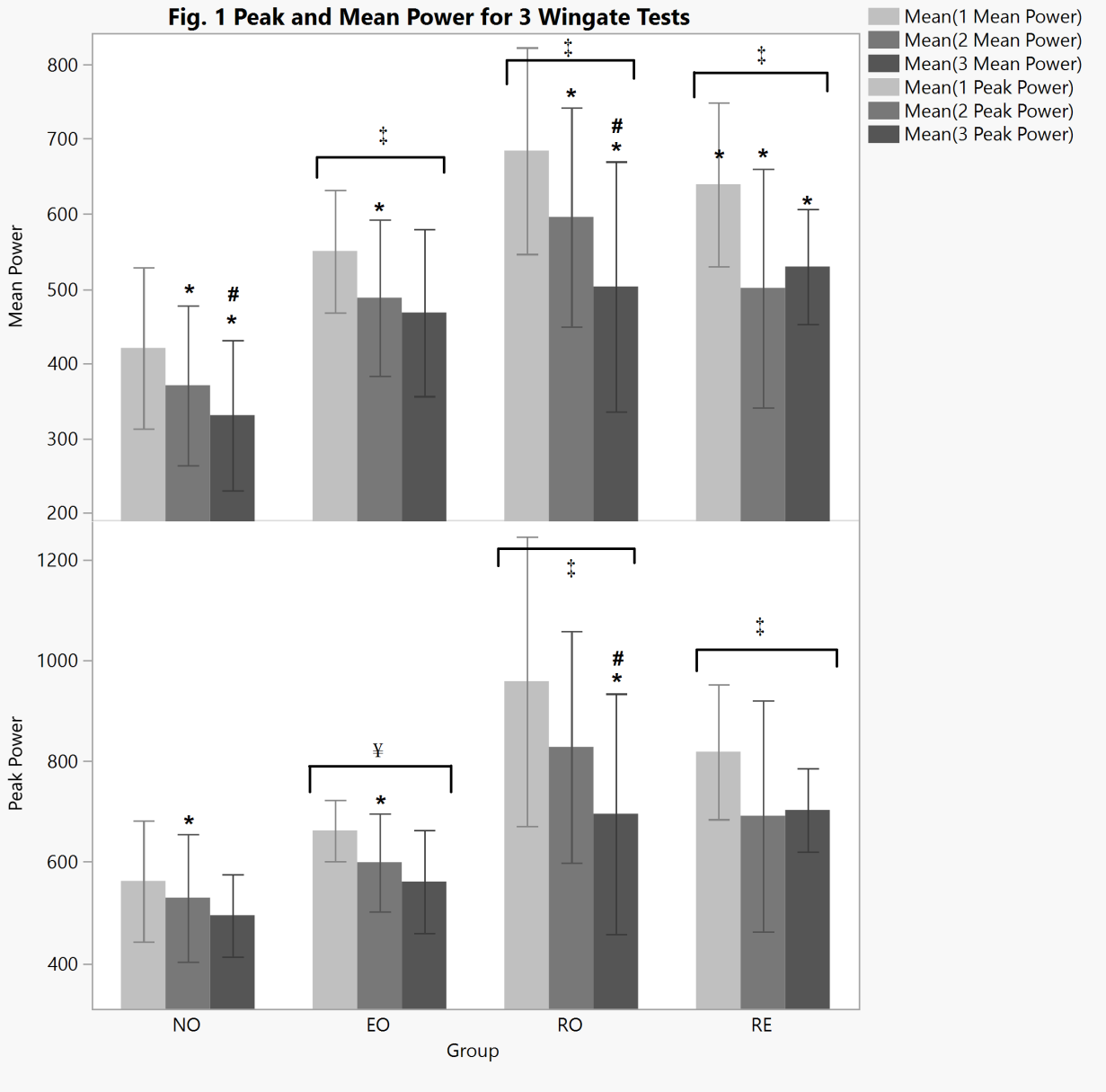

Fig. 1 Error bars represent standard error. W Watts, NO no exercise, EO endurance only, RO resistance only, RE resistance and endurance exercise. * significantly different from Wingate 1 values. # significantly different from Wingate 2 values. ‡ Significantly different from NO. ¥ Significantly different from RO.

There was no significant group effect for blood lactate ($F = 0.68$; $p = 0.57$), or blood pH ($F = 1.35$; $p = 0.28$). The effect for time for lactate and pH were significant ($F = 129.89$; $p < 0.01$ and $F = 80.62$; $p < 0.01$, respectively).

