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The effects of sr2w-1 supplementation on cycling performance and muscle fatigue

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THE EFFECTS OF SR2W-1 SUPPLEMENTATION ON CYCLING PERFORMANCE AND MUSCLE FATIGUE

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ABSTRACT

Purpose: The purpose of this investigation was to examine the potential effects of SR2W-1 supplementation on cycling performance, central fatigue, and a variety of physiological parameters, including blood lactate, blood pH, heart rate (HR), rating of perceived exertion (RPE), oxygen consumption (VO_2), expired ventilation (VE), and respiratory exchange ratio (RER). **Methods:** Five recreational cyclists (38 ± 8 yr, 168.4 ± 4.3 cm, 68.8 ± 5.6 kg, and 54.4 ± 2.6 mL/kg/min) performed 20-min of steady-state cycling (~85% VO_{2max}) followed by three 1-min high intensity intervals at VO_{2max} with 30-sec active recovery periods at 100 W, 15-min passive recovery period, and a ride to fatigue (or < 50 RPM) at VO_{2max}. Subjects were randomly assigned to receive either 1000mg/d of SR2W-1 (EXP) or 1000mg/d placebo (PLA). The supplement was distributed in a double-blind, crossover fashion. The twenty-one day supplementation periods were separated by a 14-day "washout" period. Change-scores from pre- to post-supplementation were calculated for all parameters, under both PLA and EXP conditions. Wilcoxon Signed-Rank Tests were utilized to compare change-scores between PLA and EXP conditions. **Results:** There were no differences between PLA and EXP for any of the dependent variables; however several variables approached significance ($p < 0.1$). Specifically blood glucose levels, HR and ventilation increased to a greater extent with EXP, compared to PLA throughout the 20-min ride and high intensity intervals. **Conclusion:** Notwithstanding the small sample size, 3 weeks of herbal supplementation (SR2W-1) does not appear to aid in cycling performance, attenuate skeletal muscle fatigue, or modify the general physiological responses to exercise.

CHAPTER ONE

INTRODUCTION

A multitude of research regarding exercise physiology, focus on the manipulation of certain variables to optimize performance. High intensity endurance performance (i.e. cycling and running) is naturally limited by factors that contribute to skeletal muscle fatigue. In 1954, Merton proposed that fatigue results from inconsistent and inadequate blood supply to the working muscle. The potential role of blood in the development of muscle fatigue is not limited to Merton's hypothesis but also includes exercise-induced variations in the concentration of certain blood parameters, namely hydrogen ions (pH) and lactate (22, 25, 27, 38, 40, 42).

Hermansen and Osnes were among the first to demonstrate the inverse relationship between pH (increase in hydrogen ion concentration) and exercise intensity (25). When ATP demands become increasingly high, in accordance with exercise intensity, glycolysis generates pyruvate at rates that exceed pyruvate oxidative capacity. In this scenario, accumulating amounts of pyruvate are converted into lactic acid, which then disassociates into lactate and H^+ ions (i.e. metabolic acidosis) (10, 51). Despite these events, it has been suggested that proton accumulation during high intensity exercise results from ATP hydrolysis, not lactic acid formation (9, 10, 51, 59). Although still unsettled, lactate dynamics of McArdle's disease patients exemplify the role that lactic acid plays in metabolic acidosis. McArdle's patients are not able to metabolize muscle glycogen and consequently have a limited ability to produce lactic acid and hydrogen ions (61).

Regardless of the derivation of the hydrogen ions, there is a clear directional relationship between exercise intensity, blood pH, and skeletal muscle function. The direct impact that pH has on muscle performance is best illustrated by experimental manipulations of pH facilitated by the ingestion of ammonium chloride (NH_4Cl) or sodium bicarbonate ($NaHCO_3$), as the two treatments have opposite effects of blood pH buffering. Skeletal muscle power output was impacted by both conditions. Specifically, $NaHCO_3$ ingestion enhanced muscle power output (increased pH), whereas NH_4Cl impaired power output (decreased pH) (27). Raymer et al. found that slight alterations in blood pH via induced alkalosis led to a longer time to exhaustion during progressive wrist flexions to fatigue (50). Additionally, Lindh and colleagues reported significantly faster swimming time-trials for the 200-m freestyle with acute ingestion of sodium bicarbonate (200 mg/kg of body weight) 60-90 minutes prior to performance (36). Denning et al. concluded that any decrement in HCO_3 in the blood led to an impaired ability to buffer H^+ ions during high intensity exercise (leading to acidosis and muscle fatigue) (17).

While the general relationship between skeletal muscle performance and pH are clear (28), underlying mechanisms for this remain uncertain (52, 54). In addition to the aforementioned peripheral factors that are known to inhibit muscle function, fatigue has also been correlated with neuromuscular dynamics (26). Reductions in muscle fiber conduction velocity and motor drive have been linked to central nervous system fatigue (CNS) (45, 49). Specifically, Racinais et al. was able to display a decrement in motor drive after repeated bouts of interval sprints, in addition to peripheral fatigue (49). However, in a state of induced alkalosis, both nerve/synapse activity and muscle fiber conduction velocity were augmented, when compared to normal conditions (26, 60).

This finding suggests that fatigue is, to a greater extent, attributable to CNS fatigue rather than peripheral fatigue under these conditions (26, 60). Specifically, elevated serum K^+ levels in a state of alkalosis improves muscle fiber conduction velocity, thereby preserving contractile properties in the sarcolemma (i.e. attenuation of central fatigue) (45). Additionally, muscle fiber conduction velocity is sustained throughout prolonged submaximal cycling in a state of alkalosis, thus providing greater resistance to fatigue (26). As for the portioning of central vs. peripheral fatigue, Kent-Braun demonstrated that there is a strong non-linear association between pH and force ($r = 0.95$), 20% of which is attributable to central fatigue, with the remaining attributed to peripheral fatigue (28). However, it remains unclear whether central or peripheral fatigue is most effected by induced alkalosis, and if duration or intensity of exercise dictate the source of fatigue that is effected (52, 54).

Recently researchers have examined the possible ergogenic effects of herbal supplements that are in accordance with NCAA and IOC regulations and associated with minimal side effects. As explained by L.R. Kleijnen et al. (30), ergogenic herbs are classified as adaptogens, which reportedly relieves central nervous system stress rather than providing a stimulatory effect, much like other ergogenic aids. However, if taken in excess, herbal supplements may cause hypertension, irritability, insomnia and gastrointestinal distress (37). Although not unanimous, some studies have demonstrated that herbal supplementation improves gains in muscular strength, maximal oxygen uptake, and lactate handling (11, 18, 31). Various forms of ginseng and mushroom are some of the more popularly researched herbs, both of which are ingredients in commercially available SR2W-1 (11). These key ingredients have been shown to aid in

lactate maintenance and exercise performance (1, 5, 31, 64). Further, there have been at least two reports that SR2W-1 supplementation improves performance, in both humans and mice (1, 31). The mechanism of action remains unclear and further research on herbal supplementation is warranted, and may be the key to exposing factors involved in muscle fatigue.

Purpose of the Study

The primary aim of this study was to examine the potential effects of 21-days of SR2W-1 supplementation on cycling performance, muscle fatigue, and various other physiological parameters (i.e. blood lactate, HR, RER, VE, VO_2) with a randomized, double-blinded, crossover study design.

Aims and Hypotheses

Aim 1- To examine the impact of 21 consecutive days of SR2W-1 supplementation on high-intensity time to exhaustion cycling performance.

Hypothesis 1- SR2W-1 supplementation will improve time to fatigue compared to a placebo supplement.

Aim 2- To examine the impact of 21 consecutive days of SR2W-1 supplementation on blood lactate during 24 minutes of high-intensity cycling and after a high-intensity time to exhaustion performance.

Hypothesis 2- SR2W-1 supplementation will elicit lower lactate levels during the cycling protocol and during the recovery period in comparison to placebo supplementation.

Aim 3- To determine the impact of 21 consecutive days of SR2W-1 supplementation on central nervous system (CNS) fatigue after 24 minutes of high-intensity cycling.

Hypothesis 3- SR2W-1 supplementation will allow for better resistance to fatigue after a bout of high-intensity cycling, in comparison to a placebo supplementation.

Aim 4- To determine the impact of 21 consecutive days of SR2W-1 supplementation on peripheral fatigue after 24 minutes of high-intensity cycling.

Hypothesis 4- SR2W-1 supplementation will allow for better resistance to fatigue after a bout of high-intensity cycling, in comparison to a placebo supplementation.

Significance

The findings from this study will further our understanding of physiological mechanisms involved in fatigue during intense cycling exercise. Recent research on athletic performance indicate that metabolic acidosis via anaerobic glycolysis, is at least a contributing factor to fatigue development. A host of research has demonstrated the positive effects of pre-exercise bicarbonate supplementation on pH and lactate levels and sports performance. However, little is known about how herbal supplementation influences acid-base balance during vigorous exercise. The benefits of natural aids include legal use in competition along with few side effects, but the ergogenic efficacy is less clear. Some research has shown that supplementation, in the days leading up to an exercise trial, increases muscular strength, while others have shown a longer time to fatigue. The present study is designed to examine the potential effects of 21-days of SR2W-1 supplementation on cycling performance, muscle fatigue, and various other

physiological parameters (i.e. blood lactate, HR, RER, VE, VO_2) with a randomized, double-blinded, crossover study design.

CHAPTER TWO

LITERATURE REVIEW

Objectives

The objectives of this chapter are to provide an overview of: 1) the relationship between blood lactate and blood pH during exercise, 2) the effect of blood pH/lactate levels on muscle fatigue, 3) central nervous system fatigue/ interpolated twitch technique, 4) the effect of blood pH/lactate levels on cycling performance, 5) the effect of blood pH/lactate levels on blood flow, 6) the effect of SR2W-1 on exercise performance, 7) and the effect of SR2W-1 on blood lactate handling.

The Relationship between Blood Lactate and Blood pH

Blood lactate and blood pH are two of the most observed physiological parameters in exercise research. Medbo et al. demonstrated that there is linearity between blood pH and lactate levels during high intensity exercise, such that as lactate increases, and blood pH decreases (39). The strong relationship between these blood parameters has been consistently reproduced so much that it is considered paradigmatic within the field of exercise science, as shown in Table 2.1 (25, 39, 42, 54, 59). As explained by Morris & Shafer, anaerobic glycolytic rates parallel exercise intensity (42). Mechanistically, as ATP demands escalate, the rate of pyruvate production via glycolysis exceeds pyruvate oxidative capacity. In this environment, much of the produced pyruvate is not being oxidized, and is alternately reduced to lactate and disassociated hydrogen ions (i.e. metabolic acidosis) (10, 51).

Pharmacological strategies to modify blood pH, further illustrates the relationship between lactate and pH. Specifically, in order to prevent marked disruptions in acid-base balance, the blood has a bicarbonate (HCO_3^-) blood buffering system. With any reduction in buffering capacity, much like is the case during high intensity exercise, H^+ ions accumulate and blood pH declines (17). By inducing alkalosis through bicarbonate ingestion (enhancing blood buffering capacity), McCartney found significantly lower lactate levels throughout exercise (38). These findings are supported some (17, 46), but not all (50) comparable studies. Interestingly, Potteiger et al., demonstrated improved performance by inducing alkalosis, but lactate levels were also elevated (46). The authors suggested that the elevated lactate levels are due to the shunt of lactate from working muscle into the blood, aiding in better performance.

Table 2.1 Relationship between Blood Lactate and Blood pH

Author	Subjects	Design	Performance Criterion	Findings
Dennig, 1931 (17)	N=1 (M)	2 experiments- • 2days of 15g ammonium chloride • 1 day of 10g sodium bicarbonate	• 7.4kph to fatigue (ammonium chloride) • run to fatigue at 9.3kph (sodium bicarbonate)	↓ blood buffering capacity w/ ↓ decrease HCO_3^- concentration
Hermansen & Osnes, 1972 (25)	N = 13 (2 F, 11 M); students (untrained)	• blood pH before, during and after exercise • muscle pH via before, during and after exercise	• Short term max exercise, intensity not specified-continuous (2min to exhaustion) • intermittent (40-60s work to exhaustion: 4 min rest, repeated 5 times)	↓ pH w/ both protocols; ↓ pH up to 4min post exercise
McCartney, 1983 (38)	N = 6 (M)	Supplements (0.3 g/kg): • $CaCO_3$ (C) • $NaHCO_3$ (alk) • NH_4Cl (acd) • 5% CO_2 - 21% O_2 - 74% N_2 (RAC)	30s at max on cycle ergometer at 100 rpm	↓ [LA] in acd and RAC
Usaj & Starc, 1995 (59)	N = 9 (gender not specified); COMP to REC marathon runners	•blood pH/lactate taken post-exercise	2 protocols: • 5-8 runs of 1200m at constant pace of 3.4-4.8 m/s (REC) • 4.2-5.6m/s (COMP) • 8 runs of 2000m at constant pace	pH was better parameter for short term endurance performance than lactate

M = male, F = female, yo = years old, s = seconds, min = minutes, h = hours, C = control, alk = metabolic alkalosis, acd = metabolic acidosis, RAC = respiratory acidosis, ME = maximal effort; [LA]= lactate concentration, COMP = competitive, REC = recreational, ↔ = no difference, ↑ = increase ↓ = decrease

Table 2.1 Relationship between Blood Lactate and Blood pH (continued)

Author	Subjects	Design	Performance Criterion	Findings
Potteiger, 1996 (46)	N = 8 (M) trained cyclists	•placebo • 0.5g/kg sodium citrate	30km cycling TT	↑pH during exercise; ↑[La]
Stepto, 2001 (54)	N = 7; trained cyclists	• Venous blood samples: after each bout, pre/post exercise protocol • Muscle biopsy: pre/post exercise protocol	8 x 5min cycling at 86% VO_{2max}	↔ blood pH, ↓ muscle pH
Raymer, 2004 (50)	N = 6 (M), REC exercisers	Ingested 1.5h prior to trial: • $NaHCO_3$ (alk) or • placebo (C)	Continuous wrist flexion to fatigue in supine position	↔ blood [LA] between conditions
Medbo, 2009 (39)	N = 7 (M)	Blood taken at 30s, 60s, 90s post warm up and 30s, 1,3,6,10,15,20, 30,45,60min post exhaustive exercise	• 15min warm up at 50% VO_{2max} • ~2min cycle to exhaustion at max VO_{2max}	pH and [LA] have a direct linear relationship
Morris & Shafer, 2010 (42)	N = 13 (10 M, 3F) trained cyclists and triathletes	Blood pre- exercise, $1/3^{rd}$, $2/3^{rd}$ during exercise, post-exercise	• Graded submax test to determine PLT, PLT1, PpHT • discontinuous exercise test (9min bouts) to determine PMLSS • 20km TT to find mean power output	↑ intensity led to ↑ reliance on anaerobic glycolysis

M = male, F = female, yo = years old, s = seconds, min = minutes, h = hours, C = control, alk = metabolic alkalosis, acd = metabolic acidosis, [LA]= lactate concentration, TT = timed trial, PLT = highest power output with no lactate increase from baseline, PLT1 = highest power output with no increase in lactate above 1mmol/L, PpHT = highest power output with no decrease in pH below baseline, PMLSS = highest power output during a 9-min steady state with no increase in lactate above 1mmol/LCOMP = competitive, REC = recreational, ↔ = no difference, ↑ = increase, ↓ = decrease

Effect of Blood pH/Lactate Levels on Muscle Fatigue

As previously stated, blood pH/lactate levels parallel exercise intensity. Generally, when blood pH levels drop and blood lactate/ H^+ ions accumulate, muscle fatigue ensues (55, 63). Muscle fatigue can be broadly defined as "an exercise-induced reduction in maximal voluntary muscle force" (22). Due to the causal relationship between muscle fatigue and exercise performance limitations, it is of interest to further explore this relationship by focusing on contributing factors such as blood pH/lactate.

In an exercise scenario, blood buffering capacity (HCO_3) is a prominent contributor to muscle fatigue. A low buffering capacity impairs the blood's ability to buffer H^+ ions during high intensity exercise, ultimately impairing exercise performance (17). Along with an accumulation of H^+ ions, pH declines. To further display the importance of pH and muscle fatigue, Coyle et al. took two groups of cyclists, one group with low lactate thresholds and another high lactate thresholds (16). The authors reported that cyclists with higher lactate thresholds were able to ride two times longer than those with low lactate thresholds (16). In retrospect, these results present the importance of maintaining low lactate levels, as to delay muscle fatigue and increase the duration of performance. Additional research has displayed similar findings by inducing alkalosis and observing changes in skeletal muscle fatigue and exercise performance (17, 26, 60, 63).

In addition to the effects of induced alkalosis on general muscle fatigue, studies have also examined the effects of alkalosis on neuromuscular physiology. While in a state of induced alkalosis, both nerve/synapse activity and muscle fiber conduction velocity were augmented, when in comparison to normal conditions (26, 60). This finding suggests fatigue is, to a greater extent, attributable to CNS fatigue rather than

peripheral fatigue under these conditions (26, 60). Interestingly, Patterson et al examined the composition of sweat during exercise and found that there was a reduction of serum K^+ levels with the ingestion of $NaHCO_3$ (45). This finding leads to the assumption that serum K^+ levels are better retained with alkalosis, and can be used as a loose reflection of blood composition (45). In application, consistent serum K^+ levels can be used to represent resistance to diminishing contractile properties in the sarcolemma during strenuous exercise, with neuromuscular function linked to the degree of conductance along the neural axon.

Table 2.2 Blood pH/Lactate and Muscle Fatigue

Author	Subjects	Design	Performance Criterion	Findings
Dennig, 1931 (17)	N=1 (M)	2 experiments: • 2days of 15g ammonium chloride • 1 day of 10g sodium bicarbonate	• 7.4kph to fatigue (ammonium chloride) • run to fatigue at 9.3kph (sodium bicarbonate)	↓ blood buffering capacity with a decrease in HCO_3^- concentration w/ subsequent muscular fatigue
Coyle, 1988 (16)	N = 14 (M), cyclists	• low LT • high LT	TTE @ 88% (stop when < 87%)	↑ 2x TTE w/ high LT, ↓ [LA] post- exercise w/ high LT
Verbitsky, 1997 (60)	N = 6 (M), REC exercisers	400mg/kg ingestion of $NaHCO_3$ (alk) 1h prior to trial	3 tests: • cycling at max • 2 supramax (max + 17%) tests C & alk, IT pre/post tests	↑ peak and residual knee torque in alk
Westerblad, 1997 (63)	Mice	alk, acd, C @ 12°C, 22°C, 32°C	Max force of single muscle fiber measured during tetanus	↓ muscle contraction w/ ↑ temp and ↓ pH
Vissing, 1998 (61)	N = 7 (3M, 4F) with McArdle's Disease	MSNA taken, no other medications were taken	Static handgrip exercise at 30% VO_{2max} to exhaustion	↑ pH and MSNA despite ↔ [LA]

M = male, F = female, s = seconds, min = minutes, h = hours, C = control, alk = metabolic alkalosis, MVC = maximal voluntary contraction, TTE = time to exhaustion, LT = lactate threshold, IT = interpolated twitch with isometric MVC, MSNA = muscle sympathetic nerve activity, TT = timed trial, [LA] = lactate concentration, ↔ = no difference, ↑ = increase ↓ = decrease

Table 2.2 Blood pH/Lactate and Muscle Fatigue (continued)

Author	Subjects	Design	Performance Criterion	Findings
Patterson, 2002 (45)	N = 10 (M), peak VO_{2max} 4.01 L/min	Supplements (0.3g/kg): • $NaHCO_3$ (alk) • placebo (C) • ingested in 6 equal doses over 2h, 1h prior to trial	TT for 90min at 62.5% VO_{2max}, self-selected rpm; each condition tested 7 days apart	↓ serum K^+ in alk throughout exercise
Saldanha, 2004 (52)	N = 8 (M) distance runners	• pre/post MVC and LOA • no blood parameters	• running 2h at 75% VO_{2max} with 1 degree incline • IT pre/post run	↓ MVC and LOA post-run found through IT (central fatigue)
Surenkok, 2006 (55)	N = 16 (M)	• pre/post fatigue [LA]	knee extensor fatigue induced via isokinetic dynamometer protocol	↑ [LA] w/fatigue
Theurel & Lepers, 2008 (58)	N = 10 (M), trained cyclists	Pre/post-exercise muscle function test; HR & [LA] immediate post-exercise	• 33 min cycle @ 70% or • 20sec high intensity intervals of 200, 150, 100% VO_{2max} x 3 min rest @ 50% x10 (total of 33min)	↓ MVC, % activation, pH during intervals (peripheral & CNS)

M = male, F = female, s = seconds, min = minutes, h = hours, ME = max effort, C = control, alk = metabolic alkalosis, MVC = maximal voluntary contraction, LOA = level of activation, IT = interpolated twitch with isometric MVC, TT = timed trial, [LA] = lactate concentration, ↔ = no difference, ↑ = increase ↓ = decrease

Table 2.2 Blood pH/Lactate and Muscle Fatigue (continued)

Author	Subjects	Design	Performance Criterion	Findings
Hunter, 2009 (26)	N = 8 (M) trained cyclists	Supplements (0.3 g/kg): • $NaHCO_3$ (alk) • placebo (C) • ingested 3h prior to trial	• 50min of submax cycling • 50s sustained isometric contraction	↑ muscle fiber conduction velocity in alk
Cordova, 2010 (15)	N = 15 semi-professional cyclists (5 yr experience)	7 blood draws throughout test and 30s post-exercise	2 tests: • incremental max test at incline of 5.84 • uphill 12min field test • 3 knee extensions with dynamometer pre/post- exercise	↔
Morris & Shafer, 2010 (42)	N = 13 (10 M, 3F) trained cyclists and triathletes	• Blood pre-exercise, $1/3^{rd}$, $2/3^{rd}$ during exercise, post-exercise	• Graded submax test to determine PLT, PLT1, PpHT • discontinuous exercise test (9min bouts) to determine PMLSS • 20km TT to find mean power output	↔ pH while ↑[LA] during TT; lactate not attributed to fatigue
Tenan, 2010 (57)	N = 8 (4 M, 4 F)	• normal condition • glycogen reduced condition	Discontinuous GXT	↓ [LA] in glycogen reduced; ↔ fatigue

M = male, F = female, s = seconds, min = minutes, h = hours, ME = max effort, C = control, alk = metabolic alkalosis, TT = time trial, MVC = maximal voluntary contraction, LOA = level of activation, IT = interpolated twitch with isometric MVC, GXT = graded exercise test, TT = timed trial, [LA] = lactate concentration, ↔ = no difference, ↑ = increase, ↓ = decrease

Central Nervous System Fatigue/Interpolated Twitch Technique

The factors involved in skeletal muscle fatigue can be put into one of two categories: central fatigue or peripheral fatigue. With respect to central fatigue, researchers have examined possible interference in efferent/afferent signaling between the central nervous system (CNS) and muscle fibers. Peripheral factors contributing to fatigue include a malfunction in contractile properties within the sarcomere. Known to limit exercise performance, targeting the source of fatigue has become important in performance enhancement.

As a potential limitation to exercise performance, CNS fatigue has been extensively researched with the use of muscle function tests. In addition to the use of iEMG readings, the interpolated twitch technique (ITT) assesses the extent of muscle activation before and after exercise. By sending a superimposed twitch through a set of surface electrodes placed directly above the targeted muscle during a maximal voluntary contraction (MVC), it is assumed that 100% muscle fiber recruitment is achieved. The difference between peak torque and peak torque + superimposed twitch (100% recruitment) is used to determine fatigue, by comparison of pre/post-exercise muscle function testing. Although only three of the nine relevant studies (Table 2.3) reported greater CNS fatigue than peripheral fatigue, all of the studies found that skeletal muscle fatigue results from elements of both peripheral and central fatigue (4, 33, 49, 56). According to Kent-Braun, around 20% of fatigue from exercise can be attributed to CNS, while the remaining 80% due to peripheral/contractile fatigue (28). In contrast, 3 of the 9 studies failed to observe changes in post-exercise CNS function (32, 34, 47). In support of peripheral fatigue being a more sizable factor than CNS function, Presland & Dowson found that even with a lower MVC post-exercise, there were no changes in CNS fatigue,

leading to the assumption that fatigue was due to peripheral factors (47). Despite equivocal findings over the development of CNS and peripheral fatigue, the origin of fatigue may be time and intensity dependant. Specifically, Lepers et al. found that throughout a 5 hour cycling ride at 55% VO_{2max}, peripheral fatigue increased primarily throughout the 1^{st} hour, while CNS fatigue played a more prominent role in the later stages of the ride (33). This suggests that fatigue during high intensity exercise may result from peripheral factors, while CNS fatigue becomes more apparent during longer duration exercise.

Table 2.3 Central Nervous System Fatigue/Interpolated Twitch Technique

Author	Subjects	Design	Performance Criterion	Findings
Kent- Braun, 1999 (29)	N = 9 (5 M, 4 F) healthy subjects	Pre/post- exercise tests: • 3 CNS tests- central activation ratio (peak MVC/peak total force) • compare MVC w/ titanic force, compare iEMG signal w/ change in activation potential • 1 peripheral test- magnetic resonance spectroscopy	4 minute sustained isometric MVC of ankle dorsiflexors	Central factors 20%, peripheral 80% of muscle fatigue
Lepers, 2001 (34)	N = 8(M) trained triathletes	Pre/post MVC (isometric and concentric) via EMG & IT	• 30min cycle at 80% VO2 (freely chosen cadence-FCC) • 30min cycle at 80% VO_{2max} at FCC+20% • 30min cycle at 80% VO_{2max} at FCC-20%	↔ CNS fatigue between 3 cadences
Lepers, 2002 (33)	N = 9 (M), highly trained cyclists/triathletes	ITT before start of each hour and 30min into recovery	5 hour cycle @ 55%	↑ peripheral fatigue in 1 hour, ↑ CNS fatigue in later stages
Lattier, 2003 (32)	N = 8 (M)	• Blood pH/lactate samples post-exercise • pre/post neuromuscular tests	Ten 1min runs @ max aerobic velocity (18% grade) w/ 2min rest periods	↔ CNS fatigue; ↓ peripheral fatigue

M = male, F = female, s = seconds, min = minutes, h = hours, ME = max effort, C = control, MAL = metabolic alkalosis, MVC = maximal voluntary contraction, LOA = level of activation, IT = interpolated twitch with isometric MVC, CNS = central nervous system, TT = timed trial, [LA] = lactate concentration, ↔ = no difference, ↑ = increase ↓ = decrease

Table 2.3 Central Nervous System Fatigue/Interpolated Twitch Technique (continued)

Author	Subjects	Design	Performance Criterion	Findings
Presland & Dowson, 2005 (47)	N = 15 (M), REC	Pre/post isometric MVC with right leg; 3 times w/1min rest interval	70% VO2 to exhaustion	↓ MVC by 30%; ↔ CNS fatigue w/ performance time
Lepers, 2008 (35)	N = 8 (M), triathletes	Pre/post MVC, activation level, excitation-contraction coupling process of knee extensors	Two 30 minute trials: variable power and constant power (75%)	↔ MVC (peripheral and CNS)
Racinais, 2007 (49)	N = 9 (M)	• ITT pre/post-exercise • muscle oxygenation recorded via near-infrared spectroscopy	Repeated sprints 10 x 6 seconds @ 0.9 N/kg w/ 30 second recovery	↓ motor drive post-exercise, ↓ muscle oxygenation w/sprints (peripheral fatigue)
Aman & Dempsy, 2008 (4)	N = 8 (M) competitive cyclists	Pre/post exercise function tests	5km TT w/ varying starting % of fatigue (0, 67, 83%)	↓ performance w/ ↑ CNS fatigue
Swart, 2009 (56)	N = 8	2 groups: • placebo (C) • 10mg methylphenidate	Cycle @ RPE of 16 (Borg scale) until PO < 70% of initial PO	↑ time 32% with methylphenidate

M = male, F = female, s = seconds, min = minutes, h = hours, ME = max effort, C = control, MAL = metabolic alkalosis, MVC = maximal voluntary contraction, LOA = level of activation, IT = interpolated twitch with isometric MVC, CNS = central nervous system, TT = timed trial, [LA] = lactate concentration, ↔ = no difference, ↑ = increase ↓ = decrease

Blood pH/Lactate Levels and Exercise Performance

As previously discussed, there is a causal relationship between muscle fatigue and exercise performance; therefore it is logical to expect that blood pH and lactate's effect on muscle function will similarly impact performance. Many studies have examined this potential relationship between the two blood parameters and exercise performance (Table 2.4 and Table 2.5). While earlier research explored the general relationship between blood lactate/ pH and exercise performance, more recent research has focused on whether or not the manipulation of these parameters impact athletic performance. It is well documented that metabolic acidosis and metabolic alkalosis yield divergent performance outcomes, such that metabolic acidosis impairs performance and metabolic alkalosis improves performance (13, 27, 36, 38, 45, 46, 50, 60). Further, of these studies, all but a few have found significant correlations between higher blood pH/ lower lactate levels and improved exercise performance.

Research has consistently displayed in the now predictable relationship between pH, exercise intensity and exercise duration, with a rise in pH during high exercise intensities (25, 42, 54, 59). As this concept became better established, attempts to manipulate pH via sodium bicarbonate ($NaHCO_3$) ingestion became increasingly popular in order to study the effects of pH on performance (13, 17, 27, 36, 38, 45, 50, 53, 60). Results of these studies seem to consistently show a state of induced alkalosis leading to elevated pH levels being maintained throughout exercise and into recovery (17, 45, 46, 60). Presumably as a result of elevated pH levels, subjects were able to maintain high power outputs, delayed fatigue, and improved time-trial performance (27, 36, 46, 50, 60). In contrast, separate investigations reported that ingesting 0.3 g/kg $NaHCO_3$ had no effect on time trial performance at 80% VO_2max, 9-min sprint performance, or pH (13, 53).

Although few in number, the studies that have found a correlation between induced alkalosis and lactate levels, have shown dissimilar results. Raymer et al. found that with continuous wrist flexion to fatigue, despite $NaHCO_3$ ingestion 1.5 hours prior to the trial, there were neither significant differences in lactate levels, nor exercise performance, in comparison to the control group (50). Jones et al. found that induced alkalosis, despite enhancements in performance, resulted in higher lactate levels throughout exercise (27). Without any supplementation, generally lower blood lactate corresponds with better performance (16, 42). In conclusion, as blood pH decreases and blood lactate elevates, exercise performance is impaired.