Group by time interaction effect for lactate was not significant (0.54; p = 0.84) and the group by time interaction effect for blood pH was not significant (F = 0.83; p = 0.59). Data for blood lactate and pH are provided in figure 2.

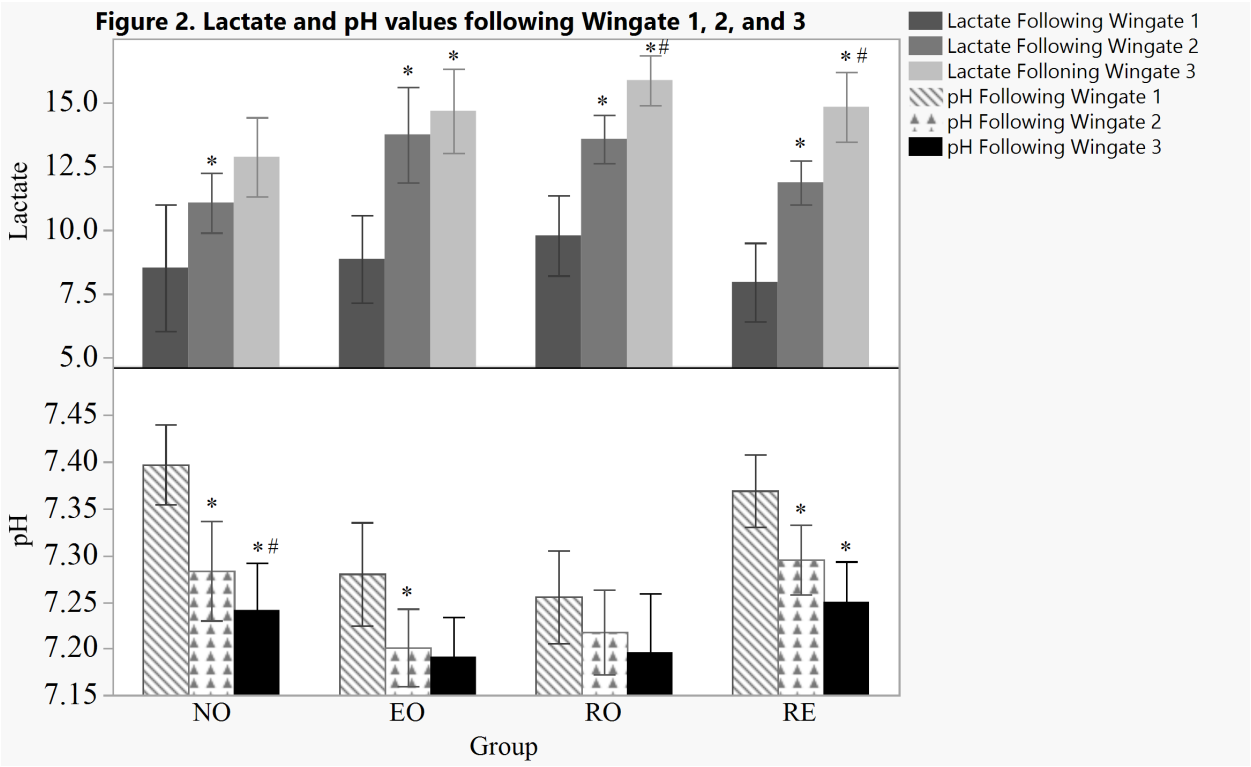

Fig. 2 Error bars represent standard error. NO no exercise, EO endurance only, RO resistance only, RE resistance and endurance exercise. * Significantly different from Wingate 1 values. # Significantly different from Wingate 2 values.

There was no significant group effect for plasma ammonia (F = 0.41; p = 0.74). Plasma ammonia significantly increased from pre- to post exercise (F = 18.01; p < 0.01), but there was no significant group by time interaction effect (F = 0.94; p = 0.43). Data for ammonia are provided in Figure 3.