Table 2.4 Blood pH and Exercise Performance

Author	Subjects	Design	Performance Criterion	Findings
Jones, 1977 (27)	N = 5 (M); healthy; training not specified	Supplements (0.3g/kg): • $CaCO_3$ (C) • $NaHCO_3$ (alk) • NH_4Cl(acd); • double blind fashion • taken 3h preceding trial	3 trials for each subject: • cycling at 33% (20m) • 66% (20m) • 95% (to exhaustion)	↑ endurance time and ↑power output w/ alk (visa versa with acidosis)
McCartney, 1983 (38)	N = 6 (M); age 19-23 yo	Supplements (0.3 g/kg): • $CaCO_3$ (C) • $NaHCO_3$ (alk) • NH_4Cl (acd) • 5% CO_2 - 21% O_2 - 74% N_2 (RAC)	30s ME on cycle ergometer at 100 rpm	↓ ME in acd, RAC
Potteiger, 1996 (46)	N = 8 (M) trained cyclists	• placebo (C) • 0.5g/kg sodium citrate	30km cycling TT	↑TT w/ sodium citrate
Verbitsky, 1997 (60)	N = 6 (M), REC exercisers	400mg/kg ingestion of $NaHCO_3$ (alk)	3 tests: • cycling at max • supramax (max + 17%) • alk 1h prior to supramax	↑quad torque in alk
Patterson, 2002 (45)	N = 10 (M), peak VO_{2max} 4.01 L/min	Supplements (0.3g/kg): • $NaHCO_3$ (alk) • placebo (C) • ingested in 6 equal doses over 2h (1h prior to trial)	• Cycle for 90min at 62.5% VO_{2max} • self- selected rpm • each condition tested 7 days apart	↑pH in alk throughout exercise compared to C

M = male, F = female, yo = years old, s = seconds, min = minutes, h = hours, ME = max effort, C = control, alk = metabolic alkalosis, acd = metabolic acidosis, RAC = respiratory acidosis, TT = timed trial, PLT = highest power output with no lactate increase from baseline, PLT1 = highest power output with no increase in lactate above 1mmol/L, PpHT = highest power output with no decrease in pH below baseline, PMLSS = highest power output during a 9-min steady state with no increase in lactate above 1mmol/L, COMP = competitive, REC = recreational, ↔ = no difference, ↑ = increase ↓ = decrease

Table 2.4 Blood pH and Exercise Performance (continued)

Author	Subjects	Design	Performance Criterion	Findings
Stephens, 2002 (53)	N = 6 (M) trained cyclists/triathletes	Supplements (0.3g/kg): • $NaHCO_3$ (alk) • placebo (C) • ingested 2h prior to trial	30min cycling at 77% VO_{2max} followed by TT at 80% VO_2max	↔
Raymer, 2004 (50)	N = 6 (M), REC exercisers	Supplements (0.3 g/kg): • $NaHCO_3$ (alk) • placebo (C) • ingested 1.5h prior to trial	Continuous wrist flexion to fatigue in supine position	↑ TTE in alk
Mora-Rodriguez, 2006 (41)	N = 9	VE, VCO2, PO at ventilatory threshold • blood pH, lactate, PO2 taken @ 175 watts-300 watts	Continuous, incremental TTE (↑ 25W every 3 min) @ 80, 100, 120 rpm	↓ PO w/ ↑ [LA] @ 120 rpm; ↔ pH b/t cadences
Lindh, 2008 (36)	N = 9 (M) elite swimmers	Supplements (300mg/kg): • $NaHCO_3$ (alk) • placebo (C) • ingested 1.5h prior to trial	200m free-style swim at ME	↑ performance time in alk
Aisbett, 2009 (2)	N = 26 (M), trained cyclists	• 3 blood draws (n = 12) @ pre-exercise, 25% of trial, post-exercise for pH, bicarbonate, lactate	6 sessions: • incremental cycle TTE • 2 self-paced FAM trials • 3 TT (fast, slow and even paced starting strategies)	↓ finish time, ↑ PO w/ fast-paced starting strategy; ↔ pH & [LA]
Cameron, 2010 (13)	N = 25 (M) rugby players	• placebo (C) • 0.3 g/kg $NaHCO_3$	• 25min warm up • 9min high intensity sprint test	↔

M = male, F = female, yo = years old, s = seconds, min = minutes, h = hours, ME = max effort, C = control, alk = metabolic alkalosis, acd = metabolic acidosis, [LA] = lactate concentration, TTE = time to exhaustion, PO = power output, REC = recreational, FAM = familiarization trial, ↔ = no difference, ↑ = increase ↓ = decrease

Table 2.5 Blood Lactate and Exercise Performance

Author	Subjects	Design	Performance Criterion	Findings
Jones, 1977 (27)	N = 5 (M); healthy; training not specified	Supplements (0.3g/kg): • $CaCO_3$ (C) • $NaHCO_3$ (alk) • NH_4Cl(acd); • double blind • taken 3h preceding trial	3 trials for each subject: • cycling at 33% (20m) • 66% (20m) • 95% (to exhaustion)	↓ plasma [LA] associated with acidosis and shorter endurance time; ↑ plasma [LA] associated with alkalosis and longer endurance time
Coyle, 1988 (16)	N = 14 (M)	• low LT • high LT	TTE @ 88% (stop when < 87%)	↑ 2x TTE w/ high LT, ↓ [LA] post- exercise w/ high LT
Verbitsky, 1997 (60)	N = 6 (M), REC exercisers	400mg/kg ingestion of $NaHCO_3$ (alk) 1h prior to trial	tests: • cycling at max • 2 supramax (max + 17%) tests C & alk, IT pre/post tests	↑ peak and residual knee torque in alk
Stepto, 2001 (54)	N = 7; trained cyclists	• Venous blood samples after each bout and pre/post exercise • muscle biopsy pre/post exercise protocol	8 x 5min cycling at 86% VO_2max	↔ blood [LA], ↑ muscle [LA], significant correlation between average blood [LA] during exercise and post-exercise muscle [LA]
Raymer, 2004 (50)	N = 6 (M), REC exercisers	Supplements: • $NaHCO_3$ (alk) • placebo (C) • ingested 1.5h prior to trial	Continuous wrist flexion to fatigue in supine position	alk: ↑ TTE by 12%, ↑ PO

M = male, F = female, yo = years old, s = seconds, min = minutes, h = hours, ME = max effort, C = control, [LA] = lactate concentration, alk = metabolic alkalosis, acd = metabolic acidosis, RAC = respiratory acidosis, ME = maximal effort, REC = recreational, TTE = time to exhaustion, PO = power output, ↔ = no difference, ↑ = increase ↓ = decrease

Table 2.5 Blood Lactate and Exercise Performance (continued)

Author	Subjects	Design	Performance Criterion	Findings
Guellich & Seiler, 2010 (24)	N = 51 (M) trained track cyclists	Arterialized blood samples taken from earlobe after each stage	Intermittent cycle ergometer test pre/post- 15wk training period	Training at intensities 3-6mmol/L [LA] led to poor ergometer testing due to ↑[LA]
Morris & Shafer, 2010 (42)	N = 13 (10 M, 3F) trained cyclists and triathletes	• Blood pre- exercise, 1/3rd , 2/3rd during exercise, post-exercise	• Graded submax test to determine PLT, PLT1, PpHT • discontinuous exercise test (9min bouts) to determine PMLSS • 20km TT to find mean power output	↔ pH while ↑[LA] during TT; lactate not attributed to fatigue
Tenan, 2010 (57)	N = 8 (4 M, 4 F)	• normal conditions • glycogen reduced conditions	Discontinuous GXT	↓ [LA] in glycogen reduced

M = male, F = female, yo = years old, s = seconds, min = minutes, h = hours, ME = max effort, C = control, [LA] = lactate concentration, alk = metabolic alkalosis, acd = metabolic acidosis, PLT = highest power output with no lactate increase from baseline, PLT1 = highest power output with no increase in lactate above 1mmol/L, PpHT = highest power output with no decrease in pH below baseline, PMLSS = highest power output during a 9-min steady state with no increase in lactate above 1mmol/L, TT = time trial, ↔ = no difference, ↑ = increase ↓ = decrease

The Effects of Blood Flow on Blood pH/Lactate Levels

In general, blood flow increases along with exercise intensity (12, 44, 48). With an increase in blood flow during exercise, better transportation is provided for lactate clearance during heavy exercise. However, Bangsbo displayed contradicting results with a higher blood flow and lower lactate clearance from muscle (8). Bangsbo et al. explains that a shortened mean transit time in the capillary bed that results from high blood flow, leads to a sub- optimal amount of time for exchange of lactate from the muscle to capillary blood, resulting in higher blood lactate levels (8). A prominent variable effecting blood flow is hydration status, of which Gonzalez et al. found influenced velocity of blood flow in a negative manor. Another variable effecting of blood flow during exercise is vein diameter (23). Venodilation is known to occur in linearity with blood flow. Calbet et al. provided evidence of this in both the subclavian and femoral veins with ergometer cycling (12). During exercise, both venodilation and increased blood flow are known mechanisms that aid in lactate clearance from working muscles (12, 23, 44).

Table 2.6 Effects of Blood Flow on Blood pH/Lactate Levels

Author	Subjects	Design	Performance Criterion	Findings
Bangsbo, 1993 (8)	N = 6 (M)	• Blood samples at 0,1,3,5,10 and recovery • blood flow pre/post exercise via thermodilution	• One legged exercise at high intensity (59±5.3 W) for 10min • 7 bouts of either high intensity (90W) or low intensity (13W) for 15s	↑ BF led to ↓ lactate transport
Gonzalez, 1998 (23)	N = 7 (M), trained cyclists	•leg blood flow measured via infusion thermodilution technique	•cycle ~61% VO_{2max} to exhaustion while dehydrated • 1 week later, cycle ~61% VO_{2max} for same time as first ride hydrated	↑ BF w/ exercise (hydrated), ↓ BF with exercise (dehydrated)
Calbet, 2007 (12)	N = 9 (M)	Pre/post femoral and subclavian venous blood flow	• upright cycle ergometer and arm cycle ergometer for 90min at 60/20% VO_{2max} respectively • 1 hr rest • Lower body cycle to fatigue • 30min rest • Upper body cycle to fatigue	↑ BF and vasodilation w/ ↑ exercise intensity
Osada & Radegran, 2009 (44)	N = 9 (M)	Doppler ultrasound of femoral artery (above bifurcation)	1 legged knee extensions at 30 and 60 CPM at work loads 10, 20, 30, 40 watts	↑ arterial BF w/ increased workload

M = male, F = female, s = seconds, min = minutes, h = hours, CPM = contractions per minute, BF = blood flow, [LA] = lactate concentration, ↔ = no difference, ↑ = increase, ↓ = decrease

Herbal Supplementation/SR2W-1 and Exercise Performance

Of the very few studies conducted on herbal supplementation and exercise performance, results have been equivocal (1, 3, 5, 6, 14, 19-21, 31, 62). Specifically, SR2W-1 has been shown to increase time to exhaustion in mice and improved cycling time trial performance in humans (1, 31). Of the ingredients in SR2W-1, ginseng has been the most extensively studied for possible ergogenic effects; however, results of improved performance are inconsistent (5, 6, 18-21, 62). Only one of the eight studies reviewed in Table 2.7 reported improved exercise time to exhaustion (5). Other studies looking at various forms ginseng have implemented quantities of supplementation ranging from 200-1200mg/day, and supplementation periods lasting anywhere from 7 days to 8 weeks, all of which have shown no effect of ginseng on performance (6, 18-21, 62). Another ingredient found in SR2W-1, Cordyceps sinesis, has also been a point of interest in the pursuit of herbal supplementation's effect of exercise performance. Colson et al (14), implemented a 13-day supplementation phase (2,000mg/day) of Cordyceps sinesis-rhodiola rosea and was unable to find any significant increases in time to fatigue in cycling performance. Despite contradictory findings, promising research that has shown benefits of herbal supplementation continues to reinforce interest.

Table 2.7 Herbal Supplementation/SR2W-1 and Exercise Performance

Author	Subjects	Design	Performance Criterion	Findings
Asano, 1986 (5)	N = 6 (M)	• 300mg/d ES Maxim for 8days • Placebo (C) • SB	•First, 3 GXT cycling on 3 consecutive days •Second, GXT cycling post 8d C •Third, GXT cycling post 8d ES	↑ TTE, ↔ VO_{2max}
Unknown, 1997 (1)	N = unknown; mice	Placebo (C) & 21 day supplement SR2W-1	Mice exercise, then swim to exhaustion	↑ TTE
Allen, 1998 (3)	N = 28 (20M, 8F)	• 200mg of 7% Panax ginseng for 3 weeks • Placebo (C)	Graded ergometer exercise test to max	↔
Lahr, 1999 (31)	N = 12	• 950mg/d of SR2W-1 for 5 weeks • placebo (C)	Post 5-week supplementation period, cycle at LT for 20min, 12min recovery, 60,000 J TT	trend of ↓ TT time w/ herbal supplement
Eschbach, 2000 (21)	N = 9 (M), cycle trained	• 1200mg/d leaf of ES for 7days • Placebo (C) •DB, CX, 7d washout	Cycle 120min at ~60% VO_{2max} followed by 10-km TT	↔ TT
Engels, 2001 (20)	N = 19 (F)	• 400mg/d Panax ginseng for 8 weeks • Placebo (C)	Supramaximal Wingate cycling protocol followed by a controlled recovery	↔TTE, power output, post exercise HR recovery

M = male, F = female, s = seconds, min = minutes, h = hours, d = day, caps = capsules, C = control, ES = Eleutheroccocus senticosus, TTE = time to exhaustion, LT = lactate threshold, TT = time trial, SB = single blind, DB = double blind, CX = crossover design, GXT = graded exercise test, ↔ = no difference, ↑ = increase, ↓ = decrease

Table 2.7 Herbal Supplementation/SR2W-1 and Exercise Performance (continued)

Author	Subjects	Design	Performance Criterion	Findings
Engels, 2003 (19)	N = 27 (active college students)	• 2g/d Panax ginseng for 8 weeks • Placebo (C)	Intervals of three 30-s sprint cycle x 3min recovery	↔ PO, HR recovery
Earnest, 2004 (18)	N = 17 (M) amateur cyclists	Placebo (C) & ginseng (loading phase of 4d-6caps/d and maintenance phase of 11d- 3caps/d)	Graded cycling ergometer test to max pre/post 14d supplementation period	↔TTE, PO
Colson, 2005 (14)	N = 8 (M)	• 13-day supplementation of 2,000mg Cs-Rr • Placebo (C)	Pre/post GXT on cycle ergometer	↔ TTE
Aziz, 2006 (6)	N = 19 (M) Field hockey players	• 31-day supplementation 440mg/d of lingzhi extract (EXP) • Placebo (C)	Pre/post body fat percentage, maximal oxygen uptake, 30s anaerobic Wingate cycling test, handgrip test	↔
Wasuntarawat, 2010 (62)	N = 35 (M) untrained	• Acute supplementation of 1.35g Kaempferia Parviflora, a.k.a. Thai Ginseng (90min prior to exercise) • Placebo (C)	Two tests: •intervals of three 30-s sprint cycle x 3min recovery •submax cycle to fatigue	↔

M = male, F = female, s = seconds, min = minutes, h = hours, d = day, caps = capsules, C = control, TTE = time to exhaustion, LT = lactate threshold, PO = power output, Cs-Rr = Cordyceps sinesis – rhodiola rosea, ↔ = no difference, ↑ = increase, ↓ = decrease

Herbal Supplementation/SR2W-1 and Blood Lactate Handling

Very little research has been conducted on the impact of herbal supplementation's influence on blood lactate production and/or clearance during exercise. Three of six relevant studies (Table 2.8) reported that herbal supplementation reduces blood lactate levels during recovery from exercise, compared to the control, one specifically pertaining to SR2W-1 (31, 43, 64). Wu et al. was able to provide evidence that reduction in lactate levels during recovery from exercise were actually accelerated with 800mg/day of leaf of Eleutheroccocus senticosus (a form of the extract found in SR2W-1) for 14 days (64). With 950mg/day of SR2W-1 for 5 weeks, lower lactic acid levels were also accompanied by faster subsequent time trial performance (31). Conversely, Morrissey et al. found that with the ingestion of 1000mg of SR2W-1, a reduction in blood lactate levels due to better lactate clearance were apparent following two weeks of ingestion, and even more so with an additional two additional weeks supplementation (43). However, others have failed to observe changes in any blood parameter or performance with the ingestion of herbal ginseng supplementation (3, 18). Mixed results could be a result of the length and type of supplementation. As provided in Table 2.8, three of the six studies with a longer supplementation period have shown positive results (31, 43, 64).

Table 2.8 Herbal Supplement/SR2W-1 and Blood Lactate Handling

Author	Subjects	Design	Performance Criterion	Findings
Wu, 1996 (64)	N = 8 (M)	• 800mg/d leaf of (ES) for 14days • Placebo (C)	Submaximal cycling test	↑↑ reduction in HR and lactate recovery from exercise
Morrissey, 1998 (43)	N = 30 (M)	3 groups: • placebo (C) • 500mg SR2W-1 • 1000mg SR2W-1	Computerized treadmill, increasing level of difficulty	↓ [LA] w/ 1000mg group (better lactate clearance) after 2 week supplementation and again with an additional 2 weeks
Lahr, 1999 (31)	N = 12	• 950mg/d of SR2W-1 for 5 weeks • placebo (C)	Cycle 20min at LT	↓[lactic acid] and change in pH during recovery w/ SR2W-1
Allen, 1998 (3)	N = 28 (20M, 8F)	• placebo (C) • 200mg of 7% Panax ginseng for 3 weeks	Graded ergometer exercise test to max	↔ [LA]
Eschbach, 2000 (21)	N = 9 (M), cycle trained	• 1200mg/d leaf of ES for 7days • Placebo (C) •DB, CX, 7d washout	Cycle 120min at ~60% VO_{2max} followed by 10-km TT	↔ [LA]
Earnest, 2004 (18)	N = 17 (M) amateur cyclists	• placebo (C) • experimental 14d supplementation period (loading phase of 4d-6caps/d and maintenance phase of 11d-3caps/d)	Graded cycling ergometer test to max pre/post supplementation period	↔[LA]

M = male, F = female, s = seconds, min = minutes, h = hours, C = control, DB = double blind, CX = crossover design, TT = time trial, TTE = time to exhaustion, LT = lactate threshold, [LA] = lactate concentration, ES = Eleutheroccocus senticosus, ↔ = no difference, ↑ = increase, ↓ = decrease, ↑↑ = accelerated

Summary

There is an eminently strong relationship between blood pH and lactate, especially during exercise. As exercise intensity increases, blood pH decreases and lactate increases (i.e. metabolic acidosis). When metabolic acidosis occurs, muscle fatigue is induced, thereby limiting exercise performance. Due to positive findings of the herbal supplement SR2W-1 on blood lactate levels, and its subsequent influence on exercise performance and muscle fatigue, the current study is designed to examine the influence of SR2W-1 on blood pH/lactate levels, cycling performance, and central versus peripheral fatigue. Whether central or peripheral fatigue is responsible for decrements during intense exercise, performance remains uncertain. Regardless, lactate and pH has been shown to have an effect on muscle fatigue. Thus, due to the potential effects of SR2W-1 on lactate handling and muscle fatigue, supplementation may have an ergogenic effect on cycling performance, and positive effect on muscle fatigue

CHAPTER THREE

METHODOLOGY

Subjects

Ten male and female recreational cyclists, aged 18-55 were recruited from James Madison University and the surrounding Harrisonburg area. Data collection on five subjects was delayed due to sickness and scheduling conflicts. Statistical analysis was therefore performed on the remaining five subjects. Subject characteristics are summarized in Table 3.1. Participants were provided with written and oral information about the experimental procedures, including potential risks, prior to informed consent (Appendix II). Prior to testing, all procedures were approved by the James Madison University International Review Board.

Table 3.1 Subject Characteristics

	Age (yrs)	Height (cm)	Weight (kg)	VO2 max (mL/kg/min)
Mean ± SE	38 ± 7.5	168.4 ± 4.3	68.8 ± 5.6	54.4 ± 2.6

Testing Procedures

Cardiorespiratory Fitness (VO_{2max})

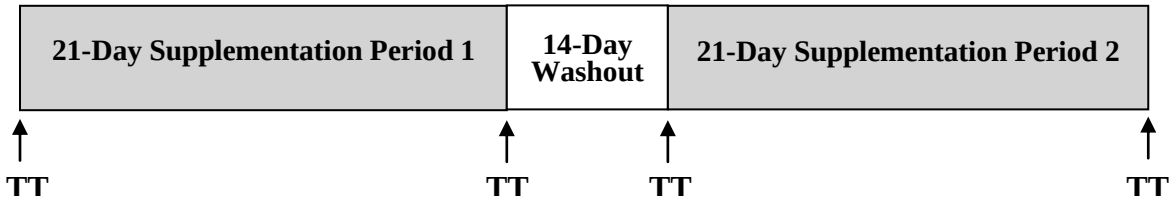
Subjects performed a graded exercise test to determine peak oxygen uptake (VO_{2max}) on a cycle ergometer (Velotron, Racermate, Inc., Seattle, WA). The initial exercise intensity was subjectively determined during a self-selected 5-minute warm-up. Subjects selected an intensity that could be maintained during a prolonged ride of "easy

to moderate intensity." Workload was then uniformly increased by 20 W every 3 minutes until volitional fatigue or until subjects were unable to maintain a pedaling cadence of ≥ 50 revolutions per minute. VO_2, VCO_2, RER, and ventilation were continuously monitored with a SensorMedics Spectra (Yorba Linda, CA) metabolic cart. A Polar (Lake Success, NY) heart rate monitor was used to determine heart rate during each test. Blood lactate levels were assessed in the final minute of each exercise stage with a blood lactate analyzer (YSI 2300 STAT glucose/lactate analyzer, Yellow Springs, Ohio), and used to determine lactate threshold (1 mmol above resting). RPE was also obtained in the final minute of each exercise stage. Peak power output was used to determine workloads during the exercise trials.

Exercise Trials

Each subject performed a total of five trials over the course of nine weeks. The initial trial served as a familiarization trial, while the remaining four trials were treatment trials (TT). The general study design is shown in Figure 3.1. The four treatment trials were conducted in a randomized, double-blind, crossover design, with the two supplementation phases separated by a 14-day washout period. The first two treatment trials were conducted before and after three weeks of supplementation with either placebo or SR2W-1. Following the subsequent washout phase, subjects completed the final two treatment trials, which were again conducted before and after three weeks of supplementation (opposite treatment- placebo or SR2W-1). All trials were performed at ambient temperatures of 21-22°C. A Home Essentials fan, set on 'medium' speed, was placed 2 meters from the handlebars of the ergometer for cooling purposes during the trial. Subjects were instructed to approach each time trial as a competitive event.

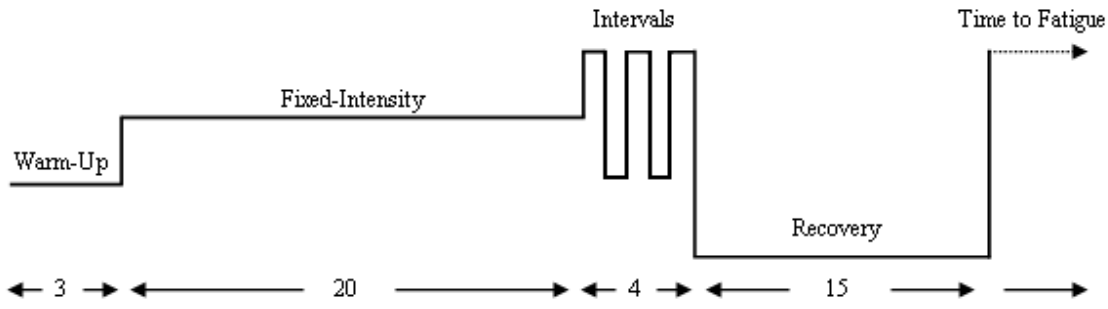
Figure 3.1 Treatment Period



TT = treatment trial

A summary of the exercise trial protocol is displayed in Figure 3.2. Subjects reported to the lab after a 10-12 hour fast overnight fast. To standardize postural-related hemodynamics, subjects sat for five minutes prior to blood flow measurements. Resting blood flow of the left femoral artery was then measured via ultrasonography (Mindray DC-6, Shenzen, Nanshan, China). Immediately following blood flow assessment and a five minute walking warm up (3.0 mph), pre-exercise muscle function was assessed via interpolated twitch, as described below. This protocol was utilized to obtain isometric peak torque (MVC) and percent muscle activation of the right leg extensors.

Figure 3.2 Treatment Trial Design



Following initial skeletal muscle function testing, subjects performed a 3-min warm-up at 100 watts, after which wattage was increased to a level that corresponded to 85% of the subject's VO_{2max} (average ± SE = 191 ± 22.2 Watts) and maintained for 20-minutes. Immediately following the 20-min phase, subjects completed three one-minute intervals at 100% of the power output obtained at VO_{2max} (average ±SE = 276 ± 31.7 Watts), with 30-seconds of active recovery (100 Watts) between each interval. Following the completion of the final interval, subjects terminated cycling and began a 15-min recovery phase during which a series of skeletal muscle function assessments were conducted. Subjects then cycled at a power output that corresponded to VO_{2max}, until fatigued (cadence ≤ 50 RPM).

Dependent Measures

Exercise Performance

Cycling duration (seconds) during the time to fatigue portion of the treatment trial was used as the performance measure.

Skeletal Muscle Function/Central Nervous System Fatigue

Prior to each treatment trial, subjects were positioned in a custom-built leg extension chair and prepared for electrical stimulation of the right knee extensor muscles. Brief electrical stimulations (i.e., paired pulses, consisting of two 0.2-ms pulses with an inter-pulse interval of 10 ms) were provided to a relaxed right knee extensor. The stimulator current was set at 90 mA for the first contraction, and progressively increased by 20 mA for each subsequent contraction, until isometric torque production of the knee

extensors has plateaued (i.e. no further increase in force production). The current that produced the greatest torque within the plateau was used as the supramaximal stimulation current for all subsequent stimulations throughout the rest of the study.

Once the supramaximal current was identified, subjects completed an interpolated twitch electrical-stimulation procedure that enabled determination of peak electrically evoked isometric torque of the knee extensors (MVC), peak electrically evoked isometric torque, and the percent muscle activation during the MVC. Subjects performed the 'interpolated twitch' procedure prior to each treatment trial, and at minutes 1 and 10 of the 15-minute recovery period. For each interpolated twitch trial, subjects performed a 3-sec isometric maximum voluntary contraction (MVC) of the knee extensor muscles; at 2.5 seconds into the contraction, paired-pulse electrical stimulation was delivered to the knee extensors to determine interpolated twitch torque (ITT). Two and four seconds following the 3-sec MVC, another paired-pulse stimulation was administered to the relaxed muscle to determine the peak electrically-evoked torque (EET). The percent muscle activation (%ACT) was estimated as 100% x (1 – ITT/EET). This procedure is an index of central nervous system fatigue.

Blood Lactate and Glucose

At the beginning of each trial, an indwelling antecubital venous catheter was inserted. After insertion, the catheter was flushed periodically with an injectable saline solution to keep the catheter patent. A total of 10 blood samples were obtained at the following time-points: 20 minute fixed intensity ride –10 and 20 minutes; intervals – immediately following the second 1-minute interval; recovery – 1.5, 3, 6, 9, 12, and 15 minutes; time-to-fatigue – 3 minutes following the time-to-fatigue. Lactate and glucose

concentrations were assessed immediately following each sample (YSI 2300 STAT glucose/lactate analyzer, Yellow Springs, Ohio). Recovery lactate was reported as area underneath the curve during the 15-min recovery period, and compared between treatments.

VO_2, RER, and Ventilation

VO_2, RER and VE were assessed at minutes 10 and 20 of the fixed-intensity ride and at the completion of the second interval of one-minute high-intensity intervals. A SensorMedics Spectra (Yorba Linda, CA) metabolic cart was used to assess breath-by-breath gas exchange and averaged over one-minute. The two minutes leading up to the desired time points during the 20-min steady state ride (minutes 10 and 20) were averaged to represent a mean for *oxygen uptake* (VO_2), *expired ventilation* (VE), and *respiratory exchange ratio* (RER; indicates relative contributions of fat and carbohydrate oxidation to total energy expenditure) at that time. Thirty seconds prior to the second completion of the second one-minute interval, VO_2, VE and RER were assessed a final time.

Heart Rate

Heart rate was recorded minutes 10 and 20 of the fixed-intensity ride, at the end of the second 1-minute interval, and 12-minutes into the recovery period, using a Polar heart rate monitor. In addition, average heart rate during the 15-minute recovery phase was recorded.

Ratings of Perceived Exertion (RPE)

Subjective ratings of exertion were obtained by having subjects point to a corresponding level of exertion on a Borg RPE scale (rated numerically from 6-20). RPE was assessed at minutes 10 and 20 of the fixed-intensity ride and at the end of the second one-minute interval.

Blood Flow

Left femoral artery diameter and velocity of blood was assessed via ultrasonography (Mindray DC-6 Shenzen, Nanshan, China). Blood flow (BF) was calculated with the following equation: $BF = \pi r^2 * (\text{blood velocity}) * 60s$. Pre-exercise flow was assessed at rest and eight minutes following the completion of the high-intensity intervals.

Supplementation

Subjects were randomly assigned to receive either 1000 mg of SR2W-1 or 1000 mg/day of Placebo. Subjects consumed 2 x 500 mg capsules every morning for 21 days. To promote supplementation compliance, subjects were required to text, e-mail, or call a specified member of the investigative team at the time of supplementation. SR2W-1 is a proprietary blend of herbs and fungi (primary ingredients: Enoki Mushroom, Eluthero Extract, Reishi Mushroom, Tangerine Extract, Cordyceps Mushroom, and Asian Ginseng). The placebo was provided by Radix BioResearch and was in capsule form identical to SR2W-1.

Dietary and Exercise Controls

Subjects were instructed to: 1) Maintain consistent dietary habits for 72 hrs prior to each trial, 2) Complete a diet record (Appendix V) for the 24 hrs preceding each trial, 3) Avoid heavy exercise for 48 hrs prior to each trial, 4) Maintain consistent physical activity habits starting 72 hrs prior to the first treatment trial until the completion of the final treatment trial (~55 days), and 5) Record all physical activity performed during the 72 hrs preceding each trial (Appendix VI). Subjects consumed their final 'self-selected' meal no less than 10 hrs prior to the start of the treatment trials (i.e. dinner on the evening prior to testing). After this time, subjects consumed only water *ad libitum* until the end of each treatment trial (a total of ~10 to 12 hrs of fasting with *ad libitum* water intake).

Statistical Analyses

Due to the small sample size used in this study, Wilcoxon Signed-Rank Tests were used to compare change-scores, for each dependent variable, from pre- to post-supplementation under both EXP and PLA conditions. Specifically, differences between pre- and post-supplementation cycling time to fatigue, MVC, percent muscle activation, blood flow, blood glucose, blood lactate, VO_2, RER, VE, HR and RPE was compared between treatments at a given time point during the exercise trials. Significance was set at $p < 0.05$, whereas p-values of < 0.1 were categorized as "approaching significance." All recovery blood lactate data is represented as areas underneath the curve. Results are reported as means ± SE.

CHAPTER FOUR

MANUSCRIPT

THE EFFECTS OF SR2W-1 SUPPLEMENTATION ON CYCLING PERFORMANCE AND MUSCLE FATIGUE

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Running Head: Herbal supplement, CNS fatigue, interpolated twitch, and cycling performance

ABSTRACT

Purpose: The purpose of this investigation was to examine the potential effects of SR2W-1 supplementation on cycling performance, central fatigue, and a variety of physiological parameters, including blood lactate, blood pH, heart rate (HR), rating of perceived exertion (RPE), oxygen consumption (VO_2), expired ventilation (VE), and respiratory exchange ratio (RER). **Methods:** Five recreational cyclists (38 ± 8 yr, 168.4 ± 4.3 cm, 68.8 ± 5.6 kg, and 54.4 ± 2.6 mL/kg/min) performed 20-min of steady-state cycling (~85% VO_{2max}) followed by three 1-min high intensity intervals at VO_{2max} with 30-sec active recovery periods at 100 W, 15-min passive recovery period, and a ride to fatigue (or < 50 RPM) at VO_{2max}. Subjects were randomly assigned to receive either 1000mg/d of SR2W-1 (EXP) or 1000mg/d placebo (PLA). The supplement was distributed in a double-blind, crossover fashion. The twenty-one day supplementation periods were separated by a 14-day "washout" period. Change-scores from pre- to post-supplementation were calculated for all parameters, under both PLA and EXP conditions. Wilcoxon Signed-Rank Tests were utilized to compare change-scores between PLA and EXP conditions. **Results:** There were no differences between PLA and EXP for any of the dependent variables; however several variables approached significance ($p < 0.1$). Specifically blood glucose levels, HR and ventilation increased to a greater extent with EXP, compared to PLA throughout the 20-min ride and high intensity intervals. **Conclusion:** Notwithstanding the small sample size, 3 weeks of herbal supplementation (SR2W-1) does not appear to aid in cycling performance, attenuate skeletal muscle fatigue, or modify the general physiological responses to exercise.

Key words: Herbal supplement, CNS fatigue, interpolated twitch, and cycling performance

INTRODUCTION

An abundance of exercise science research has focused on the manipulation of certain variables to optimize performance. High intensity endurance performance (i.e. cycling and running) is naturally limited by factors that contribute to skeletal muscle fatigue. In 1954, Merton proposed that fatigue results from inconsistent and inadequate blood supply to working muscle (40). The potential role of blood in the development of muscle fatigue is not limited to Merton's hypothesis but also includes exercise-induced variations in the concentration of certain blood parameters, namely hydrogen ions (pH) and lactate (22, 25, 27, 38, 40, 42).

Hermansen and Osnes were among the first to demonstrate the inverse relationship between pH (increase in hydrogen ion concentration) and exercise intensity (25). When ATP demands become increasingly high, in accordance with exercise intensity, glycolysis generates pyruvate at rates that exceed pyruvate oxidative capacity. In this scenario, accumulating amounts of pyruvate are converted into lactic acid, which then disassociate into lactate and H^+ ions (i.e. metabolic acidosis) (10, 51). Despite these events, it has been suggested that proton accumulation during high intensity exercise results from ATP hydrolysis, not lactic acid formation (9, 10, 51, 59). Although still unsettled, a powerful model in favor of lactate-induced acidosis includes research on individuals with McArdle's disease, a disease characterized by the inability to metabolize muscle glycogen. During strenuous activity, McArdle's patients are unable to produce lactic acid nor experience metabolic acidosis during high intensity physical activity (61).