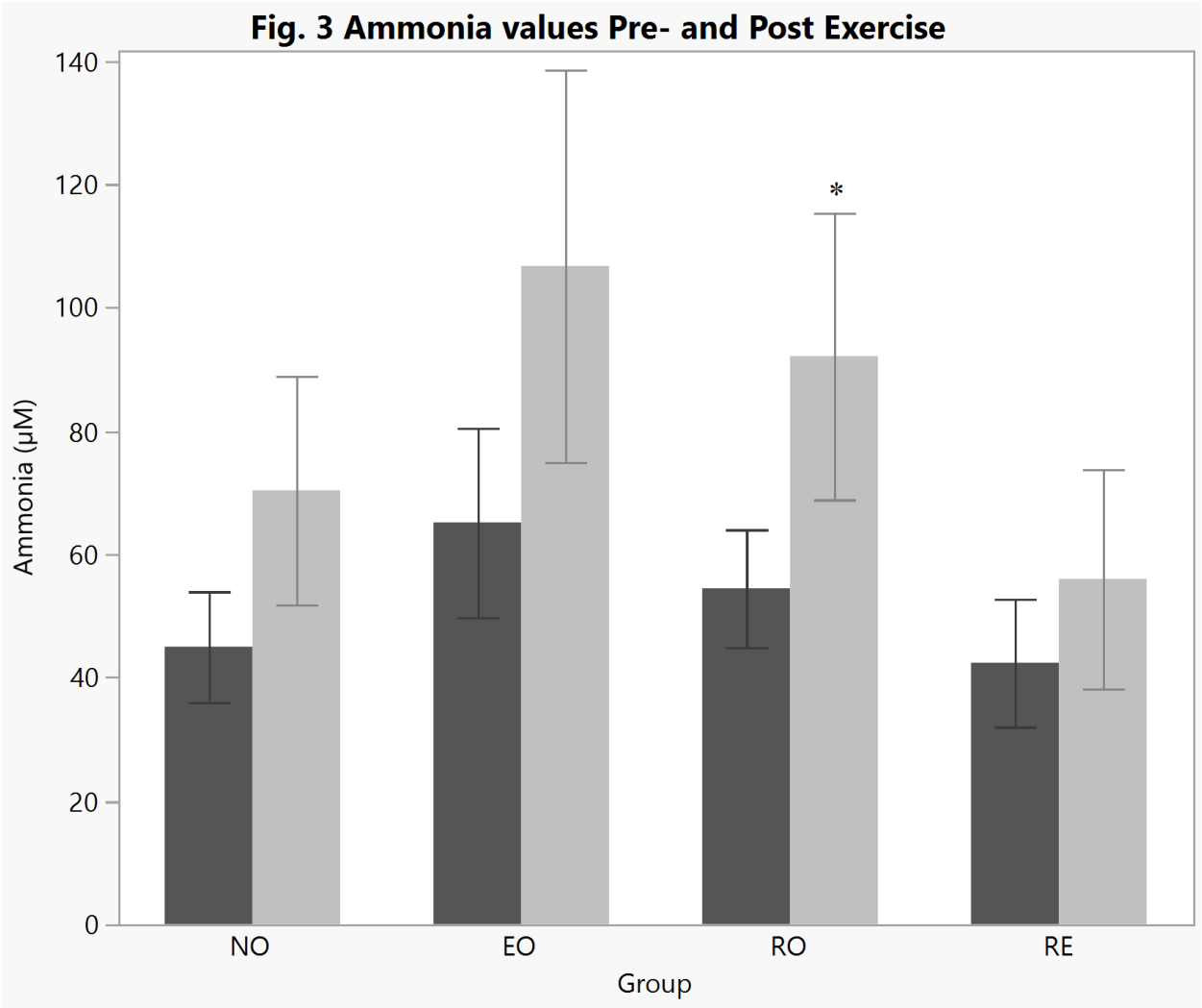

Fig. 3 Error bars represent standard error. NO no exercise, EO endurance only, RO resistance only, RE resistance and endurance exercise. * Significantly different from pre-exercise values

Pairwise comparisons of changes in peak power, mean power, lactate, pH, and ammonia did not reveal any significant correlations. Data are provided in table 3.

Table 3. Overall Pairwise Comparisons of change scores.

Outcome	Change Peak Power	Change Mean Power	Change Ammonia	Change Lactate	Change pH
Change Peak Power	1	0.82*	-0.02	-0.01	0.15
Change Mean Power	0.82*	1	-0.16	-0.12	-0.12

Change Ammonia	-0.02	-0.16	1	0.17	-0.11
Change Lactate	-0.01	-0.12	0.17	1	0.14
Change pH	-0.15	-0.12	-0.11	0.14	1

*Values represent Pearson correlation coefficients. * Significant correlation; p < 0.05*

Peak power following the first two Wingate tests compared to recovery lactate and pH did not reveal any significant correlations. Peak power following the third Wingate test compared to recovery lactate, pH, and Ammonia (which was only measured after the final Wingate) did not reveal any significant correlations. Data are provided in Figure 4.