Regardless of the derivation of the hydrogen ions, there is a clear directional relationship between exercise intensity, blood pH, and skeletal muscle function. The direct impact that pH has on muscle performance is best illustrated by experimental

manipulations of pH facilitated by the ingestion of ammonium chloride (NH_4Cl) or sodium bicarbonate ($NaHCO_3$), as the two treatments have opposite effects of blood pH buffering. Skeletal muscle power output was impacted by both conditions. Specifically, $NaHCO_3$ ingestion enhanced muscle power output (increased pH), whereas NH_4Cl impaired power output (decreased pH) (27). Raymer et al. found that slight alterations in blood pH by inducing alkalosis led to a longer time to exhaustion during progressive wrist flexions to fatigue (50). Lindh and colleagues reported significantly faster swimming time-trials for the 200-m freestyle with acute ingestion of sodium bicarbonate (200 mg/kg of body weight) 60-90 minutes prior to performance (36). Denning et al. concluded that any decrement in HCO_3 in the blood led to an impaired ability to buffer H^+ ions during high intensity exercise (leading to acidosis and muscle fatigue) (17).

While the general relationship between skeletal muscle performance and pH are clear (28), underlying mechanisms for this remain uncertain (52, 54). In addition to the aforementioned peripheral factors that are known to inhibit muscle function, fatigue has also been correlated with neuromuscular dynamics (26). Reductions in muscle fiber conduction velocity and motor drive have been linked to central nervous system fatigue (CNS) (45, 49). Specifically, Racinais et al. was able to display a decrement in motor drive after repeated bouts of interval sprints, in addition to peripheral fatigue (49). However, in a state of induced alkalosis, both nerve/synapse activity and muscle fiber conduction velocity were augmented, when compared to normal conditions (26, 60). This finding suggests that fatigue is, to a greater extent, attributable to CNS fatigue rather than peripheral fatigue under these conditions (26, 60). Specifically, elevated serum K^+ levels in a state of alkalosis improves muscle fiber conduction velocity, thereby

preserving contractile properties in the sarcolemma (i.e. attenuation of central fatigue) (45). Additionally, muscle fiber conduction velocity is sustained throughout prolonged submaximal cycling in a state of alkalosis, thus maintaining greater resistance to fatigue (26). Kent-Braun demonstrated that there is a strong non-linear association between pH and force ($r = 0.95$), 20% of which is attributable to central fatigue, with the remaining attributed to peripheral fatigue (28). However, it remains unclear whether central or peripheral fatigue is most effected by induced alkalosis, and if duration or intensity of exercise dictate the source of fatigue that is effected (52, 54).

Recent research has been conducted on the possible ergogenic effects of herbal supplements that are in line with NCAA and IOC regulations and associated with minimal side effects. As explained by L.R. Kleijnen et al., ergogenic herbs are classified as adaptogens, which reportedly relieves central nervous system stress rather than providing a stimulatory effect, much like other ergogenic aids (30). However, if taken in excess, herbal supplements may cause hypertension, irritability, insomnia and gastrointestinal distress (37). Although the data is equivocal on the effects of herbal aids, some studies have demonstrated gains in muscular strength, maximal oxygen uptake, and lactate handling (11, 18, 31). Various forms of ginseng and mushroom have been some of the more popular forms of herbal supplementation that have been researched, both of which can be found in commercially available SR2W-1 (11). These key ingredients have been shown to aid in lactate maintenance and exercise performance (1, 5, 31, 64). A limited amount of research has demonstrated positive ergogenic effects specific to SR2W-1 supplementation in both humans and mice (1, 31). Although it is still unclear as to the source of fatigue, continuing research on herbal supplementation is vital, and may

be the key to exposing factors involved with muscle fatigue. Therefore, primary aim of this study was to examine the potential effects of 21-days of SR2W-1 supplementation on cycling performance, muscle fatigue, and various other physiological parameters (i.e. blood lactate, HR, RER, VE, VO_2) with a randomized, double-blinded, crossover study design.

METHODS

Subjects

Ten male and female recreational cyclists, aged 18-55 were recruited from James Madison University and the surrounding Harrisonburg area. Data collection on five subjects was delayed due to sickness and scheduling conflicts. Statistical analysis was therefore performed on the remaining five subjects. Subject characteristics are summarized in Table 4.1. Participants were provided with written and oral information about the experimental procedures, including potential risks, prior to informed consent (Appendix II). Prior to testing, all procedures were approved by the James Madison University International Review Board.

Table 4.1 Subject Characteristics

	Age (yrs)	Height (cm)	Weight (kg)	VO2 max (mL/kg/min)
Mean ± SE	38 ± 7.5	168.4 ± 4.3	68.8 ± 5.6	54.4 ± 2.6

Testing Procedures

Cardiorespiratory Fitness (VO_{2max})

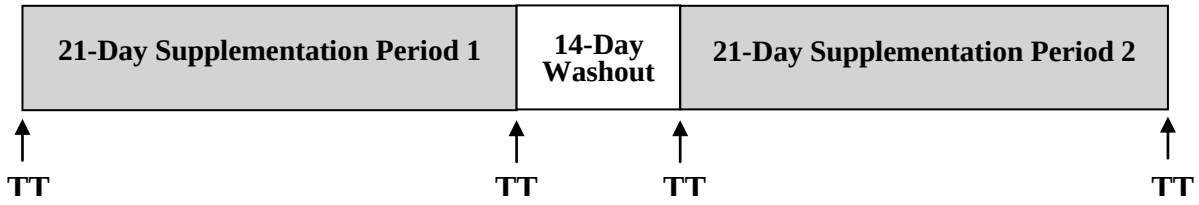
Subjects performed a graded exercise test to determine peak oxygen uptake (VO_{2max}) on a cycle ergometer (Velotron, Racermate, Inc., Seattle, WA). The initial exercise intensity was subjectively determined during a self-selected 5-minute warm-up. Subjects selected an intensity that could be maintained during a prolonged ride of "easy to moderate intensity." Workload was then uniformly increased by 20 W every 3 minutes until volitional fatigue or until subjects were unable to maintain a pedaling

cadence of ≥ 50 revolutions per minute. VO_2, VCO_2, RER, and ventilation were continuously monitored with a SensorMedics Spectra (Yorba Linda, CA) metabolic cart. A Polar (Lake Success, NY) heart rate monitor was used to determine heart rate during each test. Blood lactate levels were assessed in the final minute of each exercise stage with a blood lactate analyzer (YSI 2300 STAT glucose/lactate analyzer, Yellow Springs, Ohio), and used to determine lactate threshold (1 mmol above resting). RPE was also obtained in the final minute of each exercise stage. Peak power output was used to determine workloads during the exercise trials.

Exercise Trials

Each subject performed a total of five trials over the course of nine weeks. The initial trial served as a familiarization trial, while the remaining four trials were treatment trials (TT). The general study design is shown in Figure 4.1. The four treatment trials were conducted in a randomized, double-blind, crossover design, with the two supplementation phases separated by a 14-day washout period. The first two treatment trials were conducted before and after three weeks of supplementation with either placebo or SR2W-1. Following the subsequent washout phase, subjects completed the final two treatment trials, which were again conducted before and after three weeks of supplementation (opposite treatment- placebo or SR2W-1). All trials were performed at ambient temperatures of 21-22°C. A Home Essentials fan, set on 'medium' speed, was placed 2 meters from the handlebars of the ergometer for cooling purposes during the trial. Subjects were instructed to approach each time trial as a competitive event.

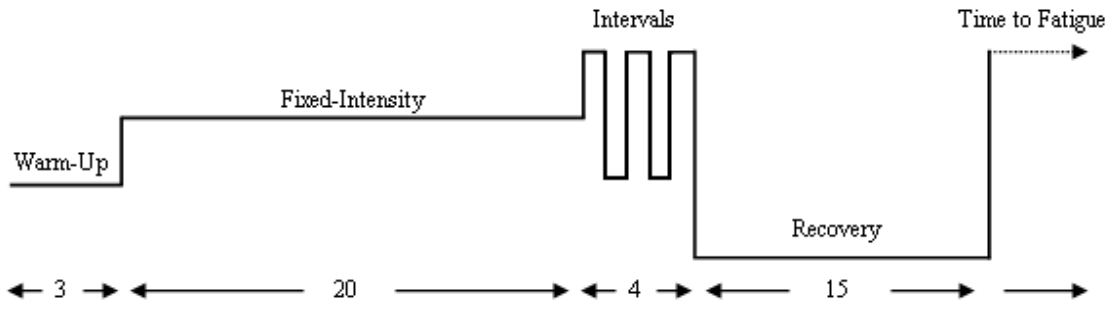
Figure 4.1 Treatment Period



TT = treatment trial

A summary of the exercise trial protocol is displayed in Figure 4.2. Subjects reported to the lab after a 10-12 hour fast overnight fast. To standardize postural-related hemodynamics, subjects sat for five minutes prior to blood flow measurements. Resting blood flow of the left femoral artery was then measured via ultrasonography (Mindray DC-6, Shenzen, Nanshan, China). Immediately following blood flow assessment and a five minute walking warm up (3.0 mph), pre-exercise muscle function was assessed via interpolated twitch, as described below. This protocol was utilized to obtain isometric peak torque (MVC) and percent muscle activation of the right leg extensors.

Figure 4.2 Treatment Trial Design



Following initial skeletal muscle function testing, subjects performed a 3-min warm-up at 100 watts, after which wattage was increased to a level that corresponded to 85% of the subject's VO_{2max} (average ±SE = 191 ± 22.2 watts) and maintained for 20-minutes. Immediately following the 20-min phase, subjects completed three one-minute intervals at 100% of the power output obtained at VO_{2max} (average ±SE = 276 ± 32 watts), with 30-seconds of active recovery (100 Watts) between each interval. Following the completion of the final interval, subjects terminated cycling and began a 15-min recovery phase during which a series of skeletal muscle function assessments were conducted. Subjects then cycled at a power output that corresponded to VO_{2max} until fatigued (cadence ≤ 50 RPM).

Dependent Measures

Exercise Performance

Cycling duration (seconds) during the time to fatigue portion of the treatment trial was used as the performance measure.

Skeletal Muscle Function/Central Nervous System Fatigue

Prior to each treatment trial, subjects were positioned in a custom-built leg extension chair and prepared for electrical stimulation of the right knee extensor muscles. Brief electrical stimulations (i.e., paired pulses, consisting of two 0.2-ms pulses with an inter-pulse interval of 10 ms) were provided to a relaxed right knee extensor. The stimulator current was set at 90 mA for the first contraction, and progressively increased by 20 mA for each subsequent contraction, until isometric torque production of the knee

extensors has plateaued (i.e. no further increase in force production). The current that produced the greatest torque within the plateau was used as the supramaximal stimulation current for all subsequent stimulations throughout the rest of the study.

Once the supramaximal current was identified, subjects completed an interpolated twitch electrical-stimulation procedure that enabled determination of peak electrically evoked isometric torque of the knee extensors (MVC), peak electrically evoked isometric torque, and the percent muscle activation during the MVC. Subjects performed the 'interpolated twitch' procedure prior to each treatment trial, and at minutes 1 and 10 of the 15-minute recovery period. For each interpolated twitch trial, subjects performed a 3-sec isometric maximum voluntary contraction (MVC) of the knee extensor muscles; at 2.5 seconds into the contraction, paired-pulse electrical stimulation was delivered to the knee extensors to determine interpolated twitch torque (ITT). Two and four seconds following the 3-sec MVC, another paired-pulse stimulation was administered to the relaxed muscle to determine the peak electrically evoked torque (EET). The percent muscle activation (%ACT) was estimated as 100% x (1 – ITT/EET). This procedure was utilized as an index of central nervous system fatigue.

Blood Lactate and Glucose

At the beginning of each trial, an indwelling antecubital venous catheter was inserted. After insertion, the catheter was flushed periodically with an injectable saline solution to keep the catheter patent. A total of 15 blood samples were obtained at the following time-points: 20 minute fixed intensity ride – 10 and 20 minutes; intervals – immediately following the first 1-minute interval; recovery – 1.5, 3, 6, 9, 12, and 15 minutes; time-to-fatigue – 3 minutes following the time-to-fatigue. Lactate and glucose

concentrations were assessed immediately following each sample (YSI 2300 STAT glucose/lactate analyzer, Yellow Springs, Ohio). Recovery lactate was reported as area underneath the curve during the 15-min recovery period, and compared between treatments.

VO_2, RER, and Ventilation

VO_2, RER and VE were assessed at minutes 10 and 20 of the fixed-intensity ride and at the completion of the second interval of one-minute intervals. A SensorMedics Spectra (Yorba Linda, CA) metabolic cart was used to assess breath-by-breath gas exchange and averaged over one-minute. The two minutes leading up to the desired time points during the 20-min steady state ride (minutes 10 and 20) were averaged to represent a mean for *oxygen uptake* (VO_2), *expired ventilation* (VE), and *respiratory exchange ratio* (RER; indicates relative contributions of fat and carbohydrate oxidation to total energy expenditure) at that time. Thirty seconds prior to the second completion of the second one-minute interval, VO_2, VE and RER were assessed a final time.

Heart Rate

Heart rate was recorded at minutes 10 and 20 of the fixed-intensity ride, at the end of the second 1-minute interval, and 12-minutes into the recovery period, using a Polar heart rate monitor. In addition, average heart rate during the 15-minute recovery phase was recorded.

Ratings of Perceived Exertion (RPE)

Subjective ratings of exertion were obtained by having subjects point to a corresponding level of exertion on a Borg RPE scale (rated numerically from 6-20). RPE was assessed at minutes 10 and 20 of the fixed-intensity ride and at the end of the second one-minute interval.

Blood Flow

Left femoral artery diameter and velocity of blood was assessed via ultrasonography (Mindray DC-6 Shenzen, Nanshan, China). Blood flow (BF) was calculated with the following equation: $BF = \pi r^2 *$ (blood velocity(cm/s)) * 60s. Pre-exercise flow was assessed at rest and eight minutes following the completion of the high-intensity intervals.

Supplementation

Subjects were randomly assigned to receive either 1000 mg of SR2W-1 or 1000 mg/day of Placebo. Subjects consumed 2 x 500 mg capsules every morning for 21 days. To promote supplementation compliance, subjects were required to text, e-mail, or call a specified member of the investigative team at the time of supplementation. SR2W-1 is a proprietary blend of herbs and fungi (primary ingredients: Enoki Mushroom, Eluthero Extract, Reishi Mushroom, Tangerine Extract, Cordyceps Mushroom, and Asian Ginseng). The placebo was provided by Radix BioResearch and was in capsule form identical to SR2W-1.

Dietary and Exercise Controls

Subjects were instructed to: 1) Maintain consistent dietary habits for 72 hrs prior to each trial, 2) Complete a diet record (Appendix V) for the 24 hrs preceding each trial, 3) Avoid heavy exercise for 48 hrs prior to each trial, 4) Maintain consistent physical activity habits starting 72 hrs prior to the first treatment trial until the completion of the final treatment trial (~55 days), and 5) Record all physical activity performed during the 72 hrs preceding each trial (Appendix VI). Subjects consumed their final 'self-selected' meal no less than 10 hrs prior to the start of the treatment trials (i.e. dinner on the evening prior to testing). After this time, subjects consumed only water *ad libitum* until the end of each treatment trial (a total of ~10 to 12 hrs of fasting with *ad libitum* water intake).