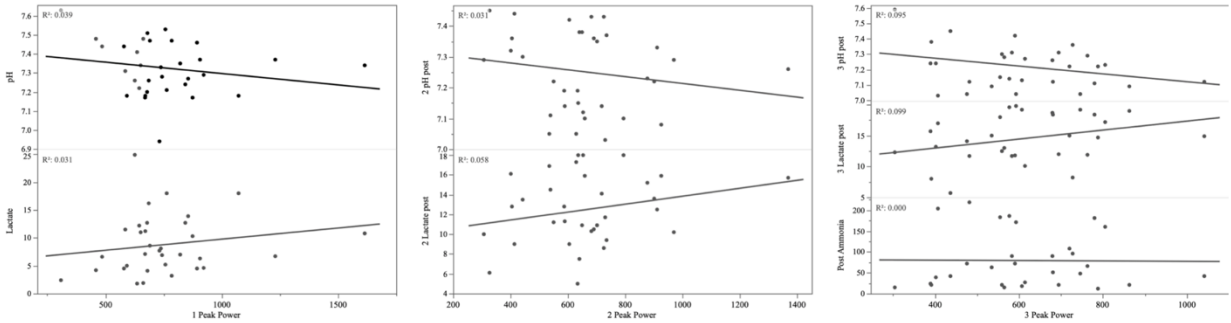

Fig. 4 Dots represent individual subjects. The line represents line best fit. R^2 values represent overall correlation. No R^2 values were statistically significant.

The supplementary data table contains data for peak power, mean power, fatigue index, lactate, pH, and ammonia for each group following each individual Wingate test.

4. DISCUSSION

Training status significantly affected peak and mean power as expected, but training status did not significantly influence blood lactate, pH, or ammonia responses to repeated Wingate tests. These results suggest that differences in performance are not attributable blood lactate, pH, or ammonia, but likely other peripheral and central fatigue mechanisms. The argument could be made that with more elite athletes, differences in blood lactate, pH, or ammonia may have been detected. Based upon previous Wingate study data, it appears that the subjects for the current study were recreationally trained, but not elite, with relative peak power (peak power divided by body

weight) averaging roughly 9 or 10 $W \cdot kg^{-1}$ compared to roughly 18 – 20 $W \cdot kg^{-1}$ seen in elite sprinters and endurance athletes (Calbet et al., 2003). The peak power, mean power, and fatigue index values seen in the current study for the recreationally trained groups fall around the 50th percentile, while the untrained group is near the lowest percentile rank based upon a review by Boone et al. (2007). Fatigue index in the aforementioned study was around 0.46 $w \cdot s^{-1} \cdot kg^{-1}$, which was similar to the findings of the current study and blood lactate levels, peaking around 15 $mmol \cdot l^{-1}$ are also in agreement with the current study. Similarly, elite sprinters and endurance athletes have exhibited similar lactate accumulation in venous blood during exercise, but elevated venous blood lactate levels in sprinters following ten minutes of recovery (Calbet et al., 2003). This suggests a greater ability of endurance athletes to remove lactate from the blood as expected. It should be noted that blood samples for the current study were collected between one to two minutes post-exercise. It has been shown that lactate levels peak immediately post-exercise and begin to drop five minutes post-exercise (Goodwin et al., 2007) and thus, differences in blood lactate were not detected between groups in the current study.

Differences in blood lactate in trained and untrained populations have been noted following submaximal exercise (Bloom et al., 1976), with aerobically trained individuals exhibiting lower blood lactate levels due to greater reliance on aerobic metabolism and greater lactate removal. Conversely, trained women have demonstrated higher blood lactate levels, peaking around 8 $mmol \cdot l^{-1}$ following repeat 24-second cycle sprints compared to untrained women, peaking around 5 $mmol \cdot l^{-1}$ (Trapp et al., 2007). These lactate levels were substantially lower than the findings of the current study. However, power outputs for trained and untrained women were 167 and 105, respectively, which were also lower than the current findings. Taken together, it appears that as previously established in longer exercise trials (Bentley et al., 2001), as absolute power output increases, blood lactate increases. However, sprinting activity is heavily reliant upon anaerobic metabolism and thus, although power output differences in recreationally trained and untrained men are obvious, blood lactate levels become elevated to a similar extent, regardless of training status. This concept is also supported in longer duration exercise, in which the authors did not find differences in blood lactate levels between trained and untrained subjects following 24 minutes of cycling (Coso et al., 2009).

Training status did not appear to affect blood pH in the current study. Coggan et al. (1993) noted similar results, with no differences in intra-muscular pH changes between trained and untrained men following a ramped resistance exercise session to volitional failure, lasting 12 – 30

minutes. Similarly, Coso et al (2009) did not find any differences in blood pH between trained and untrained subjects following 24 minutes of intense exercise. Thirty second sprints have been shown to decrease blood pH (Cheetham et al., 1985), which is aligned with the current findings. Similar to lactate, previous findings taken together with the current study data suggest that due to the anaerobic nature of repeat sprints, blood pH will decrease to a similar extent in both recreationally trained and untrained populations.