Statistical Analyses

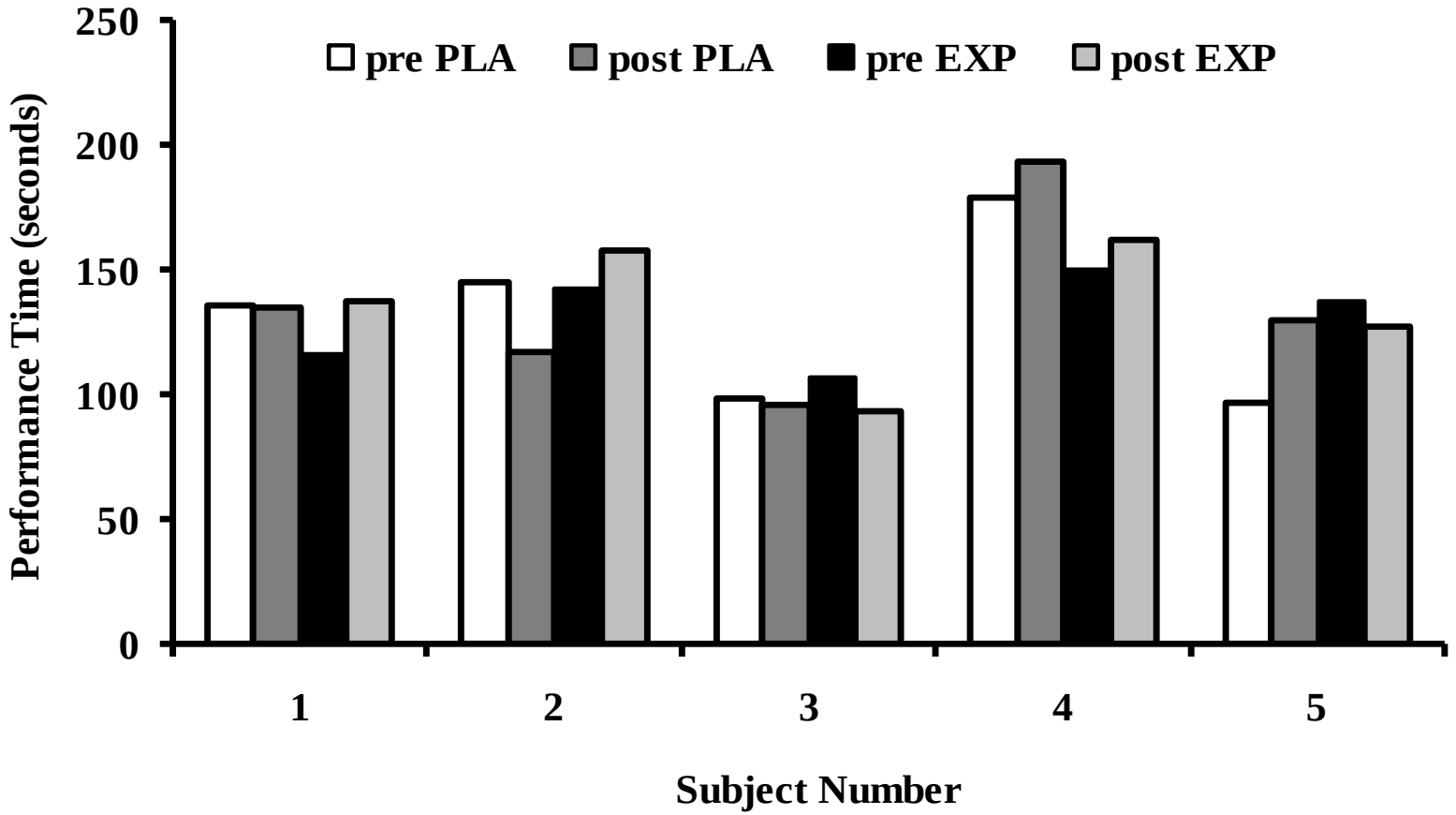
Due to the small sample size used in this study, Wilcoxon Signed-Rank Tests were used to compare change-scores, for each dependent variable, from pre- to post-supplementation under both EXP and PLA conditions. Specifically, differences between pre- and post-supplementation cycling time to fatigue, MVC, percent muscle activation, blood flow, blood glucose, blood lactate, VO_2, RER, VE, HR and RPE were compared between treatments at a given time point during the exercise trials. Significance was set at $p < 0.05$, whereas p-values of < 0.1 were categorized as "approaching significance." All recovery blood lactate data is represented as areas underneath the curve. Results are reported as means ± SE.

RESULTS

Exercise performance

Changes in time to fatigue, before and after 21 days of supplementation were not different between PLA and EXP. Individual performance times are displayed in Figure 4.3 (pre-PLA: 131.0 ± 15.4 sec; post PLA: 134.0 ± 16.2; pre-EXP: 130.0 ± 8.1; post-EXP: 135.0 ± 12.4).

Figure 4.3 Ride to Fatigue



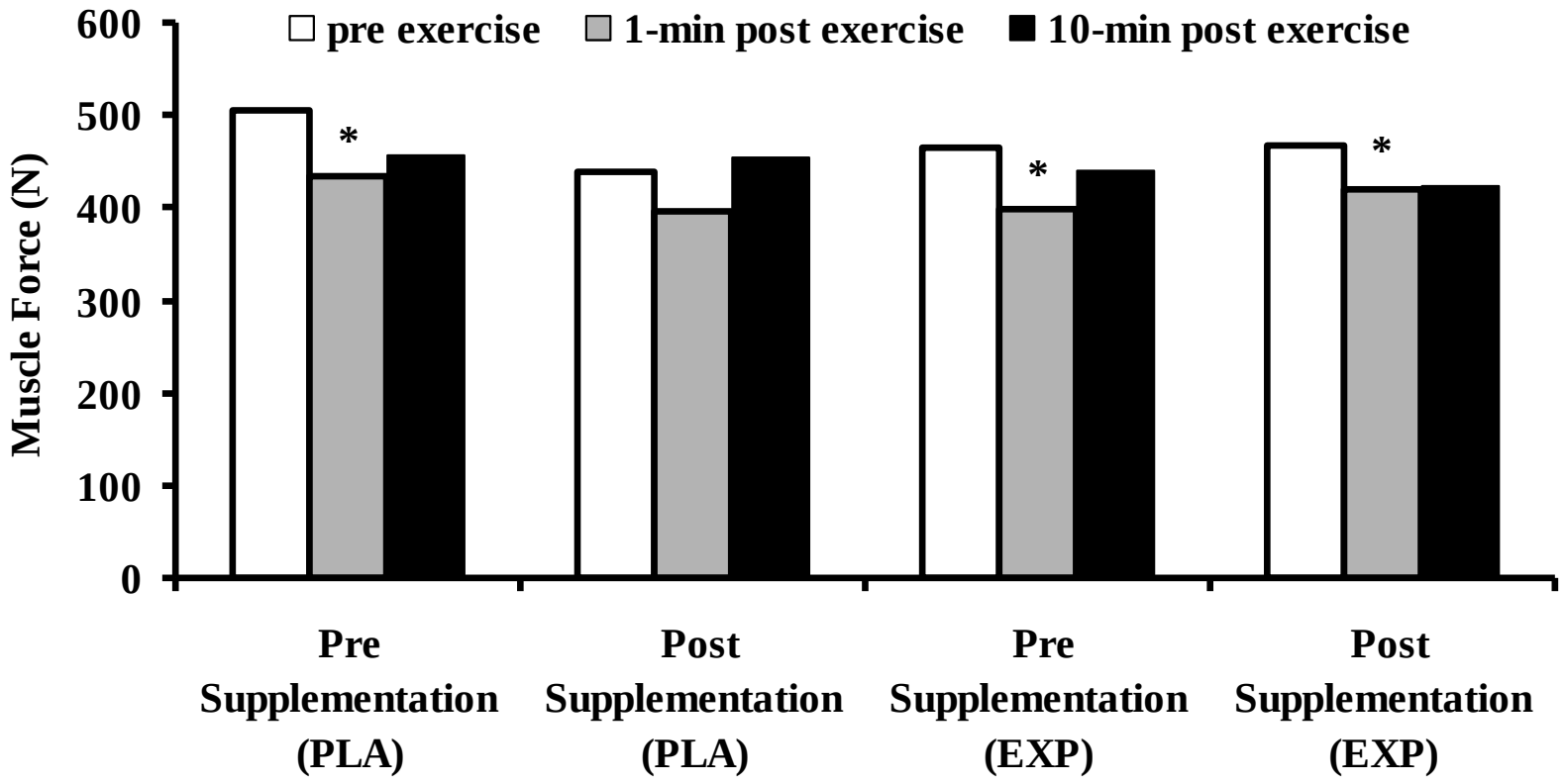
Skeletal Muscle Function/ Central Nervous System Fatigue

Changes in MVC and muscle activation resulting from exercise, before and after 21 days of supplementation were not different between PLA and EXP (Table 4.2). Average MVC data are displayed in Figure 4.4. Average percent muscle activation, pre- to post-supplementation, are displayed in Figure 4.5.

Table 4.2 Decline in Muscle Function and Activation with Exercise

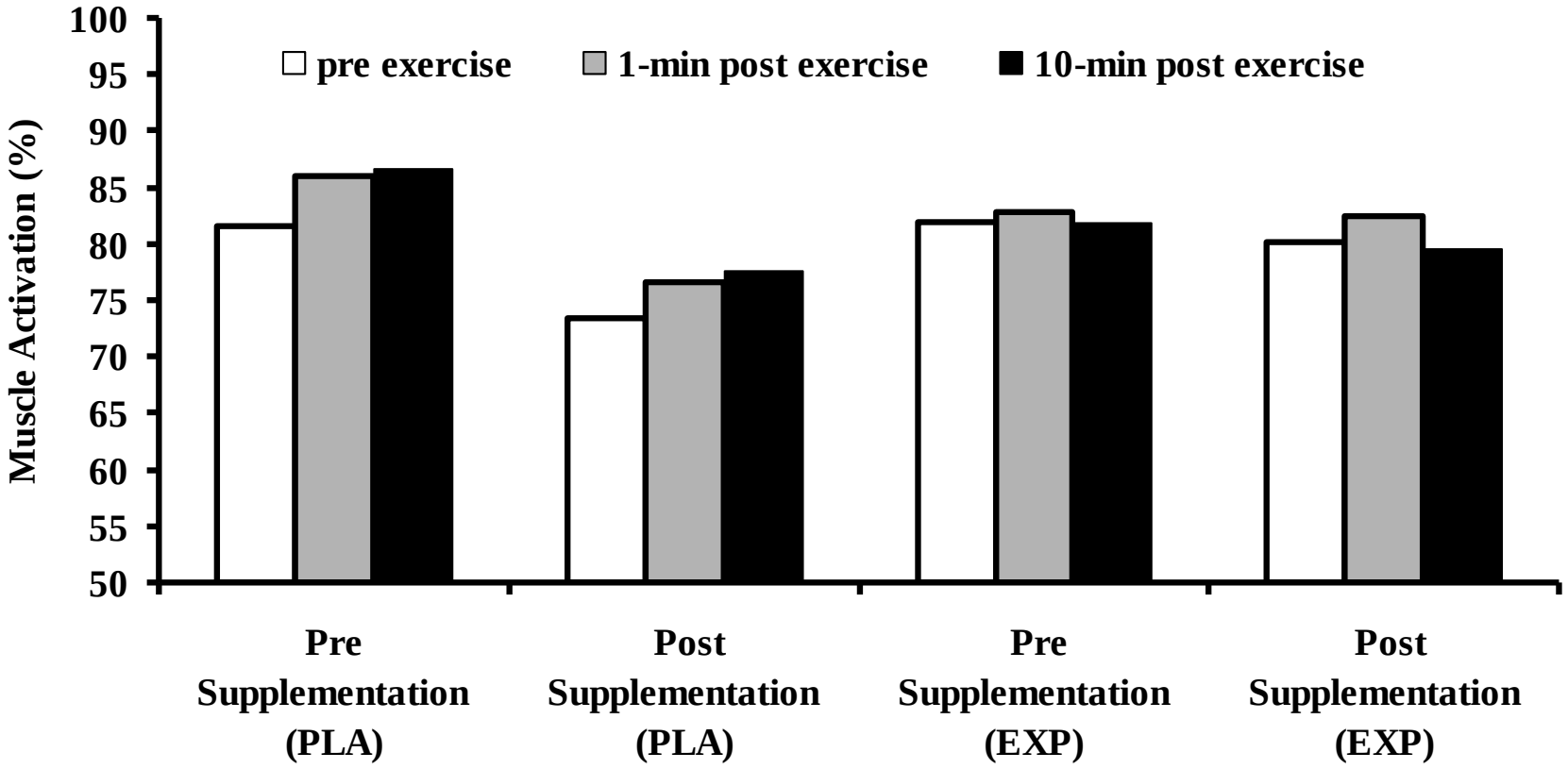
	PLA		**EXP**	
	Pre Ex to 1-min Post Ex	**Pre Ex to 10-min Post Ex**	**Pre Ex to 1-min Post Ex**	**Pre Ex to 10-min Post Ex**
Pre Supplementation Decline in Force (N)	71.2 ± 32.2	46.7 ± 21.0	67.0 ± 30.0	25.1 ± 11.2
Post Supplementation Decline in Force (N)	41.5 ±18.6	-18.0 ± -8.4	48.2 ± 21.6	42.3 ± 19.0
Pre Supplementation Decline in Muscle Activation (%)	-4.5 ± -2.0	-5.1 ± -2.3	-0.9 ± -0.4	-0.1 ± 0.0
Post Supplementation Decline in Muscle Activation (%)	-3.2 ± -1.4	-4.3 ± -1.9	-2.3 ± -1.0	0.6 ± 0.3

Figure 4.4 Peak Muscle Strength (MVC)



***** $p < 0.05$ compared to pre-exercise MVC

Figure 4.5 Percent Muscle Activation



Blood Lactate and Glucose

Changes in blood glucose and blood lactate, before and after 21 days of supplementation were not different between PLA and EXP, with the exception of minute 10. Average blood glucose and lactate levels are displayed in Table 4.3.

Table 4.3 Blood Glucose and Lactate Levels Before and Following Supplementation

		Placebo		Experimental	
Time (min)	**Variable**	**Pre**	**Post**	**Pre**	**Post**
10	Glucose† (mmol/L)	83.4 ± 2.9	83.0 ± 2.5	81.1 ± 1.7	85.3 ± 1.6
	Lactate* (mg/dL)	4.4 ± 0.8	3.9 ± 0.7	4.3 ± 0.5	4.6 ± 0.6
20	Glucose (mmol/L)	95.7 ± 5.6	92.2 ± 4.0	94.3 ± 4.9	98.3 ± 3.7
	Lactate (mg/dL)	5.9 ± 1.1	5.0 ± 0.9	5.7 ± 1.0	5.6 ± 1.0
22:30	Glucose (mmol/L)	100.9 ± 5.7	96.9 ± 4.5	97.6 ± 5.7	102.0 ± 83.5
	Lactate (mg/dL)	7.4 ± 1.0	6.5 ± 0.9	7.1 ± 1.0	6.7 ± 0.8
15-min recovery AUC	Lactate (mg/dL)	88.2 ± 13.6	76.5 ± 10.2	83.2 ± 13.7	77.6 ± 9.7

* $p < 0.05$ compared to EXP; † $p < 0.10$ compared to PLA; AUC = Area Under the Curve

VO_2, RER, Ventilation

Changes in VO_2 (L/min and mL/kg/min), RER, and VE (L/min) before and after 21 days of supplementation were not different between PLA and EXP, except for VE at minute 10. Average VO_2, RER, and VE are displayed in Table 4.4.

Table 4.4 VO_2, RER, VE Before and Following Supplementation

		Placebo		Experimental	
Time (min)	**Variable**	**Pre**	**Post**	**Pre**	**Post**
10	VO_2 (L/min) †	3.3 ± 0.3	3.03 ± 0.3	3.1 ± 0.3	3.3 ± 0.3
	VO_2 (L/kg/min)	47.9 ± 1.5	44 ± 2.7	46.5 ± 2.9	47.0 ± 1.8
	RER	0.94 ± 0.02	0.93 ± 0.03	0.93 ± 0.03	0.92 ± 0.02
	VE (L/min)*	88.2 ± 9.2	78.3 ± 8.5	80.9 ± 8.9	87.2 ± 7.6
20	VO_2 (L/min)	3.5 ± 0.4	3.2 ± 0.3	3.3 ± 0.3	3.3 ± 0.3
	VO_2 (mL/kg/min)	50.5 ± 1.5	45.9 ± 3.1	48.1 ± 2.5	48.4 ± 2.3
	RER	0.95 ± 0.02	0.92 ± 0.03	0.92 ± 0.03	0.92 ± 0.03
	VE (L/min)	110.4 ± 12.6	92.2 ± 10.6 †	97.2 ± 12.0	96.8 ± 8.4
22:30	VO_2 (L/min)	3.5 ± 0.4	3.5 ± 0.4	3.5 ± 0.4	3.5 ± 0.4
	VO_2 (mL/kg/min)	50.6 ± 2.3	49.7 ± 3.2	50.8 ± 2.8	49.8 ± 3.4
	RER	1.02 ± 0.03	1.03 ± 0.03	1.02 ± 0.04	1.01 ± 0.03
	VE (L/min)	127.2 ± 15.2	123.5 ± 16.34†	122.6 ± 15.6	129.7 ± 19.1

* $p < 0.05$ compared to EXP; † $p < 0.10$ compared to PLA

Heart Rate and Ratings of Perceived Exertion (RPE)

Changes in HR (beats per minute) before and after 21 days of supplementation were not different between PLA and EXP, the exception of minute nine into recovery from exercise. Average HR is displayed in Table 4.5. Similarly, changes in RPE before and after 21 days of supplementation were not different between PLA and EXP. Average RPE is displayed in Table 4.5.