Changes in plasma ammonia levels have been noted above 50% VO_{2Max}, these values roughly triple, and there appears to be a linear relationship between ammonia and lactate (Babij et al., 1983). All of these findings are in alignment with the data from the current study and suggest increased ammonia production or decreased clearance during high intensity exercise. There does not appear to be differences in plasma ammonia concentration in recreationally trained and untrained groups, which is in agreement with previous research following prolonged exercise (Graham et a., 1985) or maximal exercise (Vanuxem et al., 1993). However, ammonia levels have been shown to be higher following supramaximal exercise in sprinters compared to distance runners (Itoh & Ohkuwa, 1990). Interestingly, sprint training has also been shown to reduce plasma ammonia levels following a thirty second sprint (Stathis et al., 1994). These contradictory findings may suggest that plasma ammonia levels are more likely to be elevated in sprinters that rapidly hydrolyze ATP and rely more heavily on fast-twitch muscle fibers (Mutch & Banister, 1983), but that the type of training (e.g. repeated sprints with minimal recovery) may affect how quickly ammonia is cleared from plasma. The subjects of the current study were not sprinters, but resistance-trained or endurance-trained individuals, neither of which was likely engaged in repeat sprint training which led to a lack of group differences in plasma ammonia.

As previously established, lactate is not necessarily causative for changes in pH, but rather pCO_2 is primarily responsible for a drop in pH during exercise and initial recovery (Lindinger & Heigenhauser, 2008). In addition, hypercapnia reduces lactate and ammonia concentrations following maximal exercise (Kato et al., 2005) and therefore, pCO_2, which is not different between trained and untrained populations (Edwards et al., 1969) may be the driving factor behind blood pH, lactate, and ammonia, which explains the lack of differences between groups. It is important to note that several studies, including Kato et. al (2005) found significant correlation between pH and lactate ($r = - 0.91$) as well as pH and ammonia ($r = -0.88$). Although these relationships were stronger than the data from the current study, the results are in agreement with current findings. We identified R^2 values for

the full study sample (N = 33) of 0.29, 0.44, and 0.51 following Wingate 1, 2, and 3, respectively for pH versus lactate and -0.34, -0.61, and -0.42 following Wingate 1, 2, and 3, respectively for pH versus ammonia, all of which were statistically significant. Similarly, Roussel et al. (2003) found that blood lactate and ammonia levels measured during VO_{2Max} were correlated ($r^2 = 0.66$), peaking at 100% VO_{2max} and 5 minutes recovery, with peak lactate values of 11.3 mM and peak values ammonia values of 127 µM at VO_{2Max}, which are in agreement with the current study. The authors noted that the enzymatic activity of adenylate deaminase is increased by elevated ADP and AMP and a fall in pH, which may suggest that decreases in blood pH may contribute to an increase in ammonia. It appears that moderate exercise training does not affect this outcome.

5. CONCLUSIONS

The findings of the current study suggest recreationally resistance-trained or endurance trained individuals, much like elite athletes, do not exhibit physiologically significant differences in blood lactate, pH, or ammonia following or in-between three bouts of thirty second maximal sprints with five minutes of recovery. The lack of physiological differences can be largely attributed to the anaerobic nature of this activity. Differences in performance, as measured by peak or mean power between recreationally trained and untrained individuals is likely due to other physiological mechanisms. Fatigue was not different between recreationally trained and untrained groups, suggesting that the participants in the sample population were not trained for repeat sprints. This conclusion is further supported by the correlation analysis between peak power and pH, lactate, and ammonia, which did not reveal any significant relationships. Further examination of athletes that frequently engage in this specific type of training may reveal different outcomes to those observed in the current study of recreationally trained subjects. It is important to note that athletes training specifically for repeat sprint ability may exhibit different physiological responses to this type of exercise, which further reinforces the principle of specificity for repeat sprint training. Further research on more elite athletes is necessary to determine if pH, lactate, and ammonia responses differ in athlete populations and if these outcomes are more tightly correlated with performance outcomes (such as peak power) following repeat sprints.

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6.2 Conflict of Interest

The authors have no conflicts of interest to declare.

6.3 Contribution of Authors

GRD contributed to study conception, approvals, data collection, analysis, and writing. JP contributed to data collection and writing, DR contributed to data collection and writing, DB contributed to study conception, approvals, data collection, analysis, and writing.

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