Table 4.5 Heart Rate and RPE Before and Following Supplementation

		Placebo		Experimental	
Time (min)	**Variable**	**Pre**	**Post**	**Pre**	**Post**
10	HR (bpm) †	169 ± 6	166 ± 5†	168 ± 6	170 ± 7
	RPE	14.8 ± 0.5	15.2 ± 0.7	14.8 ± 0.8	15.0 ± 0.3
20	HR (bpm)	175 ± 4	173 ± 5	175 ± 5	175 ± 6
	RPE	17.0 ± 0.7	17.0 ± 0.7	16.8 ± 0.6	17.0 ± 0.5
22:30	HR (bpm) †	185 ± 4	182 ± 4	184 ± 5	184 ± 5
	RPE	19.2 ± 0.6	18.8 ± 0.5	19.0 ± 0.3	18.6 ± 0.4
3R	HR (bpm)	118 ± 6	111 ± 7	112 ± 50	118 ± 53
6R	HR (bpm)	105 ± 4	101 ± 6	109 ± 49	107 ± 48
9R	HR (bpm)*	107 ± 4	105 ± 5	103 ± 46	107 ± 48
12R	HR (bpm)	107 ± 5	103 ± 4^	106 ± 6	106 ± 6

* $p < 0.05$ compared to PLA; † $p < 0.10$ compared to EXP; ^ average ± SE with 4 of the 5 subjects; R = recovery period

Blood Flow

Changes in blood flow (L/min) before and after 21 days of supplementation were not different between PLA and EXP (difference in pre- to post- supplementation PLA: 1.1 ± 5.7 mL/min; difference in pre- to post- supplementation EXP: -1.7 ± 1.8). Average blood flow is displayed in Table 4.6.

Table 4.6 Blood Flow (L/min)

	PLA		EXP	
	Pre Exercise	**8-minute Post Exercise**	**Pre Exercise**	**8-minute Post Exercise**
Pre Supplementation	0.9 ± 0.4	1.2 ± 0.5	0.9 ± 0.4^	1.1 ± 0.4^
Post Supplementation	1.0 ± 0.4	1.4 ± 0.6	0.8 ± 0.5^	1.4 ± 0.6^

^ contains data from 4 of the 5 subjects

DISCUSSION

The primary aim of this study was to investigate the effects of herbal supplementation on exercise performance and central nervous system fatigue. To compliment the primary measures and to gain insight into the physiological changes with herbal supplementation, blood glucose, blood lactate, VO_2, RER, VE, HR, RPE and blood flow were also assessed. Contrary to our hypothesis, the herbal treatment (EXP) had no effect on cycling performance or central nervous system fatigue. Further, EXP had little effect on the aforementioned physiological parameters. Noteworthy is a greater elevation from baseline in heart rate, VO_2, ventilation, blood glucose, and blood lactate at minute 10 during the 20-minute fixed intensity workload, after 21- days of SR2W-1 supplementation, but were all similar between treatments at 20-minutes. This suggests that it took longer for subjects to physiologically adjust to the assigned workload after supplementing with EXP. Collectively, the current preliminary data indicates 21 days of EXP supplementation did not confer any performance or physiological benefits.

The performance findings in the current study are substantiated by two comparable investigations, as they reported that one to eight weeks of herbal supplementation did not influence cycling time to fatigue (20, 21). With a 400mg/d of herbal supplementation for eight weeks, Engels et al. found no effect on time to fatigue during a supramaximal Wingate Cycling protocol (20). Eschbach et al. also reported no effect on time to fatigue, with one week of 1200mg/day (21). Additional research on herbal supplementation and exercise performance has also shown no difference in exercise performance following supplementation (3, 6, 14, 18). Although performance was not enhanced by herbal supplementation in the aforementioned studies, this finding is not unanimous. Specifically, 21 days of SR2W-1 supplementation prolonged

swimming time to exhaustion in mice (1). To our knowledge, only one study reported that performance is enhanced with herbal supplementation in humans (31). The authors reported that cycling time trial performance was augmented following 5 weeks of supplementation (31). A common aspect of the studies reporting positive effects on exercise performance seems to be duration of the herbal supplementation period. A duration ranging between 8 days to 5 weeks have shown positive results (1, 5, 31), however there were a few studies with similar supplementation periods that reported null findings (3, 6, 14, 18).

In contrast to the current results, there is fairly strong evidence that herbal supplementation can favorably influence blood lactate levels during recovery from exercise (31, 43, 64). However, several other studies have failed to confirm this effect (3, 18, 21). Specifically, Allen et al. and Earnest et al. both found no differences in recovery lactate levels with 2-3 weeks of herbal supplementation (3, 18). Results in favor of herbal supplementation demonstrate reduced recovery lactate in as little as two weeks (1000mg/day of SR2W-1), with even further reductions in recovery lactate with two weeks of additional supplementation (43). Additionally, Wu et al. was able to provide evidence that reduction in lactate levels during recovery from exercise were actually accelerated with 800mg/day of herbal supplementation (64). These findings demonstrate the effectiveness of herbal aids on blood lactate recovery from exercise and potentially exercise performance.

An abundance of research supports the performance benefits of lower lactate and high pH levels, most of which has been elicited by induced alkalosis. In general, with the ingestion of $NaHCO_3$ (sodium bicarbonate) prior to exercise, exercise performance is

enhanced and muscle fatigue attenuated compared to normal conditions (26, 45, 60, 63). In context, considering that levels were similar between EXP and PLA, it is not surprising that exercise performance was unaffected by EXP. This is despite marked muscle fatigue independent of the treatment.

Although 21-days of PLA and EXP supplementation had no differential impact on the physiological response to exercise, alterations in blood glucose between PLA and EXP, consistently approached significance at several different time points during the exercise trials ($p < 0.10$). In addition to blood glucose, VE, VO_2 and HR were also visually elevated during the fixed intensity ride following EXP. In contrast to our general hypotheses, these findings provide some evidence that the same standardized protocol presented more of a physiological challenge following EXP supplementation. This heightened physiological state could be attributed to a relatively long supplementation period, thereby exposing possible side effects of herbal supplementation if taken in excess. However, other research failed to observe side effects with 3-5 weeks of SR2W-1 supplementation (1, 31).

To our knowledge, we are the first to assess post-exercise peripheral blood flow in response to herbal supplementation, and no differences were detected between PLA and EXP conditions. There is a clear association between exercise intensity and post-exercise blood flow (7, 12, 23, 44). For instance, Calbet et al. demonstrated that blood flow and vessel diameter increase in linearity with exercise intensity in both the upper and lower body extremities (12). Although associated with lactate clearance, research is equivocal in its association between blood flow and increased lactate clearance (8, 12). In the

current study, the consistent post-exercise lactate dynamics with both PLA and EXP may be related to the similarities in blood flow between treatments.

Despite the encouraging nature of early reports on the effects of herbal supplementation on exercise performance and blood lactate levels, the general performance and physiological response to exercise in the current study was similar under both PLA and EXP conditions. It is worth mentioning that a major limitation of the current study is the small sample size (n = 5); however each subject acted as their own control, thereby eliminated the possibility of inter-group differences in subject characteristics. Nonetheless, the current project is severely underpowered from a statistical standpoint and despite the apparent lack of benefits conferred by the herbal supplement, it possible a larger sample size would yield different results. Regardless, the current study indicates that SR2W-1 has no impact on cycling performance and central nervous system fatigue.

CHAPTER FIVE

SUMMARY

The primary aim of this study was to examine the potential effects of 21-days of SR2W-1 supplementation on cycling performance, muscle fatigue, and various other physiological parameters (i.e. blood lactate, HR, RER, VE, VO_2) with a randomized, double-blinded, crossover study design. We hypothesized that SR2W-1 supplementation will: 1) improve time to fatigue compared to a placebo supplement, 2) elicit lower lactate levels during the cycling protocol and during the recovery period in comparison to placebo supplementation, 3) allow for better resistance to central fatigue after a bout of high-intensity cycling, in comparison to a placebo group, and 4) allow for better resistance to peripheral fatigue after a bout of high-intensity cycling, in comparison to a placebo group.

In contrast to our hypotheses, SR2W-1 was found to be ineffective in both improving in cycling performance and attenuating muscle fatigue. Additionally, there were no differences in blood lactate, blood flow, RER, RPE, VO_2 between the treatments. Noteworthy, are trends in elevated HR and VE throughout the 20-minute fixed-intensity cycling protocol, favoring EXP. Possible explanations for lack of effect include, but are not limited to, a shorter supplementation period, short duration of cycling protocol and a small sample size. Notwithstanding these limitations, results of the present study suggest that herbal supplementation with SR2W-1 is not needed to reach optimal levels of cycling performance and attenuation of muscle fatigue.

Appendix I

CYCLISTS WANTED

FOR

SPORTS SUPPLEMENT STUDY

The Human Performance Laboratory at JMU will be conducting a study examining the effects of an herbal sports supplement on cycling performance, muscle function, and lactate dynamics.

Who are we looking for?

- Males and Females
- 18-55 years old
- Cyclists (individuals performing cycling exercise on a regular basis – at least 3 x wk)

What you will be asked to do:

- Complete preliminary fitness testing/screening (60-90 minutes)
- Participate in 5 exercise protocols (60-90 minutes), each of which consists of a 3 minute warm-up, 20-minutes of hard riding, 3 x 1-minute high intensity intervals, 15 minutes of recovery, and a high intensity ride to fatigue lasting approximately 5 minutes (~30-35 of total exercise)
- Receive laboratory assessments (including small blood draws, a muscle function test **or** small muscle biopsy) during each trial
- Supplement for 21 days with an all-natural sports formula and a placebo (each 21-day supplementation period is separated by 10 days)

What are the benefits of participation?

- Free evaluation of aerobic capacity (VO_{2max}) and physiological data
- $150 to $250 (biopsy subjects) for study completion

For more information, please contact Dr. Nick Luden at ludennd@jmu.edu or Dr. Mike Saunders at saundemj@jmu.edu

Appendix II

James Madison University

Department of Kinesiology

Consent for Investigative Procedure

Purpose

You are being asked to volunteer for a research project conducted by Dr. Nick Luden, Dr. Michael Saunders, Dr. Christopher Womack, Kevin Murach, and Tara Ata from James Madison University titled "The Effects of an Herbal Supplement on Human Cycling Performance, Muscle Function, and Lactate Dynamics".

The primary aim of the current project is to examine the influence that 21-days of SR2W-1 sports formula supplementation has on cycling performance, muscle function, and lactate dynamics. Information gained from this project will help determine the efficacy of SR2W-1 as well as indicate the underlying changes that are responsible for gains in cycling performance.

Experimental Procedures

You will be asked to report to James Madison University's Human Performance Laboratory (Godwin 209) for a total of six trials. Specifically, you will be asked to report to the laboratory for one pre-testing/screening trial, one familiarization trial, and four treatment trials. Each of the six trials will last approximately 60-90 minutes in duration. Detailed information for each of these trials is provided below:

<u>Pre-testing/Screening</u> (n = 1 trial)

Before any physical evaluation is given, pre-screening forms will be completed to ensure that you meet the study criteria, that you do not have any risk factors for heavy exercise, and that you do not have any known allergies to local anesthesia. In the process of filling out these forms, you will be asked to share information regarding your general health and lifestyle with the researchers. If you meet the criteria for the study, the researchers will measure your height and weight and you will perform a cardiorespiratory fitness test. During this assessment, an exercise test will be conducted to determine your maximal oxygen uptake (VO_{2max}). To do this, you will ride a stationary cycle at an initial workload that is 'fairly easy'. Workload will be increased every few minutes during the test. You will be encouraged to continue to cycle until you request to stop due to fatigue or are unable to continue at a cadence >50 revolutions per minute. Finger sticks to obtain blood for analysis of glucose and lactate will be taken every two minutes during exercise until fatigue. In order to be included as a participant in the study, you must achieve a VO_{2peak} of $\geq$ 40 ml/kg/min if female or $\geq$ 45 ml/kg/min if male. If you meet these criteria, you will be asked to report back to the laboratory for a familiarization trial.

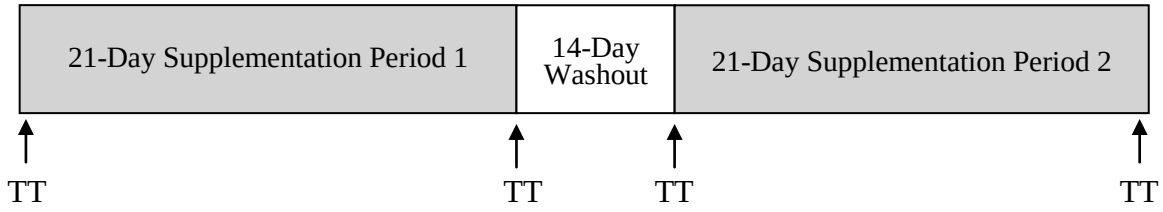
Familiarization Trial: (n = 1 trial)

Within 14 days of the pre-testing trial, you will be asked to report to the laboratory for a familiarization trial. This trial will be identical to the treatment trials, with the exception that no blood or muscle biopsy samples will be taken. Please see the detailed description of the treatment trials for a more complete understanding of the familiarization trial, noting again that no blood or muscle biopsy samples will be taken.

Treatment Trials: (n = 4 trials)

Within 5-14 days of the familiarization trial, you will be asked to perform the first of four treatment trials (TT). The treatment trials will take place before and after 21 days of sports formula supplementation and before and after 21 days of placebo supplementation. You will be randomly assigned to receive either 1000 mg/day of SR2W-1 or 1000 mg/day of placebo. The SR2W-1 supplement is an all-natural sports formula comprised of herbal and fungi extracts and the placebo capsule will look identical but will be filled with flour. You will be asked to ingest 2 x 500 mg capsules every morning for 21 days. To confirm compliance to supplementation, you will be asked to text, e-mail, or call a specified member of the investigative team once a day after the second ingestion (42 separate contacts). Ten days following the first 21-day supplementation period (and treatment trial) you will be asked to replicate the first portion of the study with the alternative supplement (placebo or SR2W-1). See next page for a general study schematic. For each treatment trial, you will be asked to report to the laboratory in the morning after 10-12 hours of overnight fasting. You will be permitted to drink water during the fast.

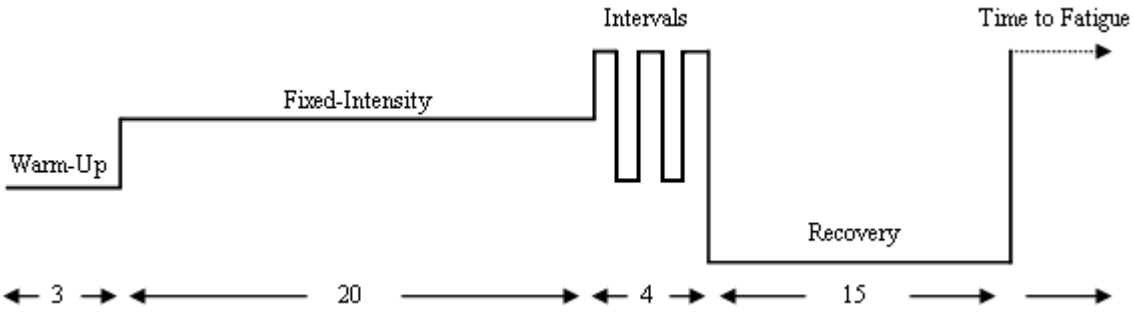
Treatment Periods:



Exercise Protocol

Each trial will last approximately 60-90 minutes in duration with the exercise protocol (shown below) lasting approximately 50 minutes. Following a 3-minute warm-up, you will ride for 20-minutes at a hard intensity (approximately 85% VO_{2peak}), upon which you will be asked to complete 3 x 1 minute intervals at an intensity associated with your VO_{2peak} (determined during the pre-testing trial). Each of these intervals will be separated by 30 seconds of riding at the same intensity as the preceding 20-minute ride. Following the final interval you will be provided with 15 minutes of recovery. The recovery phase will be followed by a ride until fatigue performed at an intensity associated with your VO_{2peak}. You are encouraged to treat this aspect of the trial as if it is a competitive event and to ride until you voluntarily stop or until you can no longer continue at a cadence of 50 revolutions per minute.

Exercise Protocol:



Blood Draws

A catheter will be inserted into a vein in the upper forearm approximately 10 minutes prior to the exercise protocol. 15 blood draws will be performed at various timepoints during the protocol and the catheter will remain in place until after the final blood draw. The catheter minimizes the number of times that a needle is inserted. During each sample, small amounts of blood (~3 milliliters) will be obtained and utilized to measure lactate, pH, and glucose. The total amount of blood obtained during each trial will be approximately 39 ml or 180 ml over the course of the entire study. This amount is similar to 50% of a can of soda or 38% of the amount given when donating blood in a single session.

Metabolic Measurements

Metabolic measurements such as oxygen uptake, ventilation, etc. will be measured using a SensorMedics metabolic cart. To do this, you will be asked to breathe through a mouthpiece/breathing apparatus that collects your expired breath during the entire 20-minute fixed-intensity ride.

Ratings of Perceived Exertion

You will be asked to provide subjective ratings of your exertion level at various timepoints throughout the exercise protocol. You will do this by pointing to your corresponding level of exertion (rated numerically from 6-20) on a Borg RPE scale.

Heart Rate

Your heart rate will be measured using a Polar heart rate monitor that will be worn around your chest during each exercise session.

Skeletal Muscle Circulation

During the 15-minute recovery period, thigh blood flow may be measured with ultrasound equipment – similar to what is done during fetal examinations during pregnancy. Ultrasound gel will be applied to your quadriceps and a plastic ultrasound probe will be held in contact with the skin for only a few minutes.

In addition to the measures outlined above, you are being asked to volunteer for either a <u>muscle function - central fatigue test</u> OR a <u>thigh muscle biopsy</u>, as described below. The muscle function-central fatigue test will be performed during the familiarization trial and each treatment trial, while the muscle biopsy will only be performed during each treatment trial.

Muscle Function – Central Fatigue Test

You will be asked to complete a maximal strength test prior to each trial, immediately following the 3 x 1-minute intervals and towards the end of the 15-minute recovery period. During this test, you will be seated in a modified chair, and asked to push as hard as possible against a shin pad that will be connected to a force transducer for 4 repetitions with a minute rest in between. During this strength test, you will also perform an electrically stimulated contraction. Two adhesive patches will be placed on the skin of your thigh. These electrodes will be used to apply electrical stimulation to the thigh muscle at an intensity, which produces a contraction force equal or greater to what you can generate on your own. The stimulation intensity will be set below the maximum stimulation intensity you can tolerate, similar to a TENS unit used in physical therapy.

_____ I agree to participate in the muscle function – central fatigue test

Muscle Biopsy

A total of 4 muscle biopsies (one biopsy during each treatment trial) from the thigh will be obtained for this study protocol. Prior to each biopsy, the skin at the biopsy site will be cleaned with povidone-iodine topical anti-septic and numbed by an injection of a local anesthetic (similar to what is done at the dentist). When the area is numb (5 minutes), a small 1/4 inch incision will be made in the skin and a needle will be inserted briefly (2-3 seconds) into the muscle to remove a piece of muscle about the size of a pea. The incision will be pulled closed with a band-aid and the area over the incision will be covered with an elastic pressure bandage. The entire procedure will take a total of approximately 10-15 minutes, with the actual biopsy lasting only a few seconds.

_____ I agree to participate in the muscle biopsy procedure

Dietary and Exercise Controls

You are to maintain consistent dietary habits for 72 hrs prior to each trial, and to complete a diet record (see Attachment 4) for the 24 hrs preceding each trial. While avoiding heavy exercise for 48 hrs prior to each trial, you will also be asked to maintain consistent physical activity habits starting 72 hrs prior to the first treatment trial until the completion of the final treatment trial (~55 days), and to record all physical activity performed during the 72 hrs preceding each trial (see Attachment 5). You are to consume your final 'self-selected' meal no less than 10 hrs prior to the start of the treatment trials (i.e. dinner on the evening prior to testing). After this time, you are to consume only water *ad libitum* until the end of each treatment trial (a total of ~11 to 13 hrs of fasting with *ad libitum* water intake).

Risks

You are expected to be honest about disclosing all known risk factors to the researcher. There are no known risks associated with supplementing with the SR2W-1 sports formula. However, there are some risks associated with high does of some ingredients in isolation. SR2W-1 is a proprietary blend of herbs and fungi (primary ingredients: Enoki Mushroom, Eluthero Extract, Reishi Mushroom, Tangerine Extract, Cordyceps Mushroom, and Asian Ginseng). Although highly unlikely, it is possible that one or more of the ingredients can adversely impact pathophysiological conditions (blood clotting disorders such as hemophilia or thrombocytopenia) or medications (Coumadin, digoxin/digitalis). You will be pre-screened for each of the aforementioned conditions as well as for known allergies to the ingredients. You will also be provided with an adverse event/side effect form for you to record any adverse effects of supplementation.

According to the American College of Sports Medicine, the risks associated with maximal exercise/testing for healthy individuals are very minimal. If you do not meet the criteria for "low risk", you will not be allowed to participate in the study. In the unlikely event of cardiac or other complications during exercise, an emergency plan is in place. This includes immediate access to a phone to call emergency personnel. In addition, each of the investigators is CPR certified.

The exercise protocol may result in minor-moderate levels of muscle soreness and fatigue for 1-2 days following each exercise session. However, the level of muscle soreness is expected to be lower than levels normally experienced when people perform other 'normal' activities that are not part of their regular exercise routine (i.e. if a cyclist played a game of basketball with friends for 2 hours).

The risks of blood draws include possible mild bruising, and the risk of transfer of blood-borne pathogens. This risk is considered to be very minimal, and all safety precautions for handing blood samples will be followed according to OSHA protocols. The investigators have been trained in phlebotomy and completed JMU blood-borne pathogen training.

The risks of the interpolated twitch technique include temporary "tingling" or "pulsing" sensation (for 1-2 seconds), which you may perceive as uncomfortable.

The risks associated with the muscle biopsy technique include a possible dull pain during the administration of the anesthetic and the biopsy procedure, and delayed soreness for one to two days following the biopsy. Sterile procedures will be used during the biopsy procedure to minimize these risks. There is a small risk of bleeding, infection, and scarring of the skin. Temporary numbness of the skin near the biopsy site occurs rarely. You may feel lightheaded and there is a slight risk of fainting. Following the biopsy you will be provided with a 'biopsy care package' that will include instructions for care, band-aids, and alcohol pads. As a precaution, a member of our research team will contact you via phone or e-mail approximately 48-hrs following the biopsy to confirm that you are recovering/healing from the biopsy appropriately. You are also encouraged to contact a member of our research team if you have any concerns about your recovery. There is a small risk of an allergic reaction to the local anesthetic used during the muscle biopsy procedure. Symptoms may include an itching sensation of the skin, difficulty breathing, fainting, and shock. Allergic reactions to the local anesthetic used are extremely rare. You will be pre-screened, as part of the medical history document, for any known allergic reaction to local anesthetics.

Benefits

The benefits associated with this project include a free VO_{2max} assessment, and a $150 payment for study completion. If you volunteered to undergo the muscle biopsy procedure you will receive an additional $100 payment for study completion for a total of $250. In the case that you freely withdraw from the

study, payments will be pro-rated as follows: non-biopsy subjects = $37.50 for the completion of each treatment trial, biopsy subjects = $62.50 for the completion of each treatment trial.

Inquiries

If you have any questions of concerns, please contact Dr. Nicholas Luden at ludennd@jmu.edu or (540) 568-4069. In the case of any immediate concerns or adverse reactions during the study, contact Dr. Luden on his cell phone (540) 746-6134.

Confidentiality

All data and results will be kept confidential. You will be assigned an identification code. At no time will your name be identified with your individual data. The researcher retains the right to use and publish non-identifiable data. All data will be kept secured in a locked cabinet. Final aggregate results will be made available to participants upon request.

Freedom of Consent

Your participation is entirely voluntary. You are free to choose not to participate. Should you choose to participate, you can withdraw at any time without consequences of any kind.

I have read this consent form and I understand what is being requested of me as a participant in this study. I freely consent to participate. I have been given satisfactory answers to my questions. The investigator provided me with a copy of this form. I certify that I am at least 18 years of age.

Name of Subject (Printed)	Name of Researcher (Printed)
Name of Subject (Signed)	Name of Researcher (Signed)
Date	Date

For questions about your rights as a research subject, you may contact the chair of JMU's Institutional Review Board (IRB). Dr. David Cockley, (540) 568-2834, cocklede@jmu.edu.

Appendix III

AHA/ACSM Health/Fitness Facility Pre-participation Screening Questionnaire
Assess your health status by marking all *true* statements

History

You have had:

_____ a heart attack

_____ heart surgery

_____ cardiac catheterization

_____ coronary angioplasty (PTCA)

_____ pacemaker/implantable cardiac

_____ defibrillator/rhythm disturbance

_____ heart valve disease

_____ heart failure

_____ heart transplantation

_____ congenital heart disease

If you marked any of these statements in this section, consult your physician or other appropriate health care provider before engaging in exercise. You may need to use a facility with a ***medically qualified staff***.

Symptoms

_____ You experience chest discomfort with exertion

_____ You experience unreasonable breathlessness

_____ You experience dizziness, fainting, or blackouts

_____ You take heart medications

Other Health Issues

_____ You have diabetes

_____ You have asthma or other lung disease

_____ You have burning or cramping sensation in your lower

legs when walking short distances

_____ You have musculoskeletal problems that limit your physical activity

_____ You have concerns about the safety of exercise

_____ You take prescription medication(s)

Cardiovascular risk factors

If you marked two or more of the statements in this section, you should consult your physician or other appropriate health care provider before engaging in exercise. You might benefit from using a facility with a ***professionally qualified exercise staff*** to guide your exercise program.

_____ You are a man older than 45 years

_____ You are a woman older than 55 years, have had a hysterectomy, or are postmenopausal

_____ You smoke, or quit smoking within the previous 6 months

_____ Your blood pressure is > 140/90 mmHg

_____ You do not know your blood pressure

_____ You take blood pressure medication

_____ Your blood cholesterol level is > 200 mg/dl

_____ You do not know your cholesterol level

_____ You have a close blood relative who had a heart attack or heart surgery before age 55 (father or brother) or age 65 (mother or sister)

_____ You are physically inactive (i.e. you get < 30 minutes of physical activity on at least 3 days of the week)

_____ You are > 20 pounds overweight

You should be able to exercise safely without consulting your physician or other appropriate health care provider in a self-guided program or almost any facility that meets your exercise program needs.

"Negative risk factors"

_____ High-serum HDL Cholesterol ≥ 60 mg/dl

_____ None of the above

Appendix IV

Subject Prescreening Information

Please Complete the Following:

Gender: Male Female (circle one)

Age (yrs):

Height (inches):

Weight (lbs):

Average Exercise Habits over the Past 2 Months:

Avg. # days of exercise per week:

Avg. # of days of aerobic exercise per week:

Avg. # of days of cycling per week:

Do you have a muscle or joint injury that precludes the completion of the exercise protocol?

Do you currently use medications for relief of pain and/or soreness?

Do you have a blood clotting disorder (haemophilia, thrombocytopenia, etc)?

Do you currently use blood-thinning medications (Coumadin, etc)?

Do you currently use cardiac medications (Digoxin, Digitalis, etc)?

Are you allergic to any type of herbal supplement or one of the following substances?

Enoki Mushroom
Eleuthero Extract
Reishi Mushroom
Tangerine Extract
Cordyceps Mushroom
Asian Ginseng
Soy
Rice
Corn

Are you a vegetarian?

Are you allergic to local anesthetics (numbing agents) such as Lidocaine (Xylocaine, Novocain, etc)?

Have you had Novocain administered at the dentist?

Appendix V

24-HOUR DIET RECORD

*Subject number*_____ *Date*_____ *Day of Week*_____

Time	Food and/or Drink	Method of Preparation	Quantity Consumed	Brand Name

Adapted From: Lee RD, Nieman DC. *Nutritional Assessment.* 2nd ed. United States of America: Mosby; 1996

INSTRUCTIONS FOR KEEPING YOUR 24-HOUR FOOD RECORD

Keep your record for three days per trial. You will include the day before, the day of, and the day after each trial. Include all meals, snacks, nibbling, and beverages including water and cocktails

1. Fill out the date and day of the week at the top of food record sheet

2. Record the time you consumed your food and/or drink. To be most accurate, fill out the food record as soon as you finish eating.

3. List the first food and/or drink you consumed when you began your day and continue to record until you consume your last food and/or drink of your day (usually before bedtime)

4. List each food and/or drink on a separate line
   Example: cereal with milk, cereal and milk should each be on separate lines

   spaghetti, noodles and sauce should each be on separate lines

   Combination foods:

   List parts of food on separate lines

   Include preparation method, quantity, and brand name of each food

   Example: Sandwich (4 oz healthy choice turkey, 2 slices Sara Lee wheat bread, 1 tbsp Hellman's light mayo, 2 oz Kraft American cheese, 1 slice of red fresh tomato)

5. Record the method of preparation
   Example: fried, baked, grille, salt, oil (olive, canola, corn, other) butter or

   margarine, spices, etc.

6. Record quantity consumed

Do not record any food not eaten

Example: made two cups of vegetables but ate half so you would record one cup

Quantity of food and/or drink

Example: cups, ounces, liters, grams, each, or other unit of measure

Example: 1 cup of vegetables, 4 ounces of meat, one medium apple

7. Record brand name
   Example: fast food chain name and/or package name

   Example: Wendy's, Betty Crocker, Lean Cuisine, Gatorade, Thomas Bagel

8. Place any helpful food labels in manila envelope that is attached to folder

USE THE FOLLOWING TO HELP DETERMINE PORTION SIZES AND TYPES OF FOODS

	PLEASE SPECIFY
Beverages	Sugar or creamer? Regular or sugar-free? Alcohol content? Name of drink and ingredients (if mixed drink)
Breads	Butter or margarine added?
Cereal/Milk	Milk, sugar, or fruit added? The type of milk? (skim, 1%, 2%, whole) Cereal: dry or cooked measure?
Dairy	Is yogurt fruited or plain? % fat of milk or yogurt? Indicate brand name of cheese substitute and/or nondairy creamer.
Desserts	Whipped topping added? Frosting? Fat modified (i.e., reduced)? Sugar-free?
Eggs	Preparation method (scrambled, hard-boiled, etc)? Fat used in cooking?
Fast Food	What restaurant? If not a national fast food chain, describe food in detail Size order of fries? Super-size? Extra toppings on sandwich?
Fats/Oils	Regular or salt-free? Stick, tub, or liquid margarine? Reduced calorie or diet product?
Fish	Water or oil packed (fresh or canned)? Baked or fried (With batter or without)? Type of fat added? Raw or cooked weight?
Fruit	Sweetened or unsweetened? Fresh, canned, or frozen? With or without skin?
Meats	Visible fat removed? Light or dark meat? Raw or cooked?
Sugars and Sweets	Regular or reduced-calorie? Don't forget hard candy as well as chocolate.
Vegetables	Raw or cooked? Fresh, frozen, or canned? Low-sodium or regular? Added fat or sauce?

Helpful Hints with Portion Sizes

- 1 teaspoon (5 ml)
  - about the size of the top half / tip of your thumb
- 1oz (28g)
  - approximately inch cube of cheese
  - volume of four stacked dice
  - slice of cheese is about the size of a 3 1/2 inch computer disk
  - chunk of cheese is about as thick as 2 dominoes
  - 1 handful (palm) of nuts
- 2 ounces (57 g)
  - 1 small chicken leg or thigh
  - 1/2 cup of cottage cheese or tuna
- 3 ounces (85 g)
  - serving of meat is about the size of a deck of playing cards (3 exchanges)
  - the size of the palm of your hand
  - 1/2 of whole chicken breast
  - 1 medium pork chop
  - 1 small hamburger
  - unbreaded fish fillet
- 1/2 cup (118 ml)
  - fruit or vegetables can fit in the palm of your hand
  - about the volume of a tennis ball
- 1 cup (236 ml)
  - about the size of a woman's fist
  - breakfast cereal goes halfway up the side of a standard cereal bowl
  - broccoli is about the size of a light bulb
- 1 medium apple = A tennis ball

Appendix VI

Daily Activity Records

Subject #_____ Trial #_____ Date:_____

Date	Type of Exercise Performed	Duration of Exercise (minutes)	Intensity of Exercise (use scale below)

Intensity Scale

6
7 Very, very light
8
9 Very light
10
11 Fairly light
12
13 Somewhat hard
14
15 Hard
16
17 Very hard
18
19 Very, very hard
20

